Seasonal Fluctuations of Trace Elements from Different Habitats of Orbetello Lagoon (Thyrrenian Sea, Italy)

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Abstract This study evaluated seasonal fluctuations of trace elements of major ecotoxicological concern in sediments and their uptakes by the aquatic vegetation indifferent undisturbed habitats from the Orbetello lagoon, correlating measured levels to abiotic and biotic drivers to scale the significant of their effect on observed seasonal variability of trace elements. Results show that under natural undisturbed conditions, observed seasonal fluctuations in different habitats are statistically correlated to temperature, salinity, and turbidity of water and total nutrients in sediments. These variables and the habitat type dominated by macroalgae (*C. linum*) play a significant role as drivers of variability for measured trace elements in sediments. This study represents a reference undisturbed condition of natural seasonal trends of trace elements in different habitat types before the occurrence of numerous impacting activities on sediments and could represent a useful baseline for further management evaluations after the occurrence of sediment disturbance actions planned for the near future.

Coastal lagoons are defined physically controlled ecosystems (Sanders 1968) due to the high variability of environmental descriptors that fluctuate appreciably daily as well as yearly (Barnes 1980; Phleger 1981 Nixon 1982; Kjerfve 1994). Rivers and freshwater inputs mainly influence some lagoons, whereas others are tidal dependent and are similar to small estuaries (Coelho et al. 2007). Some common features (i.e., water depth, seawater exchanges, hydrodynamic fluxes, winds) are drivers that significantly affect the spatial and temporal scales of natural variability. Natural processes (rains, water input, erosion, and weathering) and limited lagoon/seawater exchanges drive nutrient enrichments from the surrounding watersheds. The naturally high variability favours the presence of a great number of physical and ecological gradients, which enhance biodiversity and the coexistence of numerous habitat types (Zaldívar et al. 2003). Sediments represent the final bulk compartment for numerous impact sources of both macronutrients and chemical contaminants as well as trace elements (Hedges and Keil 1995), urban impacts, municipal wastewater treatment plant effluents, inland agricultural practices, fish farm activities, industry, and many others (Kormas et al. 2001; Muslim and Jones 2003; Newton et al. 2003; Zaldívar et al. 2003). In such ecosystems, abiotic and biotic components are strongly linked together and multiple natural factors could affect and modulate ecological relationships in a very short time frame. As example, quick and progressive phytosociological changes are associated with an increase of nutrient load producing the switch from the presence of phanerogams towards microalgae proliferations within few months (Knoppers 1994; Morand and Briand 1996; Souza et al. 2003), inducing as consequence, measurable physical–chemical and biotic modifications in both water and bottom habitats (Chessa et al. 2005). Numerous studies have been performed in recent decades to understand the spatial and temporal patterns of the main abiotic drivers of water variability producing as result various classification criteria and indices concerning trophic levels, water quality, and eutrophication crisis risk assessment (Carlson 1977; Karydis et al. 1983; Herrera-Silveira et al. 2002; Gikas et al. 2006; Coelho et al. 2007). Some of these indices are a

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structural part of the EU Marine Strategy Framework Directive and the Water Framework Directive. Until recently, water trophic levels have been considered the main driver of macrophyte proliferation and species dominance in coastal lagoons (Giusti and Marsili-Libelli 2006). Nevertheless, recent papers reconsider the role of sediment, and a closer relationship between sediment descriptors and the distribution of aquatic vegetation is documented showing a strong link between abiotic and biotic components of the lagoon ecosystem. Grain-size, pH, Eh, and nutrients are key sediment drivers for the presence and establishment of seagrass in eutrophic ecosystems (Ben Charrada 1995; Plus et al. 2003; Zaldívar et al. 2003 and citations therein; Renzi et al. 2007; Giusti et al. 2010). Trace elements accumulated in sediments are remobilized by many natural processes, such as wind or bioturbation (Wainright and Hopkinson 1997), and could interact in such systems with the biotic component. A recent study found that the fate of contaminants in sediments due to interaction between the biogeochemical cycles of major elements (i.e., C, P, S, Fe, Mn) is somehow linked to seasonal dynamics occurring in sediments (Zaldívar et al. 2003). Currently, the major environmental concern in coastal lagoons is solid analysis of synergic dynamics involving sediment resuspension, dispersion and release of pollutants in water, eutrophication levels, aquatic vegetation dynamics, and trends (Zaldívar et al. 2003 and citations therein). Despite these needs, current knowledge is limited, and exhaustive multi-level statistically based research on drivers that affect trace element seasonality in undisturbed habitat have not yet been developed in coastal lagoons.

This study was designed to define seasonal natural variability of trace elements of ecotoxicological concern (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn) in surface sediments and aquatic vegetation tissues collected in different undisturbed habitat type of a coastal lagoon. Furthermore, this study evaluated aquatic vegetation uptakes and the seasonal variability and it scales, on a statistical basis, the significant of different abiotic and biotic factors as drivers of the observed seasonal variability in different habitat types.

Materials and Methods

Study Area and Sampling Strategy

A well-known coastal lagoon ecosystem characterized by a variety of habitat types and severe trace element pollution (Orbetello lagoon, Italy) was selected as a case study. The Orbetello lagoon is located in Southern Tuscany (Western Coast of Italy), between 42°25′ and 42°29′ Lat. North and 11°10′ and 11°17′ Long. East. It has an average depth of 98.2 (±31.9) cm and covers a total surface of 25.25 km^2 and it is divided into two connected basins called Western (15.25 km^2, 25–130 cm depth) and Eastern (10.00 km^2, 30–170 cm depth) by an artificial dam (Consorzio Pisa Ricerche 2002). Three communicating channels ensure lagoon–seawater exchanges, although overall water circulation is drastically reduced by the presence of the central dam and by the geomorphology of the lagoon bottoms (Consorzio Pisa Ricerche 2002; Aminti et al. 2003; Innamorati and Melillo 2004). We a priori excluded the Western basin, because it was interested over the years by harvesting boat activities to control macroalgae proliferation (Aminti et al. 2003; Di Vincenzo 2006) producing sediment disturbance and structural changes in bottoms that could affect this study (Lenzi et al. 2005). The study area is a small, 9 hectares, undisturbed area located in the Eastern basin of the Orbetello lagoon (Fig. 1) and characterized by a stable presence of three different habitat types: stable bare sediment (HT-I), phanerogams dominance (*Ruppia* spp., HT-II), dense macroalgae proliferation (HT-III) documented by literature during the sampling period (Giovani et al. 2010, 2014). Samplings were performed within 2005–2007 using a nested-random replicated sampling designed to reduce Type I and Type II errors according to a logic model and to avoid pseudoreplicates (Underwood and Beyond 1992; Underwood 1993; Underwood and Chapman 2003; Benedetti-Cecchi 2004). The logic model adopted is based on three factors: bottom type (three levels, fixed); season (number of levels based on the matrix's natural variability, random and orthogonal); and replicates (three levels, random). With regard to the season factor, water data were collected on 15 levels ($n = 180$), whereas sediment and biomass data were collected on 5 levels ($n = 60$). Sampling sites were georeferenced and marked using the *balisage* technique. Waters parameters were determined in situ. Superficial sediments (0–5 cm) were collected in three replicates at each sampling site using an 8-cm internal diameter HDPE core tube. Eh and pH values were measured in sediments using field probes (*Crison* mod. *pH25* with a combined pH electrode, and *Crison* mod. *Eh25*) as described by Giusti et al. (2010). Collected sediment samples were extracted from the core tube, homogenized, split into two aliquots and stored at +4 °C in HDPE until they were analyzed. In HT-II and HT-III, biomass samplings were performed simultaneously with sediment to determine total biomass, levels of trace element in tissues, and bioaccumulation rates. The number of sampling replicates in each sampling site was established during the preliminary survey in relation to local biomass density as per Downing and Anderson (1985). Biomass density was evaluated using a

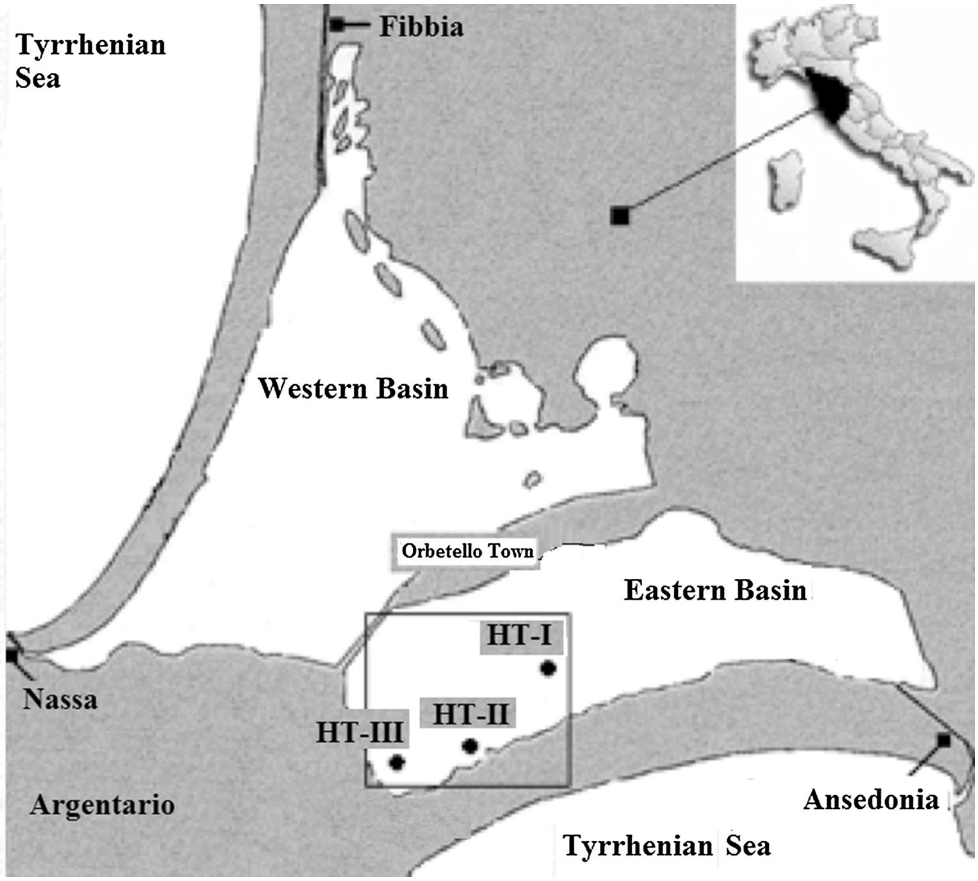


Fig. 1 Study area localization in the Orbetello lagoon. Geographical localization of the Orbetello lagoon (42°25′ and 42°29′ Lat. North and 11°10′ and 11°17′ Long. East), the study area (Eastern basin, black square) and Habitat types (HT, black dots) are represented. Sampling replicates within each HT are not represented. *Notes* HT-I = bare sediment, complete absence of aquatic vegetation during the whole year), HT-II = dominance of phanerogams (Ruppia spp.), HT-III = dominance of macroalgae (*C. linum*)

2500 cm^2 (50- × 50-cm frame) surface test in three replicates for each sampling site.

Water

In water, temperature (T, °C), pH (pH units), conductivity (SpC, $mScm^{-1}$), salinity (S, PSU), reduction–oxidation potential (ORP, mV), dissolved oxygen (DO, %; mg L^{-1}), turbidity (Turb, NTU), and total suspended solids (TDS, g L^{-1}) were measured. Data were recorded at the water/sediment interface using a multi-parameter probe (Corr-TeckHydrometria, mod. Datasonde 5A).

Sediments

pH (pH units) and reduction–oxidation potential (ORP, mV) were measured in the field. Collected sediments were wet weighed, dried, and analyzed to determine humidity (U, %), total carbon (TC, %), total organic carbon (TOC, %), total nitrogen (TN, %), total phosphorous (TP, %), total sulphur (TS, %), carbonates ($CaCO_3$, %), trace elements (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn, mg kg^{-1}), aluminium (Al, %), and iron (Fe, %). Sediment grain-size was determined according to ICRAM 2S (2001) by sieving (DIN EN ISO 9001), whereas humidity was determined according to ICRAM 1S (2001). TC, TOC, TN, and TS were quantified using a CHNS analyzer (Perkin Elmer, mod. CHN/O 200 *Analyzer* with a thermo-conductivity detector, TCD) by direct total flash combustion (ICRAM 4S 2001). TP was quantified by acid digestion and spectrophotometry (Perkin Elemer, mod. 6505 UV–Vis) after colorimetric reaction (Aspila et al. 1976). Carbonates ($CaCO_3$) were determined by titration (Hülsemann 1966). Trace elements were determined after mineralization with an H_2O_2–HNO_3 mixture in a microwave oven (Milestone, mod. ETHOS D Microwave Labstation) by US-EPA Method 3051A. Aluminium (Al), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), and nickel (Ni) were quantified by means of atomic absorption spectrometry with electrothermal atomization (GF-AAS, Perkin-Elmer, mod. AAnalyst 700). Zinc (Zn) was determined by means of atomic absorption spectrometry with flame atomization (Flame-AAS) as per US-EPA 6010B and US-EPA 7010 methods. Mercury (Hg) and arsenic (As) were determined, respectively, by atomic absorption via cold vapour generation (CV-AAS) as per US-EPA 7473 and by atomic absorption after the hydride generation as per the US-EPA 7061A method.

Aquatic Vegetation

In aquatic vegetation, total density (TD, g^{-2}), humidity (U, %), total carbon (TC, %), total organic carbon (TOC, %), total nitrogen (TN, %), total phosphorous (TP, %), total sulphur (TS, %), trace elements (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn, mg kg^{-1}), aluminium (Al, %), and iron (Fe, %) were measured. Collected biomass samples were washed with synthetic marine water to remove sediment particles. Epiphytes were manually removed from phanerogam leaves, and wet weight (g_{ww}) was determined as per Westlake (1969). On an aliquot of sample, dry weights were determined after desiccation at 75 °C for 48 h as per Elliott (1971) to evaluate tissue water content (U, %). Tissue samples were lyophilized and analyzed determine amounts of macronutrients (TOC, TN, TP, TS) and trace elements (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn), following the same analytical methods previously described for sediments.

