MDPI

Metal Bioaccumulation and Oxidative Stress in *Ulva laetevirens* in the Venice Lagoon: Early Warning Biomarker for Metal Bioaccumulation

Abdul-Salam Juhmani [1,*] , **Andrea A. Sfriso** [2] , **Mohammad Wahsha** [3] , **Alessandro Buosi** [1] , **Yari Tomio** [1] and **Adriano Sfriso** [1]

1   Department of Environmental Sciences, Informatics and Statistics, Ca'Foscari University of Venice, Via Torino 155, 30172 Venezia, Italy; alessandro.buosi@unive.it (A.B.); yari.tomio@unive.it (Y.T.); sfrisoad@unive.it (A.S.)
2   Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara 17, 44121 Ferrara, Italy; andrea.sfriso@unife.it
3   Marine Science Station, The University of Jordan, Aqaba Branch, Aqaba 77110, Jordan; m.wahsha@ju.edu.jo
*   Correspondence: abdulsalam.juhmani@unive.it; Tel.: +39-3920-175088

Abstract: Transitional water systems (TWSs) may be threatened by various metals originating from increased agricultural, industrial activities, or urban effluents. Macroalgae are one of the biological quality elements used to monitor and assess the health status of TWS due to their structural and functional key role in marine ecosystems. Here, metal accumulation from the macroalgae *Ulva laetevirens* Areschoug (1854) and oxidative stress by lipid peroxidation (LPO) biomarker were investigated during four sampling seasons from three sampling sites (SMM: Santa Maria del Mare; PM: Porto Marghera; SG: San Giuliano) of Venice Lagoon, affected by different anthropogenic stressors. The metal pollution index (MPI) scores for *U. laetevirens* increased in the order SMM < PM < SG (sea inlet < industrial area < Osellino River estuary), with average values per site of 2.99, 4.37, and 6.33, respectively. The level of LPO was statistically correlated with the concentration of toxic metal(loid)s (As, Pb, Hg) measured in macroalgae, and seasonality affected both levels of LPO and metal bioaccumulation, with peak values during spring and summer. These findings highlighted the efficiency and usefulness of the oxidative stress test (LPO) on the common macroalga *U. laetevirens* as an early warning signal for health assessment in aquatic ecosystems.

Keywords: Venice Lagoon; lipid peroxidation; oxidative stress; *Ulva*; metals

1. Introduction

Increased anthropogenic pressures over the last few decades have caused the ecological deterioration of estuarine habitats of high economic value [1,2]. Transitional lagoons are subjected to dramatic environmental changes that influence their high biological productivity [3]. TWSs are threatened by several types of potentially toxic elements (PTEs) resulting from increasing volumes of agricultural, industrial, or domestic effluents [4]. Particularly, it is well known that both macrophytes and sediments of TWSs can act as a sink for various contaminants released into the environment by freshwater inputs, for instance, metals and metalloids [5].

Macrophytes are one of the biological quality elements within the European Water Framework Directive (WFD) (2000/60/EC) used to monitor and assess the ecological status of TWS because of their structural and functional role in the aquatic ecosystems [6]. Macroalgae are sensitive to anthropogenic stressors, and they modify their structure and function accordingly [7]. Thus, macroalgae and aquatic plants are indicators that provide information on the health status of TWS ecosystems. Therefore, their use as target species for a biomarker investigation and early warning signal stress is recommended [4]. The macroalgae of the genus *Ulva* have been proposed as good pollution biosentinels

due to their exceptional capacity to tolerate a broad range of environmental conditions, e.g., [8,9]. These macroalgae are characterised by wide geographic distribution in TWS with a high propensity to accumulate metals and possibly a potential bioindicator of metal contamination [10]. Other favourable characteristics are their high surface area/volume ratio (provided by a laminar structure) and a structurally uniform and physiologically active thallus [9]. Indeed, the greater presence of nutrients in seawater, also deriving from eutrophic phenomena, favours the increase of these macroalgae, which tend to consume the excess of nutrients [11].

A great challenge of TWS health assessment is to distinguish between environmental and anthropogenic stressors [12]. In fact, the complex interactions of PTEs and biota that appear in the environment make them difficult to evaluate by means of chemical analyses only. Accordingly, the comprehensive assessment of metal contamination in TWS must involve both the quantification of metal accumulation in biota tissues and the assessment of biochemical parameters [13,14]. Metal exposure can induce excessive reactive oxygen species (ROS) production, resulting in cellular oxidative damage of lipids, proteins, or DNA [15,16]. However, oxidative stress deteriorates the structure and functionality of the cells. Fatty acid-rich structures, such as the cell membranes, may be oxidised by overproduction of ROS, causing a loss of rigidity and permeability and ultimately leading to cell death [17]. Previous studies demonstrated that the determination of the lipid peroxide (malondialdehyde (MDA)) content is a widely used technique to estimate the oxidative stress level in biological materials [17]. Indeed, these biochemical responses have been less explored in macroalgae. Most of the existing studies have been conducted under laboratory conditions [18,19], rather than in field scenarios [10,20]. In this context, this paper aims, for the first time, to evaluate the oxidative stress responses of a model primary producer, the macroalga *U. laetevirens*, to environmental metal contamination on a seasonal basis, and to evaluate the efficiency of the oxidative stress response on monitoring of TWS.

2. Materials and Methods

2.1. Study Area

The Venice Lagoon (VL) ($\approx$549 km^2) is a complex dynamic system (Figure 1), including marine and estuarine environments, salt marshes, and areas subjected to intense anthropogenic pressures, such as the historical centre of Venice and the industrial site of Porto Marghera [21]. Various pressures have threatened the lagoon ecosystem, including eutrophication [22], degradation of biota due to bioaccumulation of pollutants through the food chain [23,24], and sediment erosion [25]. The central part of the lagoon has been affected by metal contamination originating from the industrial area of Porto Marghera [26]. However, sediments and particulate matter are also potential sources of contaminants. The high rate of settled particulate matter [SPM] (600–1000 kg dwt m^2 year^{-1}) in the VL [25] can redeposit in situ or spread all around. SPM contains high nutrient and pollutant concentrations and spread the contaminants accumulated in highly polluted areas over the entire lagoon [27,28]. Three sampling sites were selected from VL, with reference to the central basin according to the variation in the source of anthropogenic pressures that were expected to affect the macroalgal assemblages.