Quality Assurance and Quality Control

Chemicals and reagents were analytical grade (Sigma-Aldrich) and glassware was carefully washed to avoid sample cross-contamination. Procedural blanks and standard reference materials (SRM) were analyzed to check recovery and reproducibility. Blanks were prepared before test samples using the same analytical procedures. A solvent/matrix blank was analyzed every 15 samples to check detector response. Sediment and biota SRMs were analyzed in statistical replicates ($n = 10$) to calculate averages and standard deviation of recoveries (SD) for each tested matrix. In Table 1, average measured value, SD, and average percentage of recovery are reported for each variable measured in sediment and biological matrices. SRM type and origin also are indicated. Analytical concentrations were not recovery corrected. Limit of quantification (LOQ) are reported in the same table.

Data Analyses

Univariate statistics (averages, standard deviation SD, maximum, minimum) were performed using GraphPad Prism (GraphPad Software, San Diego, CA, www.graphpad.com). Pearson's (1894) correlation matrix ($p < 0.01$) and box-plot representations were calculated in abiotic and biotic matrices. Student's t test and F-test were calculated to evaluate significant of observed segregations. Multivariate statistics were performed using Primer-E package v6.0 (Plymouth Marine Laboratory, UK) software (Clarke and Warwick 2001) by calculating Euclidean distances resemblance matrix after square root and $\log(x + 1)$ function transformation and normalization of field data according to literature (Clarke and Green 1988; Clarke and Warwick 2001). Principal Components Analyses (PCA) was applied to investigate correlations and similarities between variables of abiotic and biotic matrices (Chatfield and Collins 1980; Legendre and Legendre 1998). Non-metric multidimensional scaling (*nm*-MDS) ordinations were plotted (Kruskal stress formula 1; minimum stress level = 0.01; 9999 recalculations) as per Shepard (1962) and Kruskal (1964) according to Somerfield and Clarke (1995). *nm*-MDS on variables (trace elements) in sediment also was calculated to test the level of significance with regard to abiotic and biotic factors of interest. ANOSIM test one-way was performed to evaluate significant. Uptake of trace elements by aquatic vegetation (bioaccumulation factors, BaRs) was calculated for each species considered in each season as a pure ratio between the concentration of a given element measured in tissues and the concentration of the same element measured in sediment from the same HT (Usero et al. 2003).

Results

Sediments

Levels and Seasonal Trends

Principal features of sediments collected in different Habitat Types (HTs) are reported in Table 2. More than 90% of sampled sediments show a particle diameter size less than 125 μm for their total amount of dried weight. The sediment fraction lower than 63 μm of diameters is highly represented by collected samples from HT-I and HT-III. Very fine sands (63–125 μm) dominate HT-II, whereas silts (63 μm) are the highest in HT-III. In Fig. 2, levels and seasonal trends in sediment are shown by means of the box-plot technique. Nutrients in sediments (TC, TOC, TN) show similar seasonal trends and the highest variability among HTs in summer. TP, however, shows a different seasonal trend compared with other macronutrients with maximum values and highest variability among HTs recorded in autumn. Fe shows an inverse trend than TP, with the highest variability among HTs recorded in summer and the lowest in autumn. Cu, Pb, and Zn are strongly related and show wide variability among HT sand with the highest average values in summer and the lowest average value in spring. As and Cr correlate together and show the highest value in winter and the lowest in spring–summer. Hg, Mn, and Ni show very different seasonal trends: Hg and Mn show many outliers, with comparable winter and summer levels but very low autumn levels, as well as homogeneous values among HTs. Ni shows the

Table 1 Quality assurance and quality control

Matrix	Variable	Meas. unit	SRM	Note n.	Av. SRM	Av. meas.	SD	% Av. rec.
S	TOC	%	Cysteine		29.99	29.33	0.13	97.8
S	TOC	%	SRM1944	2	4.4	4.46	0.33	101.4
S	TN	%	Tibet Soil	1	0.125	0.118	0.005	94.4
S	TP	mg kg^{-1}	SRM1646a	3	270	267.5	3.3	98.9
S	TS	%	Cysteine		10.00	9.99	0.02	99.8
S	$CaCO_3$	%	Bicarbonate		5.00	4.91	0.12	98.7
S	Al	mg kg^{-1}	LGC6156	4	19,000	21,090	322	111
S	As	mg kg^{-1}	LGC6187	4	24	23	1.5	95.8
S	Cd	mg kg^{-1}	LGC6187	4	2.7	2.8	0.2	103.7
S	Cr	mg kg^{-1}	LGC6187	4	84.6	81.6	2	96.4
S	Cu	mg kg^{-1}	LGC6187	4	87.8	94.8	1.2	108
S	Fe	mg kg^{-1}	LGC6187	4	23,644	24,590	112	104
S	Hg	mg kg^{-1}	SRM2709	5	1.4	1.44	0.05	103
S	Mn	mg kg^{-1}	LGC6187	4	1241	1389	45	112
S	Ni	mg kg^{-1}	LGC6187	4	34.8	33.3	0.7	95.9
S	Pb	mg kg^{-1}	LGC6187	4	77.3	70.2	2.1	90.9
S	Zn	mg kg^{-1}	LGC6187	4	440	408	16	93
B	TP	mg kg^{-1}	SRM1946	6	1980	1933	102	98
B	As	mg kg^{-1}	SRM1946	6	0.277	0.257	0.012	93
B	Cd	mg kg^{-1}	SRM1946	6	0.0021	0.0017	0.0005	81
B	Cr	mg kg^{-1}	DORM-2	7	34.7	32.6	1.45	94
B	Cu	mg kg^{-1}	SRM1946	6	0.476	0.441	0.054	93
B	Fe	mg kg^{-1}	SR1946	6	4	3.63	0.43	91
B	Mn	mg kg^{-1}	SRM1946	6	0.07	0.065	0.01	93
B	Ni	mg kg^{-1}	DORM-2	7	19.4	18.91	1.13	97
B	Pb	mg kg^{-1}	SRM1946	6	0.7	0.65	0.06	93
B	Zn	mg kg^{-1}	SRM1946	6	3.1	2.97	0.57	96

Standard reference material (SRM) type and origin, average certified value, average measured value, measured standard deviation (SD) and average % of recovery are reported in this table for each considered variable. Limits of quantification (LOQ) were: (1) T (0.1 °C), pH (0.01), SpC (0.01 mS/cm), S (0.1 PSU), ORP (0.1, mV), DO (0.1%; mg L^{-1}), Turb (0.1 NTU), TDS (0.1 g L^{-1}), for water; (2) pH (0.01), Eh (0.1 mV), $CaCO_3$ (0.1%), for sediments; (3) TD (0.1 g^{-2}) for biomass; (4) U (0.01%), TC (0.01%), TOC (0.01, %), TN (0.01, %), TP (0.001%), TS (0.01%), Al, Fe (1 mg kg^{-1}), Cd (0.05 mg kg^{-1}), Pb, Zn (0.1 mg kg^{-1}), Cu, Hg, Mn, Ni (0.01 mg kg^{-1}), Cr, As (0.5 mg kg^{-1}), for both sediment and biomass. S = sediment; B = biomass; 1 = Institute of Environ. Chem. Acad. Sinica Beijing China; 2 = Nat. Inst. of St. and Tec.-New York/NJ waterway Sed.; 3 = National Ins. Standard and Technol.- Estuarine Sediment; 4 = Department of trade and industry as a part of the national measurement system (UK)—Harbour sediment; 5 = National Institute of Standard and Technology—Soil sediments or other materials of a similar matrix; 6 = National Institute of Standard and Technology—Lake Superior fish tissue; 7 = National Research Council Canada—Dogfish

TOC total organic carbon, *TN* total nitrogen, *TP* total phosphorous, *TS* total sulfur, *$CaCO_3$* carbonate of calcium, *Al* aluminium, *As* arsenic, *Cd* cadmium, *Cr* chromium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Ni* nickel, *Pb* lead, *Zn* zinc

highest HTs variability in spring and the lowest in autumn, while very low levels are recorded in summer.

Univariate Statistics

In Table 3, the results of univariate statistical analyses performed on physical–chemical variables in sediments are reported. Minimum (min), first quartile (1st qu.), median, average, 3rd quartile (3rd qu.), maximum (max.), and standard deviation (SD) are shown. Pearson's correlation matrix calculated ($n = 60$, $p < 0.01$) shows a strong positive correlation only between the pH-Eh (0.80) pair. pH also is significant negatively related with U (−0.49), Ni (−0.45), TOC (−0.42), Fe (−0.42), TC (−0.39), Cr (−0.37), Al (−0.34), Cu (−0.31), Mn (−0.29), As (−0.29), Pb (−0.23), and Zn (−0.15). Positive relationships occur

Table 2 Principal features of collected sediments

		HT-I	HT-II	HT-III
Northern	UTM	4,699,755	4,699,345	4,698,649
Eastern	UTM	683,835	682,611	681,602
Water depth	m	1.0	0.8	0.8
pH	pH units	7.5	7.8	7.3
Eh	mV	−320	−200	−340
Sediment Fraction Fine sands (125–63 μm)	%	55	90	20
Sediment Fraction Silt (<63 μm)	%	45	10	80

Physical–chemical characteristics of sediments are reported for each Habitat Types as average data. HT-I = bare sediment; HT-II = dominance of phanerogams (*Ruppia spp.*); HT-III = dominance of macroalgae (*C. linum*)

between Eh and U (−0.60), Al (−0.50), Cr (−0.50), whereas negative relationships occur between Eh-TN (−0.31) and Eh-TP (−0.28). Macronutrients are strongly positively related to U (>0.50) and to each other (>0.70). Average carbonate levels are not significantly correlated to the season but are significantly correlated with HTs ranging within 10.1 ± 0.4% d.w. (HT-III) and 27.0 ± 2.0% d.w. (HT-I). Season and HTs significant affect TS in sediments (approximately +100% is recorded in August in HT-I and HT-III and approximately −70% is in the same season in HT-II). Strong positive relationships also are reported between macronutrients and trace elements (always >0.60) with the exception of Ni, which is not related to organic matter. Al is positively related to organic matter (0.52) and some trace elements (Cr, Fe, Pb, Cu, Zn, As; >0.32) but any relationships observed among Al and Cd, Hg, Mn, and Ni. Iron is strongly positively related with macronutrients (Fe-TP and Fe-TC > 0.80) and almost all other trace elements.

Abiotic and Biotic Drivers of Sediment Variability

Levels and Seasonal Trends in Water

In Fig. 3, levels and seasonal trends in water variables are represented by the box-plot technique. HTs variability is strictly variable-dependent, as evidenced by the ranges of variations reported. HTs are homogeneous for some variables (i.e., T, S, SpC), whereas in other cases higher fluctuations are recorded (i.e., pH, ORP, DO, Turb). On a seasonal basis, as attended, T shows wide fluctuations, with minimal values in winter and maximum values in summer. Except for spring, the same seasonal trend is observed for S and SpC. The whole study area is characterized by very high water salinity (>40 PSU) from spring to autumn. In autumn, pH is approximately 8.1, whereas from winter to summer, levels are above 8.4, with significant differences related to HTs. ORP shows very high fluctuations with a summer range of 100–250 mV. DO and SpC show a similar seasonal trend, but DO evidences greater fluctuations linked to HTs. The highest values of Turb and TDS are recorded in autumn. These variables show comparable trends with homogeneous levels in spring and high HTs variability in all of the other seasons.

Univariate Statistics in Water

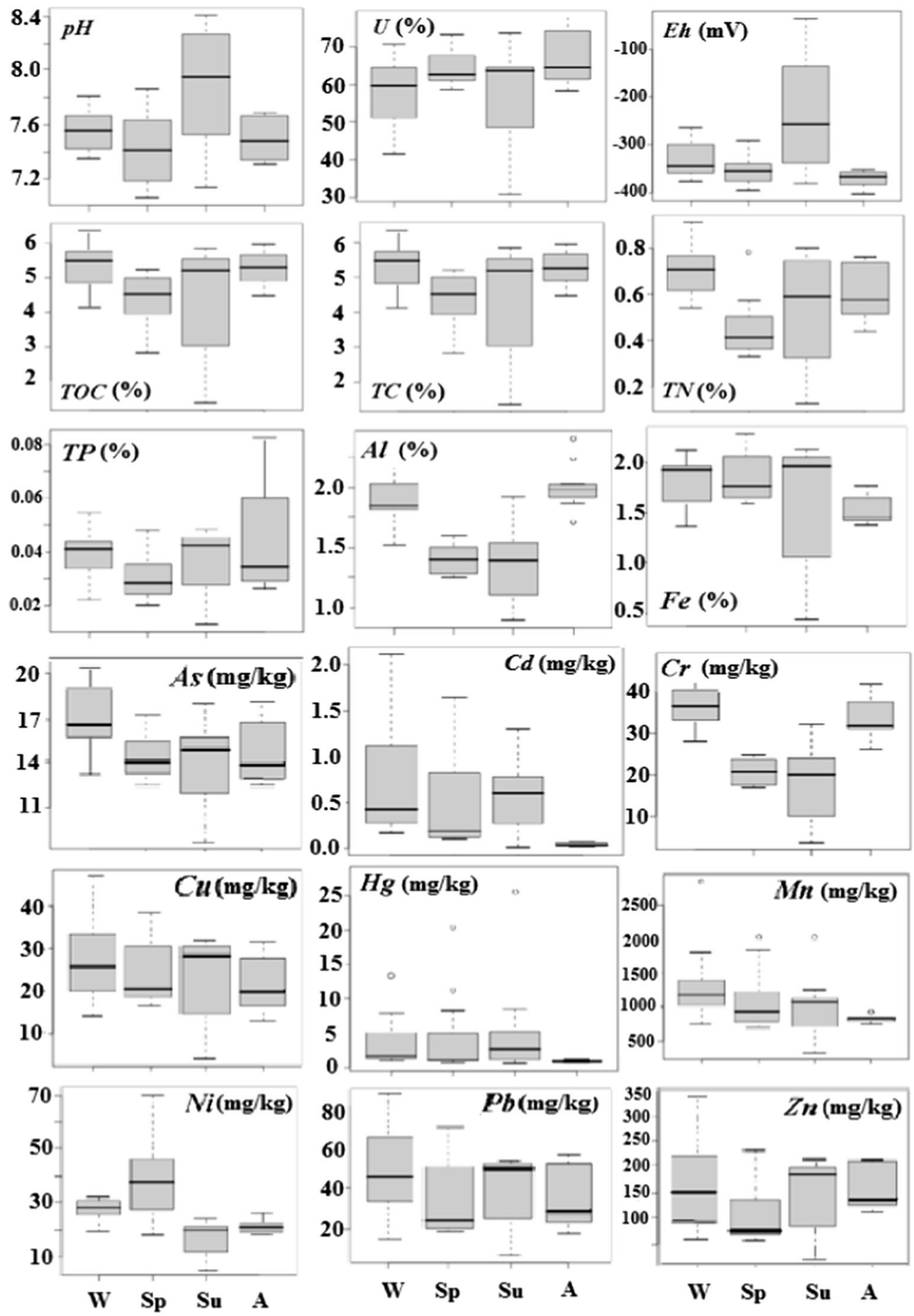
Pearson's correlation matrix calculated ($n = 180$, $p < 0.01$) shows a strong positive correlation between T-SpC (0.902), T-S (0.930), T-DO % (0.735), SpC-S (0.997), SpC-DO% (0.681), S-DO% (0.714), and TDS-Turb (0.999) pairs. Strong negative relations are recorded for the following pairs: pH-Turb (−0.825), pH-TDS (−0.830). Lower (greater than −0.460) but significant inverse relationships are reported for TDS, Turb, DO %, DO (mg L^{-1}) and between ORP-pH (−0.452).

Levels and Seasonal Trends in Aquatic Vegetation

TD recorded in sampling sites change significantly with the seasons (Fig. 4). HT-I showed no TD production during the entire study period as attended. In HT-III (*C. linum*), the maximum average production is recorded in spring ($\approx$3000 g^{-2}), whereas TD is consistently (500 g^{-2}) lower from summer to winter. In HT-II, the highest TD average value is recorded in summer ($\approx$1500 g^{-2}), whereas in winter, TD is zero. In spring and autumn, HT-II shows low TD production, below 500 g^{-2}.

In Table 4, the range of variation of macronutrients and trace elements measured in aquatic vegetation is reported for both species (HT-II and HT-III). In *C. linum* (HT-III), TC and TOC increase significantly in summer. The same trend is reported in Ruppia spp. (HT-II) for TC and TN, whereas TP changes significantly with all seasons in tissues from *C. linum* (HT-III) and in Ruppia spp. (HT-II) during autumn. In winter, As, Cr, Cu, Hg, Ni, Pb, and Zn are significantly higher in *C. linum* (HT-III). In spring, Cr, Ni, Pb, and Zn are significantly lower in *C. linum* (HT-III). In

Fig. 2 Levels and seasonal trends in sediments. Levels and seasonal trends in sediments are represented by the box-plot technique grouping *per* season the whole dataset collected in the study area. Measurement unit of each considered variable is reported in brackets. Average values, 25th and 95th percentile are represented. Hotspots are represented as dots. *W* winter, *Sp* spring, *Su* summer, *A* autumn



autumn, *C. linum* (HT-III) evidences significant decreases of Cd, Hg, and Ni. Ruppia spp. (HT-II) shows significant increases of Cd and Hg in spring, whereas As and Cr have significant increases in summer. In autumn Cd levels are significantly lower, whereas Cu and Mn levels increase notably.

Univariate Statistics in Aquatic Vegetation

Pearson's correlation matrix calculated ($n = 60$, $p < 0.01$) shows strong positive correlations among TN, TOC, TC (>0.80) and between TP and trace elements (As, Cu, Mn, Ni, Pb, Zn) (>0.70). A weak positive relationship is recorded between TD and TN (0.35). Hg is significantly correlated with Cr, Fe, and Cu (>0.60). Highly significant positive correlations are recorded between the following

Table 3 Results of univariate statistics in sediments

		Min.	1st qu.	Median	Average	3rd qu.	Max.	SD
pH	–	7.060	7.365	7.565	7.589	7.702	8.410	0.319
Eh	mV	−402.0	−374.2	−352.0	−322.2	−315.0	−36.0	82.02
U	%	30.86	60.43	63.02	61.58	65.50	77.75	10.20
TC	%	2.510	7.745	8.450	7.984	8.820	9.460	1.456
TOC	%	1.370	4.527	5.040	4.820	5.567	6.370	1.126
TN	%	0.130	0.455	0.560	0.573	0.730	0.910	0.180
TP	mg kg^{-1}	128.1	276.2	374.1	377.7	453.7	828.4	137.7
Al	mg kg^{-1}	2628	13,028	18,258	18,279	23,360	32,974	7291.4
Fe	mg kg^{-1}	4536	15,047	17,572	17,036	19,682	22,947	4015.6
Cd	mg kg^{-1}	0.010	0.058	0.255	0.455	0.655	2.110	0.532
Pb	mg kg^{-1}	7.02	22.51	40.58	39.55	51.43	85.25	19.95
Ni	mg kg^{-1}	5.36	19.20	22.57	25.78	30.60	70.12	11.38
Cr	mg kg^{-1}	3.68	20.06	27.61	27.32	34.29	47.69	10.04
Cu	mg kg^{-1}	4.20	18.00	24.97	24.28	29.73	46.75	8.94
Mn	mg kg^{-1}	342.8	823.0	992.5	1062.5	1165.5	2845.0	446.4
Zn	mg kg^{-1}	23.02	83.09	134.03	144.74	200.42	338.59	68.42
Hg	mg kg^{-1}	0.560	0.848	1.270	3.773	2.690	28.180	6.220
As	mg kg^{-1}	1.40	8.75	12.12	11.83	14.33	20.65	4.21

Univariate statistics calculated on sediments are reported for the whole dataset. Measurement units are put in brackets

Min. minimum value, *1st qu.* first quartile, *3rd qu.* third quartile, *Max.* maximum value, *SD* standard deviation, *TC* total carbon, *TOC* total organic carbon, *TN* total nitrogen, *TP* total phosphorous, *Al* aluminium, *As* arsenic, Cd cadmium, *Cr* chromium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Ni* nickel, *Pb* lead, *Zn* zinc

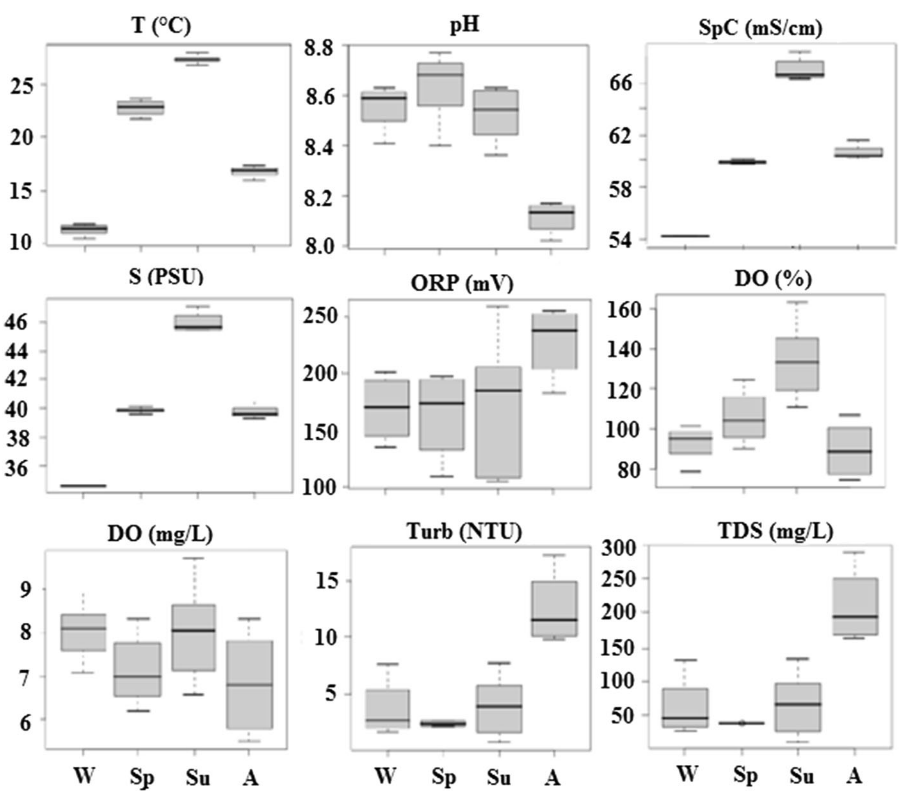


Fig. 3 Levels and seasonal trend in water. Levels and seasonal trends in water are represented by the box-plot technique grouping *per* season the whole dataset collected in the study area. Measurement unit of each considered variable is reported in brackets. Average values, 25th and 95th percentile are represented. Hotspots are represented as dots. *W* winter, *Sp* spring, *Su* summer, A autumn

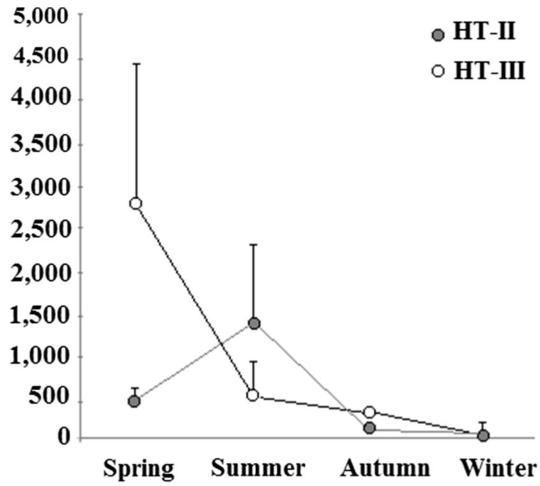


Fig. 4 Aquatic vegetation productivity in Habitat types: seasonal trends. Total biomass (TD) of aquatic vegetation is represented separately *per* each HT and season. Average values are represented + standard deviation. *Notes* HT-I = bare sediment, complete absence of aquatic vegetation during the whole year, HT-II = dominance of phanerogams (Ruppia spp.), HT-III = dominance of macroalgae (*C. linum*). Experimental data were converted using the conversion factor (4 g_{ww}) proposed by Bellan-Santini (1969) to calculate total aquatic vegetation density (TD, g^{-2})

pairs: Al–Cr (0.889), Al–Fe (0.974), Cr–Fe (0.954), Cr–Cu (0.898), Ni–Pb (0.984), Ni–Cu (0.921), Ni–Zn (0.998), Ni–As (0.981), Pb–Cu (0.962), Pb–Zn (0.976), Pb–As (0.990), Cu–Zn (0.899), Cu–As (0.960), and Zn–As (0.972), but lower and still significant positive correlations also are recorded.

Multivariate Relationships

In Fig. 5, multivariate statistics (PCA and *nm*-MDS) are shown for sediments. Two different factors (seasons and HTs) are highlighted. Concerning PCA, the first three axes account for 89.8% (respectively 64.2, 16.6, and 9.0%) of the total variance in sediment data. HT-I and HT-II are overlapping, indicating that absence of vegetation or dominance of phanerogam does not allow segregate data according to levels of trace elements in sediment. Conversely, HT-III (dominance of macroalgae, *C. linum*) segregates from others HTs suggesting an effect due to tested factor that is strongly related with Cr and Cd levels in sediments. Seasonality significantly affects similarities as evidenced by *nm*-MDS; segregations are particularly clear in autumn. In summer, samples showing the highest distance from the main group are represented by sampling replicates from HT-I.

In Table 5, results of the ANOSIM test (one-way) are reported. The significance of ANOSIM results is compared in terms of the effect on trace element assessment in sediments due to the tested drivers. Only one strongly auto-correlated variable (such as salinity or conductivity) was included in the analysis. The effect of two nested factors on total variability (i.e., the overlapped interference of seasonality and the other considered factors) also is tested by ANOSIM test (two-way). Even in this case, only one among strongly auto-correlated variables (such as salinity or conductivity) is included in the analysis. The ANOSIM test performed on the season factor nested with abiotic and biotic drivers (P/A, HT, and TD) evidences the significant of relations among tested factors and trace elements measured in sediments. A significant nested with season effect is reported for the following drivers: (1) water salinity (Global R: 0.321, significant level of sample statistic, $p = 0.01\%$); (2) water TDS (Global R: 0.650, $p = 0.01\%$); (3) sediment TN levels (Global R: 0.378, $p = 0.01\%$); (4) HT-II versus HT-III (Global R: 0.370, $p = 0.01\%$). Lower significance is reported for water DO ($p = 0.03\%$), water ORP ($p = 0.02\%$), presence/absence of aquatic vegetation (Global R: 0.119, $p = 0.02\%$).

Aquatic Vegetation Uptakes

BaRs allow the evaluation of enrichments compared with levels measured in sediments. BaR < 1 (decimal values) means tissue levels lower than sediment ones; on the contrary, BaR > 1 indicates bioaccumulation in tissues of aquatic species. In Fig. 6, BaRs are reported *per* season for the two different HTs (HT-II vs. HT-III). Results show that: (1) BaRs are element-dependent: for the same species, different trace elements show different bioaccumulation rates; (2) BaRs are species-dependent: for the same trace element, different species show different BaRs; (3) BaRs are season-dependent: the same species, for the same trace element, shows different BaRs during the year.