The first site, Santa Maria del Mare (SMM), is located near the Malamocco port entrance which experiences very effective tidal water exchanges. It is characterised by high ecological status and is poorly affected by anthropogenic pressures [27]. The second site, Porto Marghera (PM), is located close to the inner border of the lagoon, and it is considered as a site of national interest (SIN) by the Italian National Law n. 426/1998 for its unprecedented levels of contamination [29]. PM is influenced by the pollutants released from the industrial activities of the petrochemical industrial pole and by urban and agricultural water inputs that released pollutants into the lagoon [26,30,31]. The third site, San Giuliano (SG), is close to the inner border of the lagoon and has very poor water exchange with the sea (with water residence time exceeding 60 days—[32]); it is characterised by hypertrophic conditions and marked industrial and urban contamination [33,34]. SG is affected by

the freshwater inputs of the Osellino River, which carries nutrients and pollutants to the lagoon [24]. It is also characterised by the presence of anoxic sediments and persistent water anoxia in spring–summer.
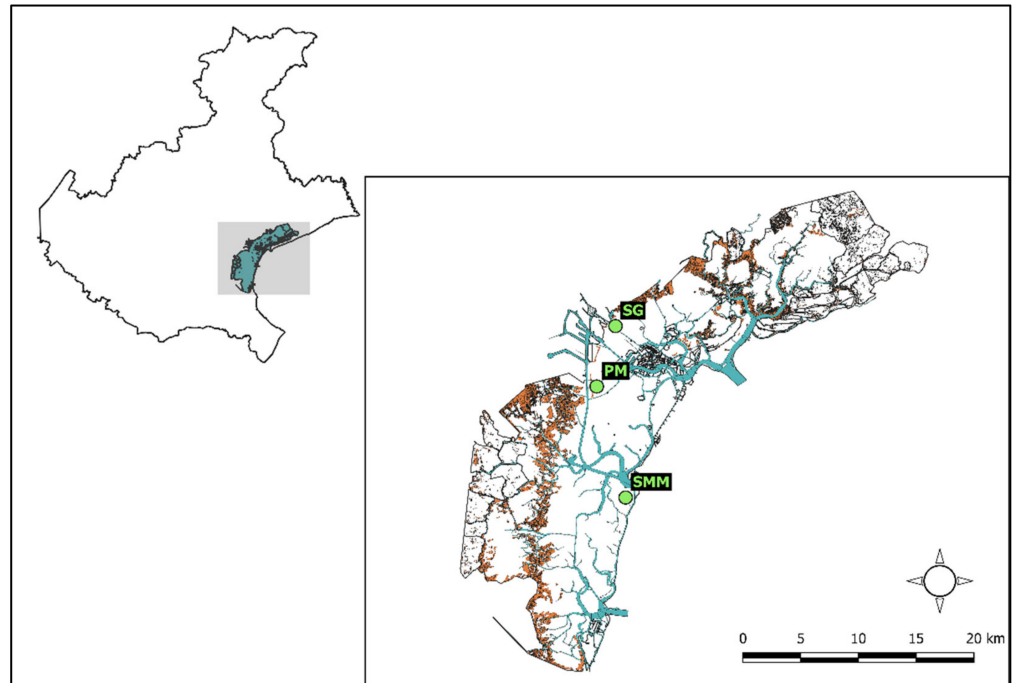

Figure 1. Sampling sites from the Venice Lagoon: SMM—Santa Maria del Mare; PM—Porto Marghera; SG—San Giuliano.

2.2. Macroalgal Collection and Preparation

Macroalgal samples of *U. laetevirens* were collected at low tide from the intertidal zone. Only thalli attached to rock/substrate were collected to verify the tissue site source. Approximately 15 to 25 equal size thalli from different areas were collected and pooled together in each sampling site. The thalli were washed on-site by seawater to eliminate the trace of sediment and epiphytes. Samples were stored at room temperature in sealed polyethylene bottles until analysis. For biochemical analyses, at least 10 equally sized thalli were sampled at each site during low tide and pooled together with the surrounding water, as described by Wahsha et al. [4]. Samples were packed in plastic bags not fully closed with a non-metallic closure (allowing gas exchange) to minimise the stress during transport to the laboratory allowing gas exchange. Before analyses, all macroalgal samples were gently washed and rinsed with standardised seawater for 10 s to avoid any appreciable stress decomposition.

2.3. Water, Sediments, and SPM Collection and Preparation

From each sampling site, surface water at a depth of 50 cm was collected and stored in polyethylene bottles. Three surficial sediments (top 5 cm) samples were collected using a Plexiglass corer (i.d. 10 cm) and mixed together according to the sampling procedure described in Sfriso et al. [25]. Water and sediment pH (accuracy $\pm 0.015\%$) and redox potential (Eh) (accuracy $\pm 0.15\%$) were measured on-site with a portable pH meter (model HD 8705, Delta HHM, Padua, Italy). A total of 20 mL water samples were collected to determine salinity by the chlorine titration method [35]. Sedimentation traps (base: 20×20 cm; mouth: 15×15 cm; height 10 cm) designed for shallow bottoms were used to collect the SPM according to the sampling procedure described in Sfriso et al. [25]. An aliquot of sediment and SPM samples were retained for physicochemical characteristics.

2.4. Physicochemical Characteristics of the Sediment and SPM

Sediment and SPM samples from each sampling site were analysed for grain-size composition and total organic matter (TOM) content. The percentage of fines (fraction < 63 μm) was obtained by sieving ca. 50 g of dried samples through 63 μm mesh sieves [25]. The TOM content was determined by loss on ignition according to Byers et al. [36], corresponding to the percentage of weight loss in 1 g of dried sediment or SPM, after 2 h of combustion at 450 °C using a muffle furnace (Muffola-ZA, Padua, Italy). The concentration of organic phosphorus (P org) and inorganic (P inorg) phosphorus in the sediment and SPM was determined by the spectrophotometric procedure according to Aspila et al. [37]. Total phosphorus (TP) was extracted with 1 N hydrochloric acid after ignition at a high temperature (550 °C), whereas P org was determined by the difference in phosphorus content of the 1 N hydrochloric acid extract measured before and after ignition of the dry sediments at 550 °C. Total nitrogen (TN) was determined by CHNS Elemental Analyser (Vario-MICRO, Elementar CHNS, Langenselbold, Germany) after sediment pulverisation with a sediment mill according to the procedure previously described by Sfriso et al. [38].

2.5. Metal(Loid) Determination in Sediment, SPM, and Macroalgal Tissues

The concentration of metal(loid)s was measured in the surface sediment layer, SPM, and macroalgal tissues. Macroalgal samples were freeze-dried, pulverised, and sieved (Retsch Sieve shaker, AS200, Haan, Germany) to a particle size of <500 μm. Aliquots of approx. 100 mg of the lyophilised sediment, SPM, and macroalgae were completely mineralised by a mixture of nitric acid, perchloric acid, distilled water in closed Teflon bombs and digested at 130 °C for 2 h. in a microwave oven (CEM MDS-2000, Naples, Italy). Each digest was brought to 50 mL with Milli-Q water. The extractions were performed in triplicate on different days to ensure the reliability and reproducibility of the extraction method [28]. Procedural blanks were prepared using the same analytical procedure and reagents without the sample. The quantification of metal(loid)s in the extracts was determined by atomic absorption spectrometry (SpectrAA-250, Plus VARIAN spectrometer, CA, USA). Results were expressed as microgram per gram of dry weight ($\mu g\ g^{-1}$). The accuracy of the analytical procedure was verified through the analysis of certified reference materials, MESS-3 (Marine Sediment Reference Materials for Trace Metals—National Research Council Canada) and CRM N° 0974 (Olea Europaea) that had undergone the same extractions of the samples. Metal(loid) recoveries were higher than 94%, while the mean coefficient of variation was < 8%. All the quantifications were performed with samples, and sample blanks were run in parallel. The sample blank was subtracted from each sample, and the mean values and respective standard deviations were calculated [39].

2.6. Biochemical Analysis in Macroalgal Tissues

The LPO level (as MDA) was determined by the thiobarbituric acid reactive substance (TBARS) reaction as described by Wahsha et al. [17]. About 0.10 g fresh weight of the macroalgal tissues was homogenated in a 5 mL solution of 0.25% thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA). The sample was incubated at 95 °C for 30 min, followed by quick cooling for 1 min, and centrifuged at $10,000\times g$ for 10 min. The absorbance of the supernatant was measured by spectrophotometer (V-10 plus, ONDA, Carpi, Italy) at 532 nm, and correction for unspecific turbidity was performed by subtracting the absorbance of the sample at 600 nm. The concentrations of MDA were quantified and expressed using Beer's law with an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.7. Statistical Analyses

The Spearman's coefficients ($p < 0.05$) among sediment characteristics (% fines and TOM, pH and Eh), SPM (% fines and TOM), metal(loid) concentration in the sediments, SPM and macroalgal tissues, and their spatial distribution were analysed by STATISTICA software version 10.1 (Stat. Soft. Inc.,1998, Tulsa, OK, USA). Metal concentrations were reported as mean values ± standard deviation for all macroalgal, SPM, and sediment

samples. The overall metal content in sediment, SPM, or macroalgae was measured for different sites to compare the degree of metal pollution with the metals pollution index (MPI) using the formula reported by Usero et al. [40].

$$MPI = (Cf1 \times Cf2 \ldots Cfn)^{1/n}$$

where Cfi = concentrations of the metal 'i' in the sample.

The concentration of metal(loid)s in macroalgae ($\mu g\, g^{-1}$ dw) used for the determination of the biota-sediment accumulation factor (BSAF) as in Velez et al. [41].

$$BSAF = \frac{Cf\ Ulva}{C\ s}$$

where Cf Ulva = total concentration of a given metal(loid) in the macroalgae tissue, and C s = concentration of that metal(loid) in the sediment or SPM.

3. Results

3.1. Environmental Parameters for Water, Surface Sediments, and SPM

Physicochemical characteristics of the surficial water, superficial sediments, and SPM from the VL sampling sites are shown in Table S1. Water samples from SMM were characterised by the highest pH and salinity (8.3 ± 0.14 units, 26.6 ± 4.37 psu, respectively) and the lowest average water temperature (19.7 ± 4.53 °C). Sediment from PM and SG showed more reduced conditions ((−80)–(−193) mV) and presented lower pH values (7.4–7.9) in comparison to SMM (168 mV, 8.1 units for Eh, pH, respectively). Both sediment and SPM showed an increasing percentage of fines and organic matter (TOM) from SMM to PM and SG.

In general, TN concentrations increased from SMM to SG and were higher in SPM than in surface sediments. At SG, the highest TN values were 1.4 mg/g in surface sediments and 2.3 mg/g in SPM. Total phosphorus (TP) (sum of Pinorg and Porg) showed the lowest concentrations at SMM in both sediments and SPM. TP in SPM showed an increasing trend from SMM to SG, whereas in surface sediment, the highest concentration was recorded at PM but with the lowest Porg value.

3.2. Metal(Loid)s in Sediments and SPM

Metal(loid) concentrations ($\mu g\, g^{-1}$ dw) in the sediments and SPM are shown in Table 1. Most of the metal(loid)s (As, Cu, Mn, Pb, V, and Zn) showed higher concentrations in SPM in comparison to sediment.

Table 1. Metal(loid) concentrations (mean ± SD) ($\mu g\, g^{-1}$ dw) in surface sediments and SPM collected at LV: SMM—Santa Maria del Mare; PM—Porto Marghera; SG—San Giuliano; MPI—metal pollution index.

Site		As	V	Ni	Mn	Zn	Cu	Pb	Hg	MPI
SMM	Sediment	1.00 ± 0.14	14.0 ± 0.21	8.65 ± 1.63	258 ± 1.77	17.3 ± 1.13	6.40 ± 0.84	4.75 ± 0.07	<d.l.	10.7
PM		4.89 ± 0.21	32.5 ± 1.27	22.0 ± 0.85	242 ± 1.34	327 ± 3.39	28.4 ± 1.06	68.1 ± 1.83	1.65 ± 0.05	31.1
SG		11.2 ± 0.89	22.9 ± 0.63	15.8 ± 1.27	368 ± 28.9	428 ± 12.5	45.3 ± 2.76	56.1 ± 0.98	0.85 ± 0.02	32.9
SMM	SPM	13.6 ± 3.39	19.1 ± 0.47	14.6 ± 0.85	365 ± 0.70	46.7 ± 0.07	9.17 ± 0.22	13.3 ± 0.78	0.29 ± 0.03	14.8
PM		16.9 ± 0.49	53.6 ± 1.41	22.1 ± 0.35	485 ± 3.53	659 ± 2.12	49.1 ± 0.85	72.8 ± 0.21	1.09 ± 0.04	47.3
SG		17.8 ± 3.89	39.5 ± 0.85	23.5 ± 0.21	606 ± 2.83	325 ± 2.83	49.2 ± 0.01	45.4 ± 0.78	0.87 ± 0.05	39.9

In surface sediments, Cu, Mn, Pb, Zn, and As were significantly higher at SG (Tukey's test, $p < 0.05$), whereas Ni and V were higher at PM. Sediments from both polluted sites (SG and PM) showed concentrations of Cu, Pb, and Hg which fall into Class B of the 'Venice Special Law' [42], whilst Zn was in class C (high contamination).

In SPM, the contamination of metal(loid)s showed a similar pattern with the highest concentrations at SG and PM. The toxic metal(loid)s As, Hg, and Pb at PM and SG were in class B, whereas Cu and Zn concentrations at PM were in class C (high contamination).

Spearman's rank-order correlations of metal(loid)s from sediment and SPM are shown in Table S2.

Positive correlations (significant with $p < 0.05$) were shown for many elements, except Mn in sediments and As in SPM. The sediment concentrations of V, Pb, Hg were significantly correlated with those of SPM (r = 0.83, 0.89, and 0.87, respectively). The metal pollution index (MPI) allowed the comparison of total metal(loid)s in sediments and SPM (Table 1). The highest MPI values for both sediment and SPM were measured in the polluted sites (PM and SG), whereas at the SMM site, MPI for sediment and SPM were 10.7, 14.8, respectively. Higher concentrations of SPM metals compared with sediment resulted in the increase of MPI value at PM and SG (47.3 and 39.9, respectively).