Discussion

Brief Considerations on Measured Values

Water

Measured descriptors of water are within the range of variability recorded in the literature for the same season in the Orbetello lagoon (Innamorati and Melillo 2004; Specchiulli et al. 2008; Giovani et al. 2014). Temperature ranged from 7.20 to 28.70 °C attesting on average to 19.6 °C (±1.2 °C) in the Eastern basin (Specchiulli et al. 2008). Salinity levels evidence hypersalinity in the study area almost all year; Specchiulli et al. (2008) recorded comparable average salinity values (average 39.9 PSU) in the study area. Oxygenation of water analysis reveals wide fluctuations as reported for the literature (range

Table 4 Range of variation of variables measured in aquatic vegetation

	Winter		Spring		Summer		Autumn	
	Average	SD	Average	SD	Average	SD	Average	SD
HT-III *C. linum*								
TC (%)	26.28	0.28	24.29	7.12	34.87*	1.20	24.34	1.16
TN (%)	2.46	0.14	2.83	0.92	2.22	0.44	2.63	0.17
TOC (%)	24.85	0.88	25.57	8.45	33.29*	0.19	21.64	1.45
TP (mg kg^{-1})	1484.87*	492.31	848.80*	168.15	2132.17*	263.95	277.07*	11.35
Al (mg kg^{-1})	32,161	27,109	962*	584	7406	3573	4398*	119
As (mg kg^{-1})	27.74*	16.44	8.82	1.17	10.53	0.96	12.43	0.32
Cd (mg kg^{-1})	0.91	0.53	0.54	0.09	1.88*	2.11	0.02*	0.01
Cr (mg kg^{-1})	55.15*	46.89	1.86*	0.90	21.36*	10.51	6.12*	0.47
Cu (mg kg^{-1})	61.10*	40.79	7.73	1.98	19.77*	5.20	11.80	0.69
Fe (mg kg^{-1})	35,767	25,092	2022	932	8353	3393	5477	144
Hg (mg kg^{-1})	1.99*	0.17	0.55	0.28	2.91	2.27	0.18*	0.01
Mn (mg kg^{-1})	7666.21	5995.55	1911.00	534.62	3733.12	676.88	2165.81	31.05
Ni (mg kg^{-1})	73.31*	71.68	3.41*	0.80	23.96*	19.08	8.00*	0.61
Pb (mg kg^{-1})	91.56*	20.66	10.81*	1.67	22.52	7.72	21.14	0.75
Zn (mg kg^{-1})	304.67*	71.87	43.03*	17.57	174.20	104.51	115.36	3.62
HT-II *Ruppia spp.*								
TC (%)	–	–	36.06	0.38	30.79*	1.20	36.67	0.60
TN (%)	–	–	2.92	0.27	1.30*	0.13	2.21	0.09
TOC (%)	–	–	32.37	9.40	29.60	1.06	35.10	0.67
TP (mg kg^{-1})	–	–	2015.63	70.29	1627.53	394.95	466.45*	6.46
Al (mg kg^{-1})	–	–	445	352	71*	23	452	17
As (mg kg^{-1})	–	–	1.22	0.41	3.50*	0.00	0.97	0.12
Cd (mg kg^{-1})	–	–	2.06*	0.36	0.48*	0.10	0.05*	0.01
Cr (mg kg^{-1})	–	–	1.03	0.51	2.98*	0.85	1.27	0.15
Cu (mg kg^{-1})	–	–	7.10	1.67	8.72	4.84	11.35*	0.17
Fe (mg kg^{-1})	–	–	763	462	159*	22	545	30
Hg (mg kg^{-1})	–	–	0.34*	0.06	0.14	0.01	0.17	0.02
Mn (mg kg^{-1})	–	–	495.00	104.73	793.51	333.94	1735.09*	53.18
Ni (mg kg^{-1})	–	–	1.76*	0.43	3.81	2.34	4.61	0.37
Pb (mg kg^{-1})	–	–	3.41	0.75	2.86	0.11	8.51*	0.08
Zn (mg kg^{-1})	–	–	41.18	11.69	69.45	18.59	200.76*	14.51

Data recorded for variables considered for aquatic vegetation are reported as average and standard deviation (SD) grouping data separately *per* season and HTs. HT-II = dominance of phanerogams (Ruppia spp.), HT-III = dominance of macroalgae (*C. linum*)

TC total carbon, *TOC* total organic carbon, *TN* total nitrogen, *TP* total phosphorous, *Al* aluminium, *As* arsenic, *Cd* cadmium, *Cr* chromium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Ni* nickel, *Pb* lead, *Zn* zinc

Significant differences among season are highlighted (* Student's t test $p < 0.01$)

20.1–89.6% for the whole lagoon ecosystem and 120.9 ± 25.3%, for the Eastern basin) with values typically associated with high primary productivity rates due to photosynthesis alternating with values typical of anoxia.

Sediment

Averages of measured descriptors of sediments are within the range of values reported in previous studies for the same ecosystem. pH values recorded in this study range within 7.0–8.4, whereas Eh was always lower than −50 mV suggesting the occurrence of sediment reduction in superficial layers (0–5 cm) of all HTs (Renzi et al.

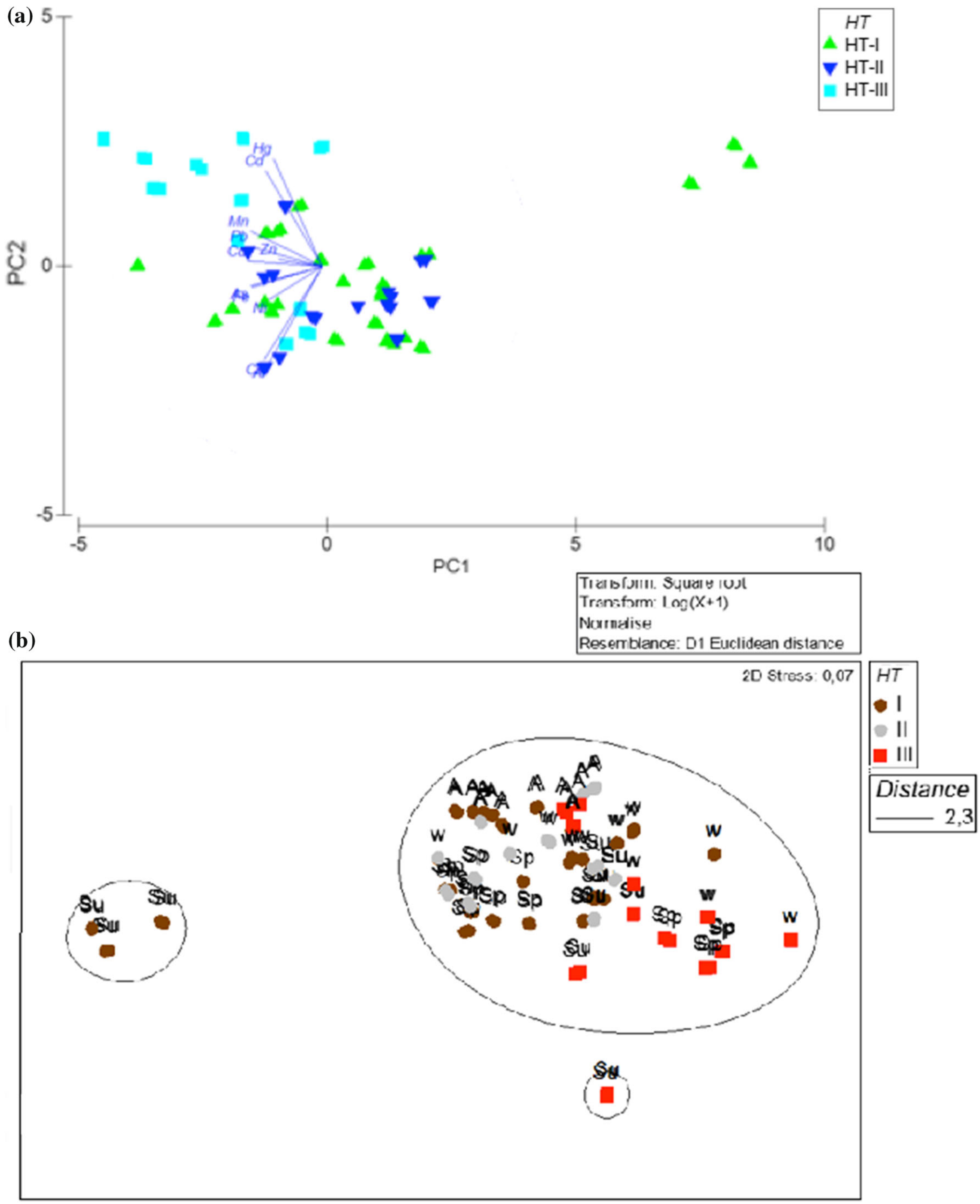


Fig. 5 Principal component analysis (**a**) and non-metric multi-dimensional scaling (**b**) on sediment data. Different habitat types (HT) (**a**, **b**) and season factors (**b**) are highlighted

2007). Concerning macronutrients (TOC, TN, TP), levels measured in this study are comparable to literature values for the same ecosystem: 2–7% for TOC (Lardicci et al. 2001; Lenzi et al. 2005), 0.01–0.91% for TN, and 0.006–0.090% for TP (Renzi et al. 2007). The strong relationship between TC-TOC levels is due to the low amount of carbonates responsible for the inorganic fraction of carbon in sediments. TOC values are high (on average 5%), representing more than 60–70% of the TC content. TOC show slow seasonality, with minimum values in spring, and wide low-spatial scale variability ranging from 1.3 to 5.3%. TIC concentrations are 2–3% on average. Trace element concentrations in sediment differ a great deal among HTs and show a seasonal behaviour that is

Table 5 ANOSIM test one-way

Matrix	Factor	Global R	NPS > R	Significance
–	**Season**	**0.387**	**0**	**0.01**
–	**Bottom type**	**0.200**	**0**	**0.01**
	HT I versus HTII	0.097	64	0.70
	HT I versus **HT III**	**0.296**	**0**	**0.01**
	HT II versus **HT III**	**0.370**	**0**	**0.01**
Water	pH	0.193	14	0.20
	S	**0.295**	**0**	**0.01**
	ORP	*0.326*	*1*	*0.02*
	DO	*0.581*	*2*	*0.03*
	TDS	**0.690**	**0**	**0.01**
Sediment	TOC	0.231	601	6
	TN	**0.378**	**0**	**0.01**
	pH	0.223	90	0.90
	Eh	0.303	37	0.40
Aquatic vegetation	P/A	0.099	13	0.10
	U	0.503	1189	11.9

The significance of different factors considered on the multivariate assessment of trace element in sediments is reported (ANOSIM test one-way). High significant effects on trace element distribution are evidenced in bold ($p = 0.01$) while significant within 0.01–0.05 are represented in italic

element-dependent. Concerning measured levels, Al and Fe are the principal components of the Earth's crust and measured values are on average within 1.5–2.0% for both of them and similar to values recorded by the literature for the Argentario Mountain area (Borghini 1998). Fe shows the highest seasonal difference among HTs in summer. As shows maximum values above 20 mg kg^{-1}. Leoni and Sartori (1997) measured higher Cr values (94–131 mg kg^{-1}) and lower Cu values (25–27 mg kg^{-1}) compared with average recorded in this study, whereas Cr and Cu values recorded in this study are comparable to results recorded by Borghini (1998), which were respectively 27–40 and 21–41 mg kg^{-1} even if notably lower levels in summer are recorded for both. Ni shows very high values and variability in spring (70 mg kg^{-1}) with minimum values recorded in summer, whereas Ni values in sediment of different HTs are homogeneous in autumn. Measured values are similar to 48–77 mg kg^{-1} recorded by Leoni and Sartori (1997) for the Elba-Argentario marine basin. Average values of Pb are similar to values recorded by the literature (19–56 mg kg^{-1} Borghini 1998; 25–64 mg kg^{-1} Leoni and Sartori 1997) even if values notably higher and lower the range reported by literature are recorded in this study (10–85 mg kg^{-1}). On the contrary, Hg, Mg, and Zn show average levels that are higher than ranges reported by the literature that are respectively within 0.30–1.70, 350–695, and 44–98 mg kg^{-1} (Borghini 1998). Concerning Zn, measured levels are higher than values reported by Leoni and Sartori (1997), which were within 88–121 mg kg^{-1}. Hg concentrations show a local hot spot with very high values (above 10 mg kg^{-1}) recorded in HT-III probably associated with the now-disused Fe–Mn mines activity in a cinnabar and pyrite area (local unpublished data; de Vivo et al. 2009). Hg accumulation in HT-III sediments is high.

Biomass

Biomass production (TD) in the study area are comparable to values recorded in the literature over a longer monitoring period (Innamorati and Melillo 2004; Lenzi et al. 2003; Lenzi et al. 2013). Nutrient and trace element levels in tissues are species-dependent, and there are few data in the literature on the studied species to perform comparisons. Concerning BaRs, little data are available on autotrophic species. Furthermore, the vast majority (approx. 90% of the total number) of studies performed on BaRs are done in freshwater and only 10% in salt water (Arnot and Gobas 2006). The largest segment of the literature concerns *P. oceanica*. Earlier research performed on the same species considered in this study showed that phanerogams (Ruppia spp.) and macroalgae (*C. linum*) can accumulate trace elements (Cu, Hg, Zn) at high efficiency (Renzi et al. 2014a). For these reasons, this study represents a baseline literature both for levels and BaRs comparisons of considered trace element in these species.

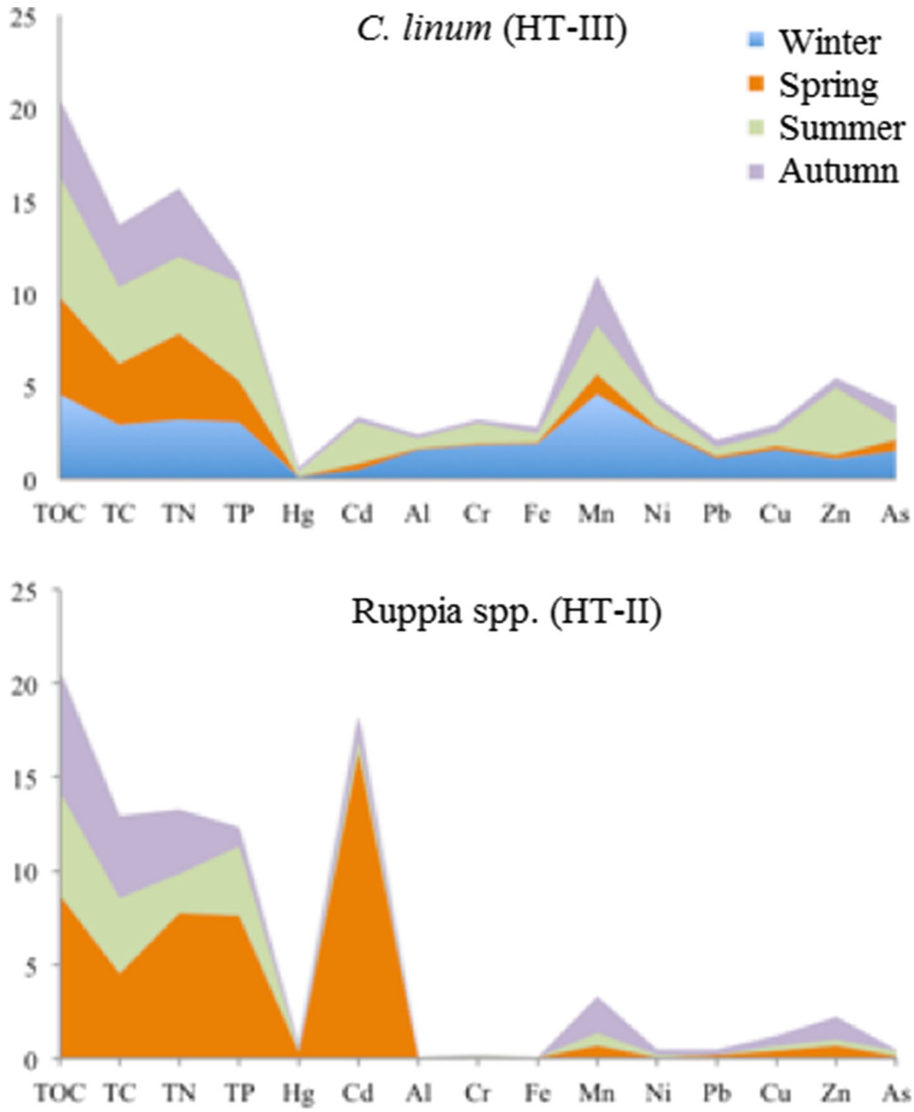


Fig. 6 Uptake of trace elements by aquatic vegetation. Bioaccumulation factors (BaRs) are calculated according to Usero et al. (2003) per HT-II (Ruppia spp.) and HT-III (C. *linum*). Results for each variable and HT are represented grouped per season. In winter HT-II was bare, so BaRs in winter are not calculated. BaRs are a pure ratio between the concentration of a given element measured in tissues and the concentration of the same element measured in sediment from the same HT

Water Variability: Internal and External Drivers

Trends

At our latitudes, solar irradiance increases from March to the end of June, peaks in July, and decreases progressively until the end of December. Air temperatures show the same trend. In lagoon ecosystems characterized by low water depth, water temperature is more strongly affected by irradiance than by atmospheric temperature. For this reason, even though average water temperatures are significantly higher in summer due to the correlated increase in air temperature and irradiance, cloudy days and light/dark cycles can rapidly affect water temperature. Fluctuations in water temperature recorded in this study are comparable to data collected during previous, more extensive sampling campaigns (Innamorati and Melillo 2004; Giovani et al. 2014). Giovani et al. (2014) reported fluctuations in average temperature from 35 °C (July 2007) to 6.5 °C (January 2007) for the same ecosystem, with comparable cycles on a yearly basis in the period 2003–2009.

Salinity values reported by the same research showed hyper-salinity during the summer season (>45 PSU) with average values from 24 to 40 PSU. Also, salinity (and consequently conductivity) is greatly affected by meteorological conditions. Evaporation rates and rains affect salinity respectively in terms of concentration and dilution of water salts. Evaporation is closely related to water temperature, and some differences in yearly trends are reported in the literature due to climate fluctuations (Giovani et al. 2014). Between 2000 and 2001, a total of 686 mm of rains were recorded within September to June (Innamorati and Melillo 2004) in the study area. Also, freshwater inputs could affect salinity through dilution processes. In the spring, salinity and conductivity are similar to values recorded in autumn, probably due to freshwater inputs in spring. Municipal wastewater effluents discharged freshwater into the Western basin until the end of 2010, when almost all municipal effluents were channelled to a pipeline discharging into the sea (Specchiulli et al. 2008; Renzi et al. 2009), with some exceptions along sandbars (Renzi et al. 2012), and the Orbetello lagoon changed from a brackish to a hyper-saline ecosystem.

Turbidity and suspended solids could significantly affect irradiance and, consequently, ecosystem productivity (Banas et al. 2005). Suspended matter in the water column of lentic systems may have various allochthonous (winds, tributaries, runoff, aerosols, etc.) and autochthonous (production or resuspension) origins. Winds could significantly affect water turbidity, inducing the increase of suspended solids up to 800 mg L^{-1} (Banas et al. 2005). The study area is dominated by NW-SSE winds with an average speed of 4.0 ms^{-1} and maximum speed of 19.8 ms^{-1} (Innamorati and Melillo 2004). Even more than suspended solids in the water column, high planktonic proliferations affect turbidity. In this case, the strong relationship observed between TDS and suspended solids suggests that turbidity is due to suspended finer sediment particles rather than proliferations of plankton.

A variety of processes drive oxygen levels in lagoon water. Temperature affects oxygen solubility in water according to Henry's Law. Winds and marine-lagoon water exchanges increase oxygen availability. Water turnover and residence times in the lagoon are principally driven by lagoon geometry and freshwater runoff, which may affect photosynthesis rates (Mee 1978; Phleger 1981; Mahapatro et al. 2013). Daily light/dark cycles affect primary producer activity, inducing hyperoxygenation during the day and oxygen depletion during the night when respiration processes are dominant (Kennish 2002). Furthermore, eutrophication induces increased organic matter decomposition rates, enhancing oxygen depletion phenomena (Kennish 1998; 2002). pH and DO are closely linked to primary production (Kennish 2002). According to Henry's Law, the amount of dissolved oxygen in water decreases as temperature increases. However, data collected in this study shows a positive correlation, and this apparent contradiction with Henry's Law is explained by the fact that the main driver of oxygen levels in water is photosynthetic activity. ORP in summer shows great variation, probably due to the low-scale spatial variability of local patchiness of bottoms made up on zones of intense primary production and other neighbouring zones characterized by high biomass decomposition rates.

Despite management plans to improve the physical–chemical properties of the lagoon water, data recorded in this study evidence a lack of positive effects of water renewal in the study area, confirmed by the fact that salinity is principally driven by temperature, except in spring when rains probably act to reduce salinity due to runoff inputs.

Sediment Variability: Internal and External Drivers

Sediments are sinks for a large part of nutrients and environmental pollutants, including trace elements (Hedges and Keil 1995). Numerous studies in the literature show that fine particles of sediment are more closely correlated to organic carbon content than coarse components (Bordovskiy 1965; Colombo 1977; Keil et al. 1994; Mayer 1994; McCave et al. 1995; Nguyen et al. 1997; Bellucci et al. 2002; De Falco et al. 2004; Carvalho et al. 2005; Como et al. 2007; Magni et al. 2008). Furthermore, recent research has shown that fine particles are responsible for the stronger phosphorus adsorption capacity and play an important role in determining phosphorus content in sediments (Lukawska-Matuszewska and Bolalek 2008; Specchiulli et al. 2010), although phosphate availability is linked to Fe and carbonate-mediated dynamics. Results obtained in this study evidence a positive correlation among silt and macronutrients and trace elements concentration in sediments according to the literature. An inverse relation between Fe and TP is recorded, supporting the occurrence of Fe modulating activity on TP.

Furthermore, results obtained in this study highlight the seasonality of the physical–chemical parameters of sediments in all sampling sites. TC and TOC show the same seasonal trend, because their inorganic component (TIC) is minimal in the study area in all HTs. Decomposition rates of organic matter increase proportionally with increases in TN and TP levels (Goldman et al. 1987). Both of them are closely correlated to finer sediment fraction (particle diameter < 63 μm). TN shows a comparable seasonal trend, with differences in spring when average levels are notably lower. TP shows maximum average values in autumn. The greatest variability between HTs occurs in summer (TN) or autumn (TP). Minimum average nutrient values are found in spring (TN, TP) when TD of aquatic vegetation is higher due to higher primary productivity rates.

Although sediments are sinks of nutrients and pollutants (Hedges and Keil 1995), they can be a source to the overlaying water. Direct and indirect abiotic adsorption/desorption processes could affect measurable levels of pollutants in sediments (Miao et al. 2006). Among others, type and amount of clay, organic matter, pH, Eh, and salinity are the main sediment properties that can affect sediment–water exchanges of nutrients and trace elements (Murray 1987). Results obtained in this study show that pH is characterized by both high values and high variability between sites in spring. Eh and pH in sediments show the same seasonal trend, confirming their relationship with organic matter degradation processes with no alteration of natural pathways due to human activity. Changes in sediment pH-Eh conditions could cause changes in metal speciation and solubility (Patrick and Jugsujinda 1992) affecting sediment–water fluxes (Pardu and Patrick 1995). In fact, redox potential (Eh) is a key factor affecting metal transformation (Guo et al. 1997). Short-term changes in speciation of metals (i.e., due to reduced sediment oxidation could release associated trace elements (Cappuyns et al. 2004). The anoxia (Eh) and bacteria associated with the sulphur cycle could mobilize mineral contaminants, such as mercury in cinnabar, and methyl-mercury (Kim et al. 2006). These mechanisms are not yet well understood, and further research is required. A strong increase in TS (+100%) in summer in HT-I and HT-III and a strong decrease (−70%) in HT-II are recorded in this study. These data are linked to the organic matter decomposition phenomenon and to the effects of rooted vegetation (Ruppia spp.) on sediment oxygenation in HT-II. Sulfide concentration in sediments is the key factor that can affect trace element solubility related to oxidation conditions. In fact, high concentration of sulfides (as in coastal sediments) induces greater solubility of trace elements under aerobic conditions. Fe and Mn oxides are excellent scavengers for metals and are affected by the Eh and pH of sediments (Murray 1987). In lagoons, the Fe cycle is significantly affected by physical–chemical fluctuations in the water column and by biogeochemical cycles (Zaldívar et al. 2003 and citations therein). Fe is a key element in coastal ponds and lagoon systems, because it is involved in TP and sulphur mobilization and fluxes at the water–sediment interface. In fact, in its ferric forms (oxides), it can adsorb orthophosphates, removing them from the water column (Zaldívar et al. 2003 and citations therein). In marine sediments, including those in lagoons and other transitional environments, sulphate-reducing (sulphate respiration) bacterial processes degrade more than 50% of organic matter (Jørgensen 1983). Eutrophic conditions lead to the production of macroalgal biomass during warmer months (Morand and Briand 1996), as in site HT-III. These conditions lead to a settling of organic matter in sediments and an increase in sulphate-reduction. Under anoxic conditions, dissolved sulphate is reduced to hydrogen sulfide gas (H_2S), which reacts with iron minerals to form iron sulfides. Iron monosulfides (FeS) form first, but are typically unstable and are usually converted into pyrite (FeS_2). This natural buffering system hindering the production of free sulfide by sulphate-reduction is opposed by the presence of ferrous and ferric ions with the production of pyrite (Berner 1983; Luther 1991; Rozan et al. 2002). The iron pool available in sediment modulates dystrophic episodes, blocking H_2S before it enters into the water column (Giordani et al. 1996). This process releases orthophosphates—previously bound to ferric oxides-hydroxides—from sediments (Rozan et al. 2002). These dynamics are linked to the seasonal Fe and TP trend observed in this study, with maximum inter-area variability in summer and minimum values in autumn. These elements also are linked to TP availability. Mn is involved in TP sorption at water-interface with haneurite formation. In fact, under anoxic conditions Fe^{3+} is reduced to Fe^{2+}iron sulfides (Roden and Edmonds 1997), the soluble form of iron leading to the release of TP into water (Miao et al. 2006) or TP enrichment in the superficial sediment layer. As far as other trace elements are concerned, Cu, Pb, and Zn show the same seasonal trends with wide fluctuations among sampling stations and average values higher in summer. Cd, As, and Cr seasonal trends are similar, with higher values recorded in winter and lower ones in spring and summer. Hg, Mn, and Ni seasonal trends are notably different from all others. Many Hg and Mn outliers are recorded; levels are similar in winter and summer, with very low and homogeneous values in autumn. The same trend is reported for Mn, whereas for Ni, higher variability among sampling stations is reported in spring, lower values in summer, and higher homogeneous levels in autumn.