3.3. Metals Accumulated in U. laetevirens

The average annual concentrations of *U. laetevirens* metal(loid)s in the VL sampling sites are shown in Table 2. The concentrations of metal(loid)s in *U. laetevirens* recorded during the four sampling seasons are reported in Table S3. The maximum average annual bioaccumulation was observed for Cu, Mn, Pb, and Zn at the SG site. This result corresponded to the highest MPI values. The MPI that summarises the total metal(loid) contamination in *U. laetevirens* was calculated for all sites and seasons. MPI scores always increased in the order SMM < PM < SG with average values per site of 2.5, 3.2, and 5.7, respectively. The highest MPI values were recorded in spring and summer (5.2 and 4.9, respectively), values almost twofold higher than autumn and winter values.

Table 2. Metal(loid) average annual concentrations (Mean $\pm$ SD) ($\mu g\ g^{-1}$ dw) in *U. laetevirens* collected at VL: SMM—Santa Maria del Mare; PM—Porto Marghera; SG—San Giuliano; MPI—metal pollution index.

Site	As	V	Ni	Mn	Zn	Cu	Pb	Hg	MPI
SMM	1.6 ± 0.7	10 ± 10.6	2.4 ± 1.0	38 ± 20.9	14.7 ± 11.1	8.6 ± 2.8	0.3 ± 0.3	0.1 ± 0.1	2.99
PM	9.9 ± 3.7	2.3 ± 2.0	1.4 ± 0.5	31.3 ± 11.6	33.3 ± 24.1	28.8 ± 30.5	0.6 ± 0.5	0.2 ± 0.1	4.37
SG	4.8 ± 1.2	1.5 ± 1.6	1.9 ± 1.0	164.8 ± 159.4	56.2 ± 35.3	29.2 ± 22.0	2.7 ± 1.3	0.3 ± 0.1	6.33

The concentrations of Cu and Zn were significantly higher in *U. laetevirens* during spring and summer. *U. laetevirens* collected at the SMM site showed a significantly higher accumulation of V in summer and autumn. The site comparison revealed significant Mn (spring) increases at SG and Cu (summer) at the PM site. The macroalgae collected at the SMM site had no seasonal differences for Cu and Ni, whereas Hg did not exhibit specific seasonal changes at all sapling sites. The toxic metal(loid) [As and Pb] in *U. laetevirens* from PM and SG showed higher accumulation during spring. Moreover, metal(loid) accumulation showed a less pronounced increase during the lowest growth period (winter), compared to the other seasons.

The BSAF values for metals in *U. laetevirens* varied between sediment and SPM (Table 3). The highest BSAF values (>1.0) in sediments were obtained for As and Cu at SMM and PM. Generally, BSAF values were higher between *U. laetevirens* and sediments than between *U. laetevirens* and SPM. The lowest accumulation factor was recorded for Pb in both sediment and SPM. Moreover, BSAF values of metal(loid)s were lower in SPM except for Hg.

Table 3. Biota-sediment accumulation factors (BSAF) for metals of sediment and SPM in *U. laetevirens* collected at LV: SMM—Santa Maria del Mare; PM—Porto Marghera; SG—San Giuliano.

Ulva/Sediment	As	V	Ni	Mn	Zn	Cu	Pb	Hg
SMM	1.62	0.72	0.28	0.15	0.85	1.35	0.05	0
PM	2.03	0.07	0.06	0.13	0.10	1.02	0.01	0.14
SG	0.43	0.06	0.12	0.45	0.13	0.64	0.05	0.30
Average	1.36	0.28	0.16	0.24	0.36	1.00	0.04	0.15
Ulva/SPM	As	V	Ni	Mn	Zn	Cu	Pb	Hg
SMM	0.12	0.53	0.17	0.10	0.31	0.94	0.02	0.44
PM	0.59	0.04	0.06	0.06	0.05	0.59	0.01	0.21
SG	0.27	0.04	0.08	0.27	0.17	0.59	0.06	0.30
Average	0.33	0.20	0.10	0.15	0.18	0.71	0.03	0.32

3.4. Oxidative Stress Biomarker (LPO)

The level of LPO (expressed as MDA content) in tissues of *U. laetevirens* in the different sampling periods is shown in Figure 2. One-way ANOVA ($p < 0.05$) results showed that the biological responses of *U. laetevirens* from polluted sites (PM and SG) were significantly different, compared to the SMM site. The maximum levels of LPO were recorded at PM and SG in spring (42.1 and 67.9 µM/g, respectively) (Table S4). Moreover, the level of LPO at the SMM site was low and showed negligible seasonal fluctuations (7.9 ± 4.3 µM/g) compared with the levels obtained from the other stressed sites during all sampling seasons.
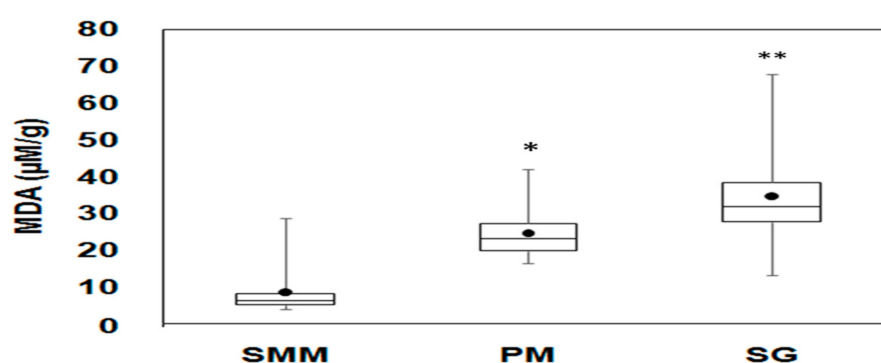

Figure 2. Boxplot with median and mean (●solid circles) of LPO (as MDA concentration) in *U. laetevirens* from the VL: SMM—Santa Maria del Mare; PM—Porto Marghera; SG—San Giuliano. *, ** denote significant difference ($p < 0.05$, $p < 0.01$, respectively) between sampling sites.

The LPO levels in macroalgae varied accordingly with the level of metal(loid)s in the tissues of the corresponding sites, indicating a close relationship between LPO and metals (Figure 3). Significant increasing coefficients were recorded for As, Pb, and Hg in *U. laetevirens* that seemed to contribute most to the LPO changes. This was supported by the significant correlation ($p < 0.05$) between these metals(loid)s accumulated in *U. laetevirens* and the LPO levels (Table S5). The scatter plot of Figure 3 showed that the highest degree of correlation was recorded between LPO and Hg concentration alone, with the steepest line. Middle scores (r value) were performed instead by the MPI averaging the concentrations of Hg, As, and Pb, the three most toxic elements. The MPI calculated on the overall metal(loid)s displayed a much lower degree of correlation with an angular coefficient that was only a small fraction of the most toxic metals. The response recorded by the LPO seems to be attributable mainly to the three toxic (As, Pb, Hg) metal(loid)s and among them, especially to Hg.
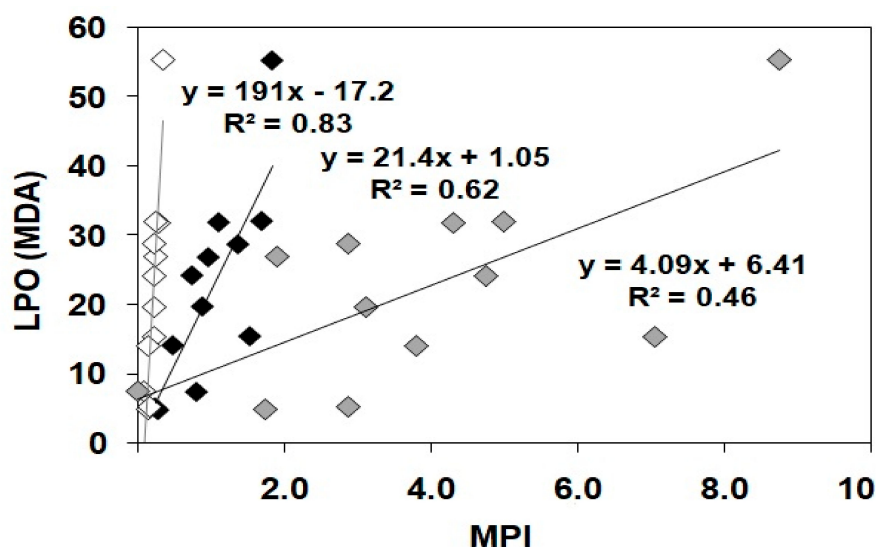
Figure 3. The plot of correlation between MPI and LPO levels for *U. laetevirens* collected in the three VL sites. Solid white squares □ indicated MPI/concentration of Hg alone, solid black squares ■ indicated MPI calculated for As, Hg and Pb together, and solid gray squares ■ indicated the MPI calculated for the overall metals.

4. Discussion

4.1. Metal Concentrations in Sediment and SPM

The highest concentrations of metal(loid)s were recorded in the sediments from PM and SG sites, whilst SMM can be considered a reference site for both sediments and SPM. Most toxic metal(loid)s (As, Hg, Pb) were abundant, especially at PM, which is continuously exposed to contaminants originating from the industrial area of Porto Marghera and the so-called oil canal for oil transport in the central lagoon. Our results are in accordance with those reported in the recent papers for VL by Masiol et al. [31] and Picone et al. [43], especially for As and Ni. In surface sediments, the metalloid As showed an increasing concentration trend on a transect from Lido (close to SMM) towards the mainland of VL [44]. However, our results showed that metalloid (As) was surprisingly high in SPM from the SMM site, which was expected to be the less polluted site than the other sampling sites. The most plausible explanation is that the biogeochemical processes of As along the sea coast of the North Adriatic Sea are different from those inside the lagoon, especially the processes related to the variability of sediment hydrodynamics and redox conditions. The aerobic conditions characterised marine sediments, favouring the precipitation of As in the sediments, bounded to Fe/Mn oxides/hydroxides [45]. Indeed, Sfriso et al. [46] found that the concentrations of As in the sands of the sea were higher than that recorded in the lagoon, also in comparison with the polluted area of PM. The SMM site is subject to sedimentation of suspended matter coming both from the polluted areas of PM during ebb tide and from the seawater entering the lagoon during flood tide.

On the other hand, the chemical composition of sediments from the inner part of the lagoon (SG, PM sites) promotes the high accumulation of organic matter in the finest sediments observed in previous studies [25,47,48]. Organic loads enter the innermost parts of the VL through river flows (Osellino and Brentella), and the weak tidal currents and long residence time in these choked areas facilitate the settling down of organically enriched particles. Indeed, the mobility of heavy metals can be remarkably affected by the changes of physicochemical parameters occurring on the surface sediments [49]. The high concentration of nutrients (TN and TP) at SG, reflects mainly the outflow of organic matter by the river Osellino into the inner part of the VL. This study shows that, in general, the study sites exhibited Hg and Pb concentrations higher than the reference values of class B and C of the 'Venice Special Law' [44]. The current results show that anthropogenic inputs are still present in these areas (SG, PM), revealing chronic contamination, while

SMM is a less contaminated area. Therefore, they should be duly taken into account in future monitoring plans.

Sediment characteristics (%fines, TOM, Eh) were also differentiated among the investigated sites. In detail, PM and SG showed the highest percentage of fines and content of TOM and the lowest Eh values. Sediment grain size and organic matter play a key role in metal–particle binding, where metal(loid)s preferentially interacts with the finest sediment surface [50] and have a great affinity for organic matter [51]. Indeed, sites characterised by high sediment and SPM percentage of fine particle and TOM content generally showed also high metal(loid) concentrations [8,52,53]. The accumulation of TOM in fine sediments and SPM of these sites was related to the urban effluents, agricultural runoff, and industrial activities, combined with the relatively long residence time of waters (especially at SG) that can reach up to 60 days [54], promoting the settlement of organically enriched particles.

4.2. Metal Levels in U. laetevirens

Our study explores whether metal accumulation in *U. laetevirens* reflects environmental contamination in VL. In general, macroalgae collected from the stressed sites (PM and SG) showed a significant metal (As, Mn, Cu, Pb, Zn) uptake, confirming the potential of *U. laetevirens* as a bioindicator for metal contamination. In this direction, an overall inter-site comparison (by MPI) revealed that the accumulation of metal(loid)s was in agreement with their environmental bioavailability (i.e., the concentration of metals in surface sediment and SPM). Metal concentrations in macroalgae from polluted sites were ca. 2–3 fold higher than samples from the less polluted SMM, in accordance with the results previously reported by Vasconcelos and Leal [55]. Overall, except for Pb, the concentrations of metal(loid)s found in *U. laetevirens* were in accordance with the results reported for macroalgae in VL by Caliceti et al. [56]. However, As was generally higher at PM. High As concentrations in the freshwater flowing into PM area, mainly in a bio-available form (soluble or adsorbed by particulate matter) were reported by Zonta et al. [29].

The metabolic increment promoted by higher water temperature [57] during spring and summer periods can be suggested as the main factor explaining the higher metal(loid) accumulation recorded at PM and SG. Current results revealed seasonal variations at SG, where the accumulation of Mn, Pb, and Zn occurred mainly in spring. Indeed, higher rates of photosynthesis were found to favour the uptake of metals by *U. laetevirens*, as occurred in summer periods [58]. Furthermore, metal(loid) accumulation can also be favoured by the highest water temperature that promotes both metabolism and protein synthesis, resulting in enhanced metal uptake [57]. Metal accumulation in biota is a complex process which depends on the metal bioavailability in the environment [59], as well as on metal toxicokinetic (regarded as a set of processes including uptake, distribution, sequestration, and elimination) in the macroalgal tissues (e.g., [60]). In fact, particular attention should be given to endogenous and exogenous factors such as nutrient availability. The highest accumulation of toxic metals (Pb and Hg) in *U. laetevirens* at PM and SG sites was consistent with higher C, N, and P concentrations in sediment and SPM. This is in agreement with the fact that higher metal bioaccumulation occurs with increasing nutrient availability from the water column, as previously found in the laboratory for *U. fasciata* by Lee and Wang [61].