Aquatic Vegetation Variability: Internal and External Drivers

Aquatic vegetation assessments and frequent changes to them in Orbetello lagoon are well documented in the literature. Water eutrophication (Lenzi et al. 2003) induces effects on spatial distributions, pathways, and total biomass production (Lenzi et al. 2003). In the study area, aquatic vegetation consists of a recently stabilized meadow of Ruppia spp. (*R. cirrhosa*) occasionally associated with algal species, such as *C. linum* and *G. longissima* in HT-II and by dense proliferations of *C. linum* in HT-III (Giovani et al. 2010, 2014). Primary productivity (TD) shows notable quantitative and qualitative differences among species. *C. linum* (HT-III) productivity is at its maximum in spring, whereas Ruppia spp. (HT-II) productivity is highest in summer. In addition, macroalgae productivity is high (3000 g^{-2}, HT-III) during a short period near the start of spring, whereas in summer phanerogams peak (1500 g^{-2}, HT-II). In the same season, macroalgae start to decompose (500 g^{-2}, HT-III). In winter and autumn, primary productivity is due only to macroalgae (100 g^{-2}). In HT-III, the species *C. linum* has almost monospecific dominance, whereas in HT-II phanerogams are dominant. TN and TP concentrations in tissues fluctuate in relation to season and taxa, in accordance with findings in the literature (Lapointe 1987; Lapointe and O'Connell 1989). Observed dynamics concerning trace elements are element-dependent, and this could be due to different uptake dynamics of trace elements and different accumulation processes within vegetal cells and structures. Furthermore, observed dynamics concerning trace elements are HT-dependent, probably due to the differences in Fe, TS, and TP, oxygen, and Eh availability between HT-II and HT-III, which affect resolubilization from sediments towards the water column and, consequently, impact uptake from aquatic vegetation.

Scaling Complex and Multi-Layer Interactions in a Complex Ecosystem

Scaling Monitoring Strategy

Physical–chemical water column descriptors have commonly been found to be highly variable on both seasonal and daily bases in transitional water ecosystems such as coastal lagoons. Natural variability of some descriptors (i.e. T, S, Turb, etc.) is also increased by the interference of different human-due factors, such as the lack of efficiency of municipal wastewater treatment plants (Renzi et al. 2009) or the increase in uncollected hot-spot discharges during the tourist season (Renzi et al. 2012). A good monitoring program should be developed to cope with spatial and temporal replicates with natural variability of descriptors of interest. For example, temperature, DO, salinity, turbidity, grain-size, nutrients and trace elements should be monitored using different temporal scales optimized to reflect the natural differences among variables. Nonetheless, a research program is usually the best compromise in terms of time and money that researchers can invest to apply a statistically solid sampling approach and obtain certain results, and the number of sampling replicates is optimized after a sort of *ad hoc* "cost–benefit" analysis. In this study, the sampling strategy adopted was sized to reduce pseudo-replicates sensu Benedetti-Cecchi (2004), collecting samples at different intervals depending on the matrix. Despite the optimized sampling strategy used in this study, results on highly variable matrices are comparable with data previously collected in the same ecosystem during extensive and more detailed sampling surveys (Innamorati and Melillo 2004; Specchiulli et al. 2008; Giovani et al. 2014) and could be considered good proxies for natural averages.

Multivariate and Multilayer Interactions

In Fig. 7, multivariate and multilevel relationships observed among trace elements in sediments and considered possible drivers of variability are represented starting from *nM*-MDS of sediment variables plotted as described in the methods paragraph. Various geomorphologic characteristics and external factors (i.e. winds, rains, hydrodynamics, human pressures, management actions) affect nutrient and pollutant inputs and determine the occurrence of substantial ecological boundaries and gradients characterizing lagoons (Kormas et al. 2001; Muslim and Jones 2003; Newton et al. 2003; Zaldívar et al. 2003). In such ecosystems, nutrient levels are the drivers of the ecological shift between macroalgae and phanerogam dominance (Knoppers 1994; Souza et al. 2003, Viaroli et al. 2008). In fact, the literature shows that in water ecosystems, the consequences of eutrophication (Morand and Briand 1996) produce changes in abiotic matrices such as the water column and surface sediments (Chessa et al. 2005) that affect zoological and phytosociological assemblages and communities (Orfanidis et al. 2008; Viaroli et al. 2008). But relationships modulating low-spatial scale differences occurring in the same ecosystems should involve more complex interactions. Relationships between sediment and aquatic vegetation are strong and self-modulating. Numerous studies report significant relationships between sediment descriptors (i.e., pH, ORP, Eh, grain-size, nutrients, and sulfide) and phanerogam spatial distribution (Ferrari et al. 1972; Goodman et al. 1995; Miller and Sluka 1999; Azzoni et al. 2001; Chau 2002; Renzi et al. 2007; Giusti et al. 2010). Sediment state appears to play a key role in plant establishment, presence, and recolonization after the occurrence of environmental crises (Plus et al. 2003), phanerogams actively contribute to the regulation of the oxidation level in sediments by spreading the oxygen produced by photosynthesis from the rhizosphere (Pedersen et al. 1998), and the reduction of system turbidity (Mannino and Sarà 2006). Furthermore, eutrophication may not be the only factor involved in aquatic vegetation dynamics. In fact, a general progressive decline in phanerogams of approximately 65% during the past 150–300 years is reported in the literature (Lotze et al. 2006) and is related to a variety of other factors, such as chemical pollution, physical impacts, changes in trophic structure, and other urban impacts (Duarte 2002; Short et al. 2006).

As previously discussed, various multifactorial and cyclic processes could significantly affect pollutants in sediments during the year, due to sorption/desorption processes involving water column/sediment interfaces. Sediment descriptors are closely linked to aquatic vegetation dynamics in such ecosystems. Low Eh and pH values induce both ammonium production (Marty et al. 1990) and nitrite increase through ammonification of organic matter, with a toxic effect on biota, and stimulate production of nitrophilic algal species. The final balance reached by the ecosystem is due to the physical–chemical properties of considered pollutants and by their relationships with different biotic components of the ecosystem itself; a significant contribution also may come from aquatic vegetation

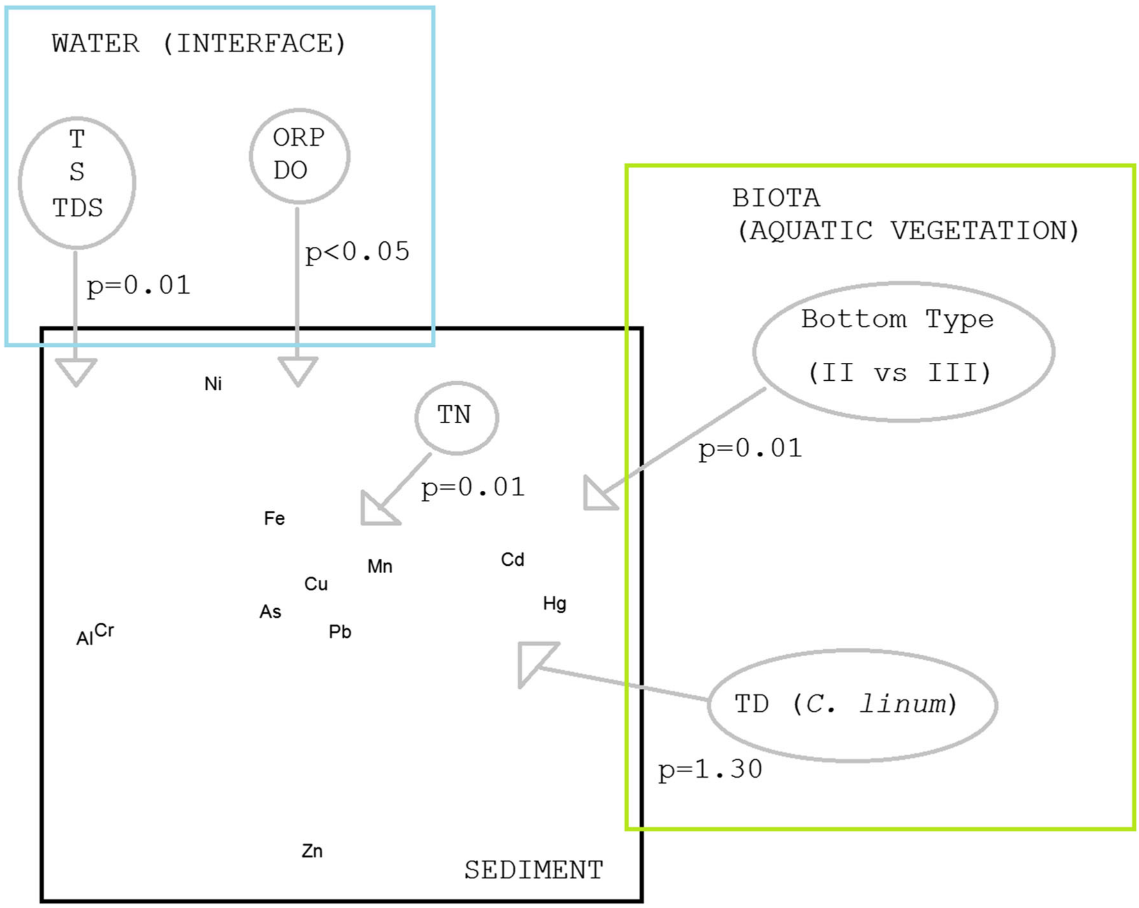


Fig. 7 Representation of *nm*-MDS performed on trace element in sediments and the significant of principal drivers affecting their seasonal behaviour. *Notes* The black square represents *nm*-MDS projection performed on sediment variables (whole dataset) to highlight multivariate relationships among variables measured in sediments. The green square represents variables from the aquatic vegetation compartment that resulted significant in affecting trace elements variability in the sediment compartment. The light blue square represents water variables resulted significant. The level of significant is reported for each variable represented

uptake. Thus, orthophosphates are mobilised into the water column where they become available to algae growth. In lagoon and estuary areas, the anoxia condition established in sediments due to high nutrient load and accumulation of organic matter produces increasing degradation and growing stress in the warm season for various populations, resulting in selection in favour of opportunistic species and in sudden and drastic changes in aquatic vegetation (Zaldívar et al. 2003). This is particularly evident for rooted plants, the development of which is curbed by bacterial and chemical conditions in sediment, by epiphyte leaf development, by phytoplankton blocking out light and by floating macroalgae masses, which can suffocate seagrass meadows (Zaldívar et al. 2003). High levels of eutrophication induce a systemic shift from seagrass to seaweed, and, if conditions worsen, to opportunistic microphytes characterized by higher turnover (Duarte 1995). As described in previous research performed at the same sampling sites, average Eh in sediments appears to have the greatest influence on HT affecting phanerogam expansion. In particular, HT-II shows positive values of ORP in the water column and positive values of Eh in the first 0–5 cm of sediment layer, whereas HT-III and HT-I are both characterized by negative ORP values in water and Eh in sediments below −200 mV (Giusti et al. 2010). Eh values below −200 mV are associated with phosphorous release from sediments (Dueri et al. 2008). Furthermore, TP and TN levels are related to sediment physical–chemical characteristics (Lapointe et al. 1992), which determine macronutrient fluxes at water–sediment interface affecting their bioavailability and thus TN and TP concentrations in tissues. Our results support general knowledge in the literature. Furthermore, this study indicates that in summer the highest segregation from the main group is due to HT-I samples. These data suggest that the complete absence of vegetation during the whole year produces a major effect on trace element concentrations in sediment during more critical seasons. These effects are related to some of the water column drivers, such as T, TDS, and Sand, to nutrients in superficial sediment layers.

Key Role of Aquatic Vegetation: Trace Element Uptakes

The possibility that aquatic vegetation accumulates bioavailable trace elements has been widely documented in the literature (Renzi et al. 2014a, b; Pergent-Martini and Le Ravallec 2007). For example, *R. cirrhosa* can efficiently accumulate trace elements from water without evident morphological alteration of tissues, which could be consumed by herbivores, carrying pollution out into the trophic web (Renzi et al. 2014a). This species could adsorb and bioconcentrates pollutants from both leaves and roots (Brinkhuis et al. 1980). Data on induced toxicity are scarce and conflicting, although it has been reported that exposure to toxicants could induce morphological and physiological stress in phanerogams and macroalgae (Renzi et al. 2014a, b). Effects are shown earlier by structurally simpler species than more complex ones, so macroalgae could show them before phanerogams do (Jochem 2000). Some recent papers have suggested that trace elements could induce stress in unicellular algae due to the alteration of the photosynthetic complex (Geider et al. 1993) and phaeophytin ratio (Ronen and Galun 1984). Trace elements affecting the health of aquatic vegetation (Pergent-Martini and Le Ravallec 2007) act directly on the photosynthetic complex. For example, Zn induces oxidative stress (Li et al. 2006) and affects photosynthesis (Nguyen-Deroche et al. 2009) and, consequently, growth rates (Stauber and Florence 1990). However, Cu acts on the permeability of cell membranes for cytoplasmic substances (Steeman-Nielsen and Wium Andersen 1971), K (Rai et al. 1981), and Mn (Sunda and Huntsman 1983). Phanerogams are key biocenosis in aquatic environments (Costanza et al. 1997). They are involved in different ecosystem functions: management of fish stocks, nurseries, shelter, and food for a large number of animal species (Boudouresque and Meinesz 1982); and play a role in regulating hydrodynamics, because they help to maintain the coastal balance (Clarke and Kirkman 1989). The leaves are this plant's most significant part from an ecological point of view. In fact, it is an annual species (Pergent-Martini and Le Ravallec 2007) that grows new leaves each year and is therefore a source of trace elements for primary consumers (Ward 1987) spreading pollution throughout the trophic web (Schlacher-Hoenlinger and Schlacher 1998). Results obtained in this study evidence a clear water driver component impacting levels of trace elements measured in sediments, probably linked to solubilisation dynamics of trace elements stored in sediments and also linked to organic matter decomposition dynamics. Biotic drivers also affect trace element in sediments and are particularly linked to the presence and accumulation of *C. linum*. Possible effects throughout trophic webs of the observed uptake dynamics affecting element-dependent, species-dependent, season-dependent, and HT-dependent trace elements should be closely studied through further research to evaluate risks for conservational and toxicological purposes and to preserve human health, given that frequently consumed fish species feed in this ecosystem.