The BSAF values correspond for each metal(loid)s calculated in both sediment and SPM to realise the processes of metal(loid) bioaccumulation in the VL sites. High BSAF values ($\geq$1) occurred only for As and Cu at PM and SMM. The highest BSAF value was observed for As at the PM site. These results were in agreement with previous studies, especially for BSAF values of As and Cu [62,63] on the capability of *Ulva* sp. to bioaccumulate these metal(loid)s. These metal(loid)s are present in a variety of association forms, with different metal–matrix bonds intensity, that exhibits a different bioavailability and a different potential for remobilisation [64]. indeed, contamination of macroalgae in TWS is related to the metal(loid) existence in the sediments and to the combining processes that influencing their bioavailability and bioaccumulation in the biota [45]. The anoxic conditions which frequently occurred during summer lead to significant changes in

environmental parameters in the inner part of VL [65]. Such changes could enhance the bioavailability of metal(loids)s at PM and SG. However, Pb and Hg were correlated with each other in the macroalgal tissues such as in sediments and SPM (Spearman's coefficients between sediment and SPM, Table S2). Despite this relationship, no bioaccumulation (BSAF > 1) was recorded either from sediments or SPM for Hg and Pb.

Referring to the limits established by the EU for certain contaminants in the foodstuff (EC, 1881/2006), it is noteworthy to observe that Pb at the SG site exceeded this limit during spring and summer. This increment can be explained by the pollution resulting from the Osellino River effluents and the engines gasoline of boats that are largely present in this area, as well as by the long residence time of waters, as previously mentioned.

4.3. Oxidative Stress and Metal Exposure

This study investigated whether metal accumulation in macroalgae and the oxidative stress response reflect the health status in the VL, particularly, the highest availability of toxic metals in areas affected by strong stressors. The significantly higher LPO levels in *U. laetevirens* collected from the contaminated sites (SG, PM), when compared with the control site (SMM), indicated an overproduction of ROS in *U. laetevirens* which reinforced the existence of oxidative damage. The current findings showed that the maximum levels of LPO were recorded at SG in accordance with the higher metal(loid)'s concentrations measured in the tissues of *U. laetevirens* (by MPI). This could suggest that the antioxidant defence of the macroalgae is not able to prevent the LPO probably induced by the metal(loid)'s exposure. Indeed, impairments of the cell defence system by chemically reactive species, which can act as antioxidant enzyme inhibitors, may reduce cell protection which becomes precarious [66]. The significant increase in LPO level was in accordance with a previous investigation [67] which showed significant increases in the LPO level in the tissues of *Ulva* spp. collected from sites characterised by a high level of anthropogenic disturbances. Moreover, significant correlations were detected between polluted sites (refineries in proximity) and the level of MDA measured in mussel *Mytilus galloprovincialis* on eastern Mediterranean coasts [68]. After one month of laboratory maintenance, MDA levels in a gill of *Mytilus edulis* were significantly reduced in sites at New Brighton, UK [69]. These results confirmed the use of LPO in the assessment of health status for aquatic environments.

Macroalgae collected during the warm period revealed significantly lower levels of LPO in comparison with the cold seasons for all sites in VL. This result was correlated with the metal accumulation in *U. laetevirens* during winter and spring. For instance, the metals Cu, Pb, and Zn, and the metalloid As were accumulated at higher concentrations during spring, in agreement with the highest level of LPO measured in *U. laetevirens*. In fact, low temperature during cold seasons reduced the metabolic rates and the enzymatic activities [70]; this could induce the ROS production and oxidation of polyunsaturated fatty acids of macroalgal cells and increase the LPO values during the colder seasons.

Strong statistical correlations were found between the toxic metal(loid)s (As, Pb and Hg) in *U. laetevirens* and the level of LPO. The induction of oxidative stress at PM and SG sites agreed with the spatial differences recorded for accumulation of As, Pb, and especially Hg in *U. laetevirens*. Under laboratory conditions, Lin et al. [71] found a significant increase in the LPO levels under high exposure of metals (Cu, Pb, and Cd) in the leaves of the seagrass *Zostera japonica*. Similarly, the pro-oxidant levels accompanied the enhanced accumulation of metal(loid)'s As and Pb in the digestive gland of the Manila clam *Ruditapes philippinarum* collected in a contaminated site in Tagus estuary [Portugal] [72]. The mussels *Mytilus galloprovincialis* exposed to seawater enriched with trace metals (Cu, Hg, and Cd) caused oxidative damage that was milder in the cases of Cu and Hg and more pronounced for Cd [73]. Significant different values of MDA were recorded after 15 days of exposure to Cd^{+2} [74].

Mercury was also reported to induce oxidative stress in microalgae [75]. It was strongly phytotoxic [76,77] and was reported as the most toxic metal for aquatic organisms [78].

The quantification and speciation of Hg in the VL was previously performed by Bloom and Lasorsa [79] and Bloom et al. [80] that reported peak values of CH_3Hg (the most toxic and bioaccumulation form of Hg) in the surface sediments in spring with higher Hg concentrations in front of PM area and in the northern part of the lagoon. The same authors highlighted as the contamination source was probably not bound to inflowing rivers or seawater but to past pollution by Hg cell chlor–alkali complex of PM and sewages from the historical centre of Venice. The changes in Hg speciation and bioavailability bound to bacterial methylation of Hg in spring could affect its uptake *by U. laetevirens* producing oxidative stress in conjunction with other metals. Peak concentrations in spring were recorded also for Pb in *U. rigida* C. Agardh from the shallow waters of Palude della Rosa, in the northern part of the lagoon, by Favero et al. [81], which reported higher metal scores in sites with lower salinity. Moreover, Sfriso et al. [82] recorded average Pb concentrations from macroalgae 6 times higher at SG than in a site (Alberoni) close to the Malamocco Sea inlet.

Undoubtedly, the effect of environmental parameters in the induction of macroalgal oxidative stress could not be excluded. However, *U. laetevirens* is a species present in a wide range of conditions [83], and it is probable that the effect of environmental parameters more than directly influencing the production of ROS alter the bioavailability of the local pollutants especially metal(loid)s that are prone to seasonal changes and uptake by macroalgae.

5. Conclusions

Despite the complexity of the processes affecting metal(loid) uptake and accumulation, the macroalgal (*U. laetevirens*) metals load consistently reflected their occurrence in sediment and SPM, especially in the sites close to the landward area of the VL. The accumulation of toxic metal(loid) in *U. laetevirens* (mainly As, Pb, and Hg) was strongly correlated with the induction of oxidative stress in macroalgal tissues. Seasonality affected both metal bioaccumulation and oxidative stress in *U. laetevirens*, with higher scores measured in spring and summer. The results of this work demonstrated the efficiency of the oxidative stress biomarker (LPO) as an early warning signal for health assessment in aquatic environments, with particular attention to seasonal changes in ecosystem assessment programs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13192626/s1, Table S1: Physicochemical parameters and nutrient concentrations for water, sediment, and SPM of VL sampling sites, Table S2: Spearman correlation matrix of metals in sediment and SPM, Table S3: Metal concentrations in *U. laetevirens* tissues within sampling seasons (µg/g dw) at LV, Table S4: Lipid peroxidation (LPO) seasonal measurement at LV sites, Table S5: Spearman correlation matrix of metals in *U. laetevirens* and oxidative stress damage (LPO).

Author Contributions: Conceptualisation, A.-S.J., A.S., and M.W.; methodology, A.-S.J. and A.A.S.; writing—original draft preparation, A.-S.J.; writing—review and editing, A.S. and M.W.; visualisation, A.B. and Y.T.; supervision, A.S. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Le Guen, C.; Tecchio, S.; Dauvin, J.-C.; De Roton, G.; Lobry, J.; Lepage, M.; Morin, J.; Lassalle, G.; Raoux, A.; Niquil, N. Assessing the ecological status of an estuarine ecosystem: Linking biodiversity and food-web indicators. *Estuar. Coast. Shelf Sci.* **2019**, *228*, 106339. [CrossRef]
2. Boerema, A.; Meire, P. Management for estuarine ecosystem services: A review. *Ecol. Eng.* **2017**, *98*, 172–182. [CrossRef]
3. Pérez-Ruzafa, A.; Marcos, C.; Pérez-Ruzafa, I.M.; Pérez-Marcos, M. Coastal lagoons: "Transitional ecosystems" between transitional and coastal waters. *J. Coast. Conserv.* **2011**, *15*, 369–392. [CrossRef]

4. Wahsha, M.; Juhmani, A.-S.; Buosi, A.; Sfriso, A.; Sfriso, A. Assess the environmental health status of macrophyte ecosystems using an oxidative stress biomarker. Case studies: The Gulf of Aqaba and the Lagoon of Venice. *Energy Procedia* **2017**, *125*, 19–26. [CrossRef]

5. Luoma, S.N.; Rainbow, P.S. Why Is Metal Bioaccumulation So Variable? Biodynamics as a Unifying Concept. *Environ. Sci. Technol.* **2005**, *39*, 1921–1931. [CrossRef] [PubMed]

6. Falace, A.; Curiel, D.; Sfriso, A. Study of the macrophyte assemblages and application of phytobenthic indices to assess the Ecological Status of the Marano-Grado Lagoon (Italy). *Mar. Ecol.* **2009**, *30*, 480–494. [CrossRef]

7. Sfriso, A.; Facca, C.; Ghetti, P.F. Rapid Quality Index (R-MaQI), based mainly on macrophyte associations, to assess the ecological status of Mediterranean transitional environments. *Chem. Ecol.* **2007**, *23*, 493–503. [CrossRef]

8. Pereira, P.; de Pablo, H.; Dulce Subida, M.; Vale, C.; Pacheco, M. Biochemical responses of the shore crab (Carcinus maenas) in a eutrophic and metal-contaminated coastal system (Óbidos lagoon, Portugal). *Ecotoxicol. Environ. Saf.* **2009**, *72*, 1471–1480. [CrossRef]

9. Villares, R.; Puente, X.; Carballeira, A. Ulva and Enteromorpha as indicators of heavy metal pollution. *Hydrobiologia* **2001**, *462*, 221–232. [CrossRef]

10. Pereira, P.; de Pablo, H.; Rosa-Santos, F.; Pacheco, M.; Vale, C. Metal accumulation and oxidative stress in Ulva sp. substantiated by response integration into a general stress index. *Aquat. Toxicol.* **2009**, *91*, 336–345. [CrossRef]

11. Sfriso, A.; Francesco Ghetti, P. Seasonal variation in biomass, morphometric parameters and production of seagrasses in the lagoon of Venice. *Aquat. Bot.* **1998**, *61*, 207–223. [CrossRef]

12. Elliott, M.; Quintino, V. The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Mar. Pollut. Bull.* **2007**, *54*, 640–645. [CrossRef]

13. Ahmad, I.; Mohmood, I.; Coelho, J.P.; Pacheco, M.; Santos, M.A.; Duarte, A.C.; Pereira, E. Role of non-enzymatic antioxidants on the bivalves' adaptation to environmental mercury: Organ-specificities and age effect in Scrobicularia plana inhabiting a contaminated lagoon. *Environ. Pollut.* **2012**, *163*, 218–225. [CrossRef] [PubMed]

14. Alves de Almeida, E.; Celso Dias Bainy, A.; Paula de Melo Loureiro, A.; Regina Martinez, G.; Miyamoto, S.; Onuki, J.; Fujita Barbosa, L.; Carrião Machado Garcia, C.; Manso Prado, F.; Eliza Ronsein, G.; et al. Oxidative stress in Perna perna and other bivalves as indicators of environmental stress in the Brazilian marine environment: Antioxidants, lipid peroxidation and DNA damage. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2007**, *146*, 588–600. [CrossRef]

15. Freitas, R.; Costa, E.; Velez, C.; Santos, J.; Lima, A.; Oliveira, C.; Maria Rodrigues, A.; Quintino, V.; Figueira, E. Looking for suitable biomarkers in benthic macroinvertebrates inhabiting coastal areas with low metal contamination: Comparison between the bivalve Cerastoderma edule and the Polychaete Diopatra neapolitana. *Ecotoxicol. Environ. Saf.* **2012**, *75*, 109–118. [CrossRef]

16. Valko, M.; Rhodes, C.J.; Moncol, J.; Izakovic, M.; Mazur, M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* **2006**, *160*, 1–40. [CrossRef]

17. Wahsha, M.; Bini, C.; Fontana, S.; Wahsha, A.; Zilioli, D. Toxicity assessment of contaminated soils from a mining area in Northeast Italy by using lipid peroxidation assay. *J. Geochem. Explor.* **2012**, *113*, 112–117. [CrossRef]

18. Malea, P.; Rijstenbil, J.W.; Haritonidis, S. Effects of cadmium, zinc and nitrogen status on non-protein thiols in the macroalgae Enteromorpha spp. from the Scheldt Estuary (SW Netherlands, Belgium) and Thermaikos Gulf (N Aegean Sea, Greece). *Mar. Environ. Res.* **2006**, *62*, 45–60. [CrossRef]

19. Nimptsch, J.; Pflugmacher, S. Ammonia triggers the promotion of oxidative stress in the aquatic macrophyte Myriophyllum mattogrossense. *Chemosphere* **2007**, *66*, 708–714. [CrossRef] [PubMed]