The uptake of trace elements by aquatic vegetation has important consequences for the management of this ecosystem; there is clearly a link between ecological complexity and human welfare. In Italy, there are approximately 150,000 ha of lagoons, half of which is exploited by humans for nursery areas or aquaculture (Cataudella et al. 1995). Worldwide, significant management efforts are underway to recuperate the extent and the water quality of transitional waters, with the goal to restore the productivity and bottom value of these important ecosystems (Lirman et al. 2008). Phytosociological dominance is a complex and not yet fully understood phenomenon that depends on multifactor levels of interaction between abiotic and biological factors. The relative importance of each variable is not completely clear, although the results obtained by this study allow us to determine the relative weights of various internal and external drivers involved in this dynamic. Vegetal biomasses naturally produced in the various HTs considered in this study were not managed during our research, because human harvesting of biomasses was performed only in the Western basin. Consequently, leaves and macroalgae biomasses decompose in situ or be transferred by currents and winds within the Eastern basin and towards the sea (Ansedonia channel). As an ecotoxicological consequence, trace elements stored in tissues of aquatic vegetation can have the following possible destinies after the vegetation's death: (1) redeposition in superficial sediments; (2) transport along the trophic web via detritivore; (3) resolubilization in water; (4) wind-driven accumulation in another site within the Eastern basin.

Approximately 9212 t per year w.w. of vegetal biomasses were harvested ($\approx$2764,000 kg d.w., 70% water content) in the Orbetello lagoon in 2005 (local municipality unpublished data). Considering average levels of trace elements measured in this study as representative of the average levels in vegetal biomasses, the possible total annual amount of trace elements removed by the Orbetello lagoon ecosystem through harvesting are: 28,612,505 kg year^{-1} for Al; 11,384,813 kg year^{-1}for Mn; 2914,891 kg year^{-1} for Zn; 248,671 kg year^{-1} for Ni; 145,000 kg year^{-1} for Pb; 84,253 kg year^{-1} for Cu; 59,013 kg year^{-1} for Cr; 57,722 kg year^{-1} for As; 3142 kg year^{-1} for Hg; 1968 kg year^{-1} for Cd. These estimations, although certainly approximate, allow us to highlight how important trace element uptake from the Orbetello lagoon ecosystem is in terms of pollution removal on a yearly basis and how crucial the question of management is in such highly polluted areas.

Conclusions

This study provides useful data concerning seasonality of trace elements in sediments in different undisturbed habitat types (HT) of a coastal lagoon. Results evidence that salinity and turbidity in water, as well as macronutrients in sediments (TN), show a significant role as drivers of observed sedimentary variability of trace element. Furthermore, also aquatic vegetation and HTs show a key role on trace elements in sediments. Total density of *C. linum* and HTs resulted significant due to the occurrence of bioaccumulation by aquatic vegetation. In fact, observed BaRs suggest a strong influence of aquatic vegetation uptakes (in particular by *C. linum*) as drivers of trace element modulation in surface sediments in undisturbed lagoon areas.

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References

Aminti PL, Cappietti L, Tecchi MG, Venturini A (2003) Hydrodynamics of lagoons, a case study: the Orbetello lagoon. In: MEDCOAST 03—of the sixth international conference on the mediterranean coastal environment, pp 2031–2042

Arnot JA, Gobas FAPC (2006) A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ Rev 14:257–297

Aspila KI, Agemiam H, Chau ASY (1976) A semiautomatic method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101:187–197

Azzoni R, Giordani G, Bartoli M, Welsh DT, Viaroli PL (2001) Iron, sulphur and phosphorus cycling in the rhizosphere sediments of a eutrophic *Ruppia cirrhosa* meadow (Valle Smarlacca, Italy). J Sea Res 45:15–26

Banas D, Grillas P, Auby I, Lescuyer F, Coulet E, Moreteau JC, Millet B (2005) Short time scale changes in underwater irradiance in a wind-exposed lagoon (Vaccarés lagoon France): efficiency of infrequent field measurements of water turbidity or weather data to predict irradiance in the water column. Hydrobiologia 551(1):3–16

Barnes RSK (1980) Coastal lagoons. Cambridge University Press, Cambridge

Bellan-Santini D (1969) Contribution al'etude des pouplements infralittoraux sursubstratrocheaux. Recl Trav Stations Mar d'Endoume 47(63):5–294

Bellucci LG, Frignani M, Paolucci D, Ravanelli M (2002) Distribution of heavy metals in sediments of the Venice lagoon: the role of the industrial area. Sci Total Environ 295:35–49

Ben Charrada R (1995) Impact des amenagement de restauration sur la qualité des eauxet des peuplement benthiques du lac de Tunis. Mar Life 5:51–64

Benedetti-Cecchi L (2004) Experimental design and hypothesis testing in ecology. Biol Mar Mediterr 11(1):407–455

Berner RA (1983) Sedimentary pyrite formation: an update. Geochem Cosmochim Acta 48:605–615

Bordovskiy OK (1965) Accumulation of organic matter in bottom sediments. Mar Geol 3:33–82

Borghini F (1998) Gli elementi in trace negli ecosistemi costieri dell'Argentario. Atti Società Toscana di Scienze Naturali Memorie 105(A):9–16 **(document in Italian language)**

Boudouresque CF, Meinesz A (1982) Découverte de l'herbier de Posidonies. Cahier Parc nation. Port-Cros 4:1–79

Brinkhuis BH, Penello WF, Churchill AC (1980) Cadmium and manganese flux in eel grass *Zoostera marina* II. Metal uptake by leaf and root-rhizome tissues. Mar Biol 58:187–196

Cappuyns V, Swennen R, Devivier A (2004) Influence of ripening on pH status leaching behaviour of heavy metals from dredged sediments. J Environ Monit 6:774–781

Carlson RE (1977) A trophic state index for lakes. Limnol Oceanogr 22(2):361–369

Carvalho S, Moura A, Gaspar MB, Pereira P, Cancela da Fonseca L, Falcão M, Drago T, Leitão F, Regala J (2005) Spatial and interannual variability of the macrobenthic communities within a coastal lagoon (Óbidos lagoon) and its relationship with environmental parameters. Acta Oecol 27:143–159

Cataudella S, Rossi R, Cognetti G (1995) Schede informative sulle lagune e sugli stagni costieri italiani. Consorzio Venezia Nuova 1–57 **(document in Italian language)**

Chatfield C, Collins AJ (1980) Introduction to multivariate analysis. Chapman & Hall, London

Chau KW (2002) Field measurements of SOD and sediment nutrient fluxes in a land-locked embayment in Hong Kong. Adv Environ Res 6:135–142

Chessa LA, Paesanti F, Pais A, Scardi M, Serra S, Vitale L (2005) Perspectives for development of low impact aquaculture in a western Mediterranean lagoon: the case of the carpet clam *Tapes decussates*. Aquac Int 13:147–155

Clarke KR, Green RH (1988) Statistical design and analysis for a "biological effects" study. Mar Ecol Progr Ser 46:213–226

Clarke SM, Kirkman H (1989) Seagrasss dynamics. In: Larkum AWD, McComb AJ, Shepherd SA (eds) Biology of seagrasses. Aquatic plant studies, vol 2. Elsevier, pp 610–634

Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. Primer-E, Plymouth

Coelho S, Gamito S, Perez-Ruzafa A (2007) Trophic state of Foz de Almargem coastal lagoon (Alarve, South Portugal) based on the water quality and the phytoplankton community. Estuar Coast Shelf Sci 71:218–231

Colombo G (1977) Lagoons. In the coastline. Wiley, Chichester, pp 63–82

Como S, Magni P, Casu D, Floris A, Giordani G, Natale S, Fenzi GA, Signa G, De Falco G (2007) Sediment characteristics and macrofauna distribution along a human-modified inlet in the Gulf of Oristano (Sardinia, Italy). Mar Pollut Bull 54:733–744

Consorzio Pisa Ricerche (2002) Studio idrodinamico e progetto preliminare degli interventi urgenti per il miglioramento della circolazione e della qualità delle acque nella laguna di Orbetello, Regione Toscana Commissario Straordinario per la Laguna di Orbetello, Pisa: 71 **(document in Italian language)**

Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P (1997) The value of the World's ecosystem services and natural capital. Nature 387:253–260

De Falco G, Magni P, Teräsvuori LMH, Matteucci G (2004) Sediment grain-size and organic carbon distribution in the Cabras lagoon (Sardinia, west Mediterranean). Chem Ecol 20(Suppl I):367377

de Vivo B, Lima A, Cicchella D, Frizzo P, Di Lella A, Raccagni L, Bove AM, Albanese S, Grezzi G, Sabatini G, Protano G, Riccobono F (2009) Geochemical environmental atlas of Italy. ARACNE editrice S.r.l. via Raffaele Garofalo, 133 A/B 00173 Roma, p 530. ISBN 978-88-548-2282-5

Di Vincenzo R (2006) La laguna di Orbetello Studi, ricerche, criteri e modalità di intervento in Quattro anni di gestione commissariale 2003–2006. In: Ludovico A (eds) Graffietti Stampati, Montefiascone (VT) **(document in Italian language)**

Downing JA, Anderson MR (1985) Estimating the standing biomass of aquatic macrophytes. Can J Fish Aquat Sci 42:1860–1869

Duarte CM (1995) Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41:87–112

Duarte CM (2002) The future of seagrass meadows. Environ Conserv 29:192–206

Dueri S, Castro-Jiménez J, Zaldìvar JM (2008) On the use of the partitioning approach to derive Environmental Water Standards (EQS) for persistent organic pollutants (POPs) in sediments: a review of existing data. Sci Total Environ 403:23–33

Elliott JM (1971) Some methods for the statistical analysis of samples of benthic invertebrates. Windermere, U.K. Freshwater Biological Association 15A:144

Ferrari C, Pirola A, Piccoli F (1972) Saggio cartografico della vegetazione delle Valli di Comacchio. Annali Università di Ferrara Nuova Serie (Sez. 1-Ecologia):35–54 **(document in Italian language)**

Geider RJ, Roche JL, Greene RM, Olalzola M (1993) Response of the photosynthetic apparatus of *Pheodactylum tricornutum* (Bacillariophyceae) to nitrate, phosphate or iron starvation. J Phycol 29:755–766

Gikas GD, Trisevgeni Y, Tsihrintzis A (2006) Water quality trends in a coastal lagoon impacted by non-point source pollution after implementation of protective measures. Hydrobiologia 563:385–406

Giordani G, Bartoli M, Cattadori M, Viaroli P (1996) Sulphide release from anoxic sediments in relation to iron availability and organic matter recalcitrance and its effects on inorganic phosphorus recycling. Hydrobiologia 329(1–3):211–222

Giovani A, Mari E, Specchiulli A, Cilenti L, Scirocco T, Breber P, Renzi M, Focardi SE, Lenzi M (2010) Factors affecting changes in phanerogam distribution patterns of Orbetello lagoon, Italy. Transit Water Bull 4(1):35–52

Giovani A, Mari E, Specchiulli A, Focardi SE, Renzi M (2014) Recent changes of macroalgae distribution patterns in the Orbetello lagoon (Italy). Transit Water Bull 8(1):10–23

Giusti E, Marsili-Libelli S (2006) An integrated model for the Orbetello lagoon ecosystem. Ecol Model 196:379–394

Giusti E, Marsili-Libelli S, Renzi M, Focardi S (2010) Assessment of spatial distribution of submerged vegetation in the Orbetello lagoon by means of a mathematical model. Ecol Model 221:1484–1493

Goldman JC, Caron DA, Dennett MR (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C: N ratio. Limnol Oceanogr 32:1239–1252

Goodman JL, Moore KA, Dennison WC (1995) Photosynthetic responses of eelgrass (*Zoostera marina* L.) to light and sediment sulphide in a shallow barrier island lagoon. Aquat Bot 50:37–47

Guo TZ, De Laune RD, Patrick WH Jr (1997) The influence of sediment redox chemistry on chemically active forms of Arsenic, Cadmium, Chromium, and Zinc in estuarine sediment. Environ Int 23(3):305–316

Hedges JI, Keil RG (1995) Sedimentary organic matter preservation: an assessment and speculative hypothesis. Mar Chem 49:81–115

Herrera-Silveira JA, Medina-Gomez I, Colli R (2002) Trophic status based on nutrient concentration scales and producers community of tropical coastal lagoons influenced by groundwater discharges. Hydrobiologia 475(476):91–98

Hülsemann J (1966) On the routine analysis of carbonates in unconsolidated sediments. J Sed Petrol 36(2):622–625

ICRAM Istituto Centrale di Ricerche Applicate al Mare (Methods 1S, 2S, 4S) (2001) Metodologie analitiche di riferimento del Ministero dell'Ambiente e della tutela del territorio—Servizio Difesa Mare, Programma di monitoraggio per il controllo dell'ambiente marino—costiero (triennio 2001–2003). Ministero Italiano dell'Ambiente e della Tutela del Territorio (Ed.), p 125 **(document in Italian language)**

Innamorati M, Melillo C (2004) Studio della Laguna di Orbetello: ecologia ed aspetti economici Laboratorio di Ecologia, Dipartimento di BiologiaVegetale, Università di Firenze (Ed.). http://eprints.unifi.it/archive/00001260/ **(document in Italian language)**

Jochem FJ (2000) Probing the physiological state of phytoplankton at single cell level. Sci Mar 64:183–195

Jørgensen BB (1983) The microbial sulphur cycle. In: Krumbein W (ed) Microbial geochemistry. Wiley, Oxford, pp 91–124

Karydis M, Ignatiades L, Moschopoulou N (1983) An index associated with nutrient eutrophication in the marine environment. Estuar Coast Shelf Sci 16:339–344

Keil RG, Tsamakis EC, Fuh CB, Giddings JC, Hedges JI (1994) Mineralogical and textural controls on the organic composition of coastal marine sediments: Hydrodynamic separation using SPLITT-fractionation. Geochim Cosmochim Acta 58:879–893

Kennish MJ (1998) Practical handbook of estuarine and marine pollution Boca Raton. CRC Press, Florida

Kennish MJ (2002) Environmental threats and environmental future of estuaries. Environ Conserv 29:78–107

Kim EH, Mason R, Porter ET, Soulen HL (2006) The impact of resuspension on sediment mercury dynamics, and methylmercury production and fate: a mesocosm study. Mar Chem 102:300–315

Kjerfve B (1994) Coastal lagoon processes. Elsevier oceanographic series, vol 60. Elsevier, New York