20. Ratkevicius, N.; Correa, J.A.; Moenne, A. Copper accumulation, synthesis of ascorbate and activation of ascorbate peroxidase in Enteromorpha compressa (L.) Grev. (Chlorophyta) from heavy metal-enriched environments in northern Chile. *Plant Cell Environ.* **2003**, *26*, 1599–1608. [CrossRef]

21. Ravera, O. The Lagoon of Venice: The result of both natural factors and human influence. *J. Limnol.* **2000**, *59*, 19–30. [CrossRef]

22. Sfriso, A.; Facca, C.; Ceoldo, S.; Silvestri, S.; Ghetti, P.F. Role of macroalgal biomass and clam fishing on spatial and temporal changes in N and P sedimentary pools in the central part of the Venice lagoon. *Oceanol. Acta* **2003**, *26*, 3–13. [CrossRef]

23. Sfriso, A.; Facca, C.; Raccanelli, S. PCDD/F and dioxin-like PCB bioaccumulation by Manila clam from polluted areas of Venice lagoon (Italy). *Environ. Pollut.* **2014**, *184*, 290–297. [CrossRef] [PubMed]

24. Sfriso, A.; Argese, E.; Bettiol, C.; Facca, C. *Tapes philippinarum* seed exposure to metals in polluted areas of the Venice lagoon. *Estuar. Coast. Shelf Sci.* **2008**, *79*, 581–590. [CrossRef]

25. Adriano, S.; Facca, C.; Marcomini, A. Sedimentation rates and erosion processes in the lagoon of Venice. *Environ. Int.* **2005**, *31*, 983–992. [CrossRef]

26. Bellucci, L.G.; Frignani, M.; Paolucci, D.; Ravanelli, M. Distribution of heavy metals in sediments of the Venice Lagoon: The role of the industrial area. *Sci. Total Environ.* **2002**, *295*, 35–49. [CrossRef]

27. Secco, T.; Pellizzato, F.; Sfriso, A.; Pavoni, B. The changing state of contamination in the Lagoon of Venice. Part 1: Organic pollutants. *Chemosphere* **2005**, *58*, 279–290. [CrossRef]

28. Rigollet, V.; Sfriso, A.; Marcomini, A.; De Casabianca, M.L. Seasonal evolution of heavy metal concentrations in the surface sediments of two Mediterranean *Zostera marina* L. beds at Thau lagoon (France) and Venice lagoon (Italy). *Bioresour. Technol.* **2004**, *95*, 159–167. [CrossRef]

29. Zonta, R.; Botter, M.; Cassin, D.; Pini, R.; Scattolin, M.; Zaggia, L. Sediment chemical contamination of a shallow water area close to the industrial zone of Porto Marghera (Venice Lagoon, Italy). *Mar. Pollut. Bull.* **2007**, *55*, 529–542. [CrossRef]

30. Raccanelli, S.; Favotto, M.; Vio, P. Estimation of PCDD/F and PCB detoxification rates in contaminated Tapes philippinarum in the Lagoon of Venice. *Chemosphere* **2008**, *73*, S166–S170. [CrossRef]

31. Masiol, M.; Facca, C.; Visin, F.; Sfriso, A.; Pavoni, B. Interannual heavy element and nutrient concentration trends in the top sediments of Venice Lagoon (Italy). *Mar. Pollut. Bull.* **2014**, *89*, 49–58. [CrossRef]

32. Guerzoni, S.; Tagliapietra, D. *Atlante della Laguna*; Venezia: Venice, Italy, 2006.

33. Guerzoni, S.; Rossini, P.; Molinaroli, E.; Rampazzo, G.; Raccanelli, S. Measurement of atmospheric deposition of polychlorinated dibenzo-p-dioxins and dibenzofurans in the Lagoon of Venice, Italy. *Chemosphere* **2004**, *54*, 1309–1317. [CrossRef]

34. Sfriso, A.; CFacca, H.; Ceoldo, S.; Marcomini, A. Recording the occurrence of trophic level changes in the lagoon of Venice over the '90s. *Environ. Int.* **2005**, *31*, 993–1001. [CrossRef]

35. Oxner, M.; Knudsen, M. *The Determination of Chlorinity by the Knudsen Method*; GM Manufacturing Company: New York, NY, USA, 1962; 63p.

36. Byers, S.C.; Mills, E.L.; Stewart, P.L. A comparison of methods of determining organic carbon in marine sediments, with suggestions for a standard method. *Hydrobiologia* **1978**, *58*, 43–47. [CrossRef]

37. Aspila, K.I.; Agemian, H.; Chau, A.S.Y. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *Analyst* **1976**, *101*, 187–197. [CrossRef]

38. Sfriso, A.; Buosi, A.; Tomio, Y.; Juhmani, A.-S.; Chiesa, S.; Greco, M.; Gazzola, C.; Mistri, M.; Munari, C.; Sfriso, A.A. Sediment carbon variations in the venice lagoon and other transitional water systems of the northern adriatic sea. *Water* **2020**, *12*, 3430. (In Switzerland) [CrossRef]

39. Figueira, E.; Lima, A.; Branco, D.; Quintino, V.; Rodrigues, A.M.; Freitas, R. Health concerns of consuming cockles (*Cerastoderma edule* L.) from a low contaminated coastal system. *Environ. Int.* **2011**, *37*, 965–972. [CrossRef]

40. Usero, J.; González-Regalado, E.; Gracia, I. Trace metals in the bivalve molluscs *Ruditapes decussatus* and *Ruditapes philippinarum* from the atlantic coast of Southern Spain. *Environ. Int.* **1997**, *23*, 291–298. [CrossRef]

41. Velez, C.; Figueira, E.; Soares, A.; Freitas, R. Spatial distribution and bioaccumulation patterns in three clam populations from a low contaminated ecosystem. *Estuar. Coast. Shelf Sci.* **2015**, *155*, 114–125. [CrossRef]

42. Ministero per l'Ambiente. *Criteri di Sicurezza Ambientale per lo Scavo, Trasporto e Riutilizzo di Sedimenti dei Canali di Venezia*; Ministero per l'Ambiente: Rome, Italy, 1993; 23p.

43. Picone, M.; Bergamin, M.; Losso, C.; Delaney, E.; Arizzi Novelli, A.; Ghirardini, A.V. Assessment of sediment toxicity in the Lagoon of Venice (Italy) using a multi-species set of bioassays. *Ecotoxicol. Environ. Saf.* **2016**, *123*, 32–44. [CrossRef] [PubMed]

44. Moschino, V.; Delaney, E.; Meneghetti, F.; Ros, L. Da Biomonitoring approach with mussel *Mytilus galloprovincialis* (Lmk) and clam *Ruditapes philippinarum* (Adams and Reeve, 1850) in the Lagoon of Venice. *Environ. Monit. Assess.* **2011**, *177*, 649–663. [CrossRef]

45. Breda, S.; Chiesa, S.; Freitas, R.; Figueira, E.; Becherini, F