Knoppers BA (1994) Aquatic primary production in coastal lagoons. In: Kjerfve B (ed) Coastal lagoon processes. Elsevier, Amsterdam, pp 243–286

Kormas K, Nicolaidou A, Reizopoulou S (2001) Temporal variations of nutrients, clorophylla and particulate matter in three coastal lagoons of Amvrakikos Gulf (Ionian Sea, Greece). Mar Ecol 22(3):201–213

Kruskal JB (1964) Multidimensional scaling by optimizing goodness of fit to a non metric hypothesis. Psychometrika 29:1–27

Lapointe BE (1987) Phosphrus and nitrogen-limited photosynhesis and growth f *Gracilariatikvahiae* (Rhodophycae) in the Florida Keys: an experimental field study. Mar Biol 93:561–567

Lapointe BE, O'Connell J (1989) Nutrient enhanced growth of *Cladophora prolifera* in Harrington Sound, Bermuda:

eutrophication of a confined, phosphors limited marine ecosystem. Estuar Coast Shelf Sci 28:347–360
Lapointe BE, Littler MM, Littler DS (1992) Nutrient availability to marine macroalgae in siliciclastic versus carbonate-rich coastal waters. Estuaries 15(1):75–82
Lardicci C, Como S, Corti S, Rossi F (2001) Recovery of the macrozoobenthic community after sever dystrophic crises in a Mediterranean coastal lagoon (Orbetello, Italy). Mar Pollut Bull 42:202–214
Legendre P, Legendre L (1998) Numerical ecology, 2nd English edition. Elsevier, Amsterdam
Lenzi M, Calmieri R, Porrello S (2003) Restoration of the eutrophic Orbetello lagoon (Tyrrhenian Sea, Italy): water quality management. Mar Pollut Bull 46:1540–1548
Lenzi M, Finoia MG, Persia E, Comandi S, Gargiulo V, Solari D, Porrello S (2005) Biogeochemical effects of disturbance in shallow water sediment by macroalgae harvesting boats. Mar Pollut Bull 50:512–519
Lenzi M, Renzi M, Nesti U, Gennaro P, Persia E, Porrello S (2013) Vegetation cyclic shift in eutrophic lagoon assessment of dystrophic risk indices based on standing crop evaluations. Estuar Coast Shelf Sci 132(1):99–107
Leoni L, Sartori F (1997) Heavy metals and arsenic distributions in sediments of the Elba-Argentario basin, southern Tuscany, Italy. Environ Geol 32(2):83–92
Li M, Hu C, Zhu Q, Chen L, Kong Z, Liu Z (2006) Copper and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in the Microalga *Pavlovaviridis* (Prymnesiophyceae). Chemosphere 62:565–572
Lirman D, Deangelo G, Serafy J, Hazra A, Hazra DS, Herlan J, Luo J, Bellmund S, Wang J, Clausing R (2008) Seasonal changes in the abundance and distribution of submerged aquatic vegetation in a highly managed coastal lagoon. Hydrobiologia 596:105–120
Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312:1806–1809
Lukawska-Matuszewska K, Bolalek J (2008) Spatial distribution of phosphorus forms in sediments in the Gulf of Gdansk (southern Baltic Sea). Cont Shelf Res 28:977–990
Luther GW (1991) Pyrite synthesis via polysulphide compounds. Geochim Cosmochim Acta 55:2829–2849
Magni P, De Falco G, Como S, Casu D, FlorisA Petrov AN, Castelli A, Perilli A (2008) Distribution and ecological relevance of fine sediments in organic-enriched lagoons: the case study of the Cabras lagoon (Sardinia, Italy). Mar Pollut Bull 56:549–564
Mahapatro D, Panigrahy RC, Panda S (2013) Coastal lagoon: present status and future challenges. Int J Mar Sci 3(23):178–186
Mannino AM, Sarà G (2006) The effect of *Ruppia cirrhosa* features on macroalgae and suspended matter in a Mediterranean shallow system. Mar Ecol 27:350–360
Marty D, Esnault S, Caumette P, Ranaivoson-Rambeloarisoa E, Bertrand JC (1990) Dénitrification, sulfate-réduction et méthanogènes dans les sediments superficiels d'un étangsaumâtre méditerranéen. Oceanol Acta 13:199–210
Mayer LM (1994) Relationships between mineral surfaces and organic carbon concentrations in soils and sediments. Chem Geol 114:347–363
McCave IN, Menighetti B, Robinson SG (1995) Sortable silt and fine sediment size/composition slicing: parameters for palaeocurrents speed and palaeo-oceanography. Paleoceanography 10:593–610
Mee LD (1978) Coastal lagoons. In: Riley J, Skirrow O (eds) Chemical oceanography, vol 7, 2nd edn. Academic Press, New York, pp 441–490
Miao S, De Laune RD, Jugsujinda A (2006) Influence of sediment redox conditions on release/solubility of metals and nutrients in Louisiana Mississipi river deltaic plain freshwater lake. Sci Tot Environ 371:334–343
Miller MW, Sluka RD (1999) Patterns of seagrass and sediment nutrient distribution suggest anthropogenic enrichment in Laamu Atoll, Republic of Maldives. Mar Pollut Bull 38:1152–1156
Morand P, Briand X (1996) Excessive growth of macroalgae: a symptom of environmental disturbance. Bot Mar 39:491–516
Murray JW (1987) Mechanisms controlling the distribution of trace elements in oceans and lakes. In: Hites RA, Eisenreich SJ (eds) Source and fates of aquatic pollutants. American Chemistry society, Washington, DC, pp 153–184
Muslim I, Jones G (2003) The seasonal variation of dissolved nutrients, chlorophyll a and suspended sediments at Nelly Bay, Magnetic Island. Esturar Coast Shelf Sci 57:445–455
Newton A, Icely JD, Falcao M, Nobre A, Nunes JP, Ferreira JG, Vale C (2003) Evaluation of eutrophication in the Ria Formosa coastal lagoon, Portugal. Cont Shelf Res 23:1945–1961
Nguyen LM, Cooke JG, McBride GB (1997) Phosphorus retention and release characteristics of sewage-impacted wetland sediments. Water Soil Air Pollut 100:163–179
Nguyen-Deroche TLN, Le TT, Bui TV, Rincé Y, Tremblin G, Morant-Manceau A (2009) Effects of copper on growth and photosynthesis in marine diatoms: a comparison between species from two different geographical areas. Cryptogam Algol 30:97–109
Nixon SW (1982) Nutrient dynamics, primary production and fishery yields of lagoons. In: Actes Symposium Interantional sur les Lagunes Côtières, SCOR/IABO/UNESCO, pp 357–381
Orfanidis S, Reizopoulou S, Basset A (2008) Transitional states in transitional waters aquatic conservation. Mar Freshwater Ecosyst 18:S1–S3
Pardu JH, Patrick WH Jr (1995) Changes in metal speciation following alteration of sediment redox status. In: Allen HE (eds) Metal contaminated aquatic sediments, CRC Press, pp 169–185
Patrick WH Jr, Jugsujinda A (1992) Sequential reduction and oxidation of inorganic nitrogen, manganese and iron in flooded soil. Soil Sci Soc Am J 56:1071–1073
Pearson K (1894) Contributions to the mathematical theory of evolution. Philos Trans R Soc Lond Ser A 185:71–110
Pedersen KL, Pedersen SN, Hojrup P, Knudsen J, Depledge MH (1998) Induction and identification of cadmium, zinc and copper metallothioneins in the shore crab *Carcinus maenas*. Comp Biochem Physiol 120:251–259
Pergent-Martini C, Le Ravallec C (2007) Guidelines for impact assessment on seagrass meadows regional activity centre for specially protected areas (RAC/SPA) Boulevard du leader Yasser Arafat B.P. 337-1080 Tunis CEDEX, 48
Phleger FB (1981) A review of some general features of coastal lagoons. In: Coastal lagoon research, past, present, and future, UNESCO technical papers in marine science, vol 33, pp 7–14
Plus M, Deslaus-Paoli JM, Degault F (2003) Seagrass (*Zoostera marina* L) bed 14 decolonisation after anoxia-induced full mortality. Aquat Bot 77:121–134
Rai LC, Gaur JP, Kumar HD (1981) Phycology and heavy metal pollution. Biol Rev 56:99–151
Renzi M, Lenzi M, Franchi E, Tozzi A, Porrello S, Focardi S, Focardi SE (2007) Mathematical modelling of sediment chemicophysical parameters in a coastal lagoon to estimate high density seagrass meadow (*Ruppia cirrhosa*) distribution. Int J Environ Health 1(3):360–373
Renzi M, Perra G, Guerranti C, Franchi E, Focardi S (2009) Abatement efficiency of municipal wastewater treatment plants using different technologies (Orbetello Lagoon, Italy). Int J Environ Health 3(1):58–70

Renzi M, Giovani A, Focardi SE (2012) Water pollution by surfactants: fluctuations due to tourism exploitation in a lagoon ecosystem. J Environ Protect 3:1004–1009

Renzi M, Giovani A, Focardi SE (2014a) Ecotoxicological effects induced by toxicant exposure on macrophytes: a mesocosm study. J Environ Protect 5:274–288

Renzi M, Roselli L, Giovani A, Focardi SE, Basset A (2014b) Early warning tools for ecotoxicity assessment based on *Phaeodactylum tricornutum*. Ecotoxicology. doi:10.1007/s10646-014-1249-z

Roden EE, Edmonds JW (1997) Phosphate mobilization in iron-rich anaerobic sediments: microbial Fe(III) oxide reduction versus iron-sulfide formation. Arch Hydrobiol 139:347–378

Ronen R, Galun M (1984) Pigment extraction from lichens with dimethylsulfoxide (DMSO) and estimation of chlorophyll degradation. Environ Exp Bot 24:239–245

Rozan TF, Taillefert M, Trouwborst RE, Glazer BT, Ma S, Herszage J, Valdes LM, Price KS, Luther GW III (2002) Iron-sulphur-phosphorus cycling in the sediments of a shallow coastal bay: implications for sediment nutrients release and benthic macroalgal blooms. Limnol Oceanogr 47(5):1346–1354

Sanders HL (1968) Marine benthic diversity: a comparative study. Am Nat 102:243–282

Schlacher-Hoenlinger MA, Schlacher TA (1998) Accumulation, contamination and seasonal variability of trace metals in the coastal zone-patterns in a seagrass meadow from the Mediterranean. Mar Biol 131:401–410

Shepard RN (1962) The analysis of proximities: multidimensional scaling with an unknown distances function. Psychometrika 27:125–140

Short FT, Koch EW, Creed JC, Magalha KM, Fernandez E, Gaeckle JL (2006) Seagrass net monitoring across the Americas: case studies of seagrass decline. Mar Ecol 27:277–289

Somerfield PJ, Clarke KR (1995) Taxonomic levels, in marine community studies, revisited. Mar Ecol Progress Series 127:113–115

Souza MFL, Kjerfve B, Knoppers B, de Souza LWF, Damasceno RN (2003) Nutrient budgets and trophic state in a hypersaline coastal lagoon: Lagoa de Araruama, Brazil. Estuar Coast Shelf Sci 57:843–858

Specchiulli A, Focardi S, Renzi M, Scirocco T, Cilenti L, Breber P, Bastianoni S (2008) Environmental heterogeneity patterns and assessment of trophic levels in two Mediterranean lagoons: Orbetello and Varano, Italy. Sci Tot Environ 402:285–298

Specchiulli A, Renzi M, Scirocco T, Cilenti L, Florio M, Breber P, Focardi S, Bastianoni S (2010) Comparative study based on sediment characteristics and macrobenthic communities in two Italian lagoons. Environ Monit Assess 160(1–4):237–256

Stauber JL, Florence TM (1990) Mechanisms of toxicity of zinc to the marine diatom *Nitzschia closterium*. Mar Biol 105:519–524

Steeman-Nielsen L, Wium Andersen S (1971) The influence of Cu on photosynthesis and growth in diatoms. Physiol Plant 24:480–484

Sunda GW, Huntsman SA (1983) Effect of competitive interactions between manganese and copper on cellular manganese and growth in estuarine and oceanic species of the diatom *Thalassiosira*. Limnol Oceanogr 28:924–934

Underwood AJ (1993) The mechanics of spatially replicated sampling programmes to detect environmental impacts in a variable world. Aust J Ecol 18:99–116

Underwood AJ, Beyond B (1992) The detection of environmental impact on populations in the real, but variable, world. J Exp Mar Biol Ecol 161:145–178

Underwood AJ, Chapman MG (2003) Power, precaution, type II error and sampling design in assessment of environmental impacts. J Exp Mar Biol Ecol 296:49–70

Usero J, Izquierdo C, Morillo J, Gracia I (2003) Heavy metals in fish (*Solea vulgaris*, *Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic Coast of Spain. Environ Int 29(7):949–956

Viaroli P, Bartoli M, Giordani G, Naldi M, Orfanidis S, Zaldivar JM (2008) Community shifts, alternative stable states, biogeochemical controls and feedbacks in eutrophic coastal lagoons: a brief overview. Aquat Conserv 18(S1):S105–S117

Wainright SC, Hopkinson CS Jr (1997) Effects of sediment resuspension on organic matter processing in coastal environments: a simulation model. J Mar Syst 11:353–368

Ward TJ (1987) Temporal variation of metals in the seagrass *Posidonia australis* and its potential as a sentinel accumulator near a lead smelter. Mar Biol 95:315–321

Westlake DF (1969) Macrophytes. In: Vollenweider RA, Talling JF, Westlake DFE (eds) A manual on methods for measuring primary production in aquatic environments including a chapter on bacteria, chapter 2.2. Wiley, Oxford, p 25

Zaldívar JM, Cattaneo E, Plus M, Murray CN, Giordani G, Viaroli P (2003) Long-term simulation of main biogeochemical events in a coastal lagoon: Sacca di Goro (Northern Adriatic Coast, Italy). Cont Shelf Res 23:1847–1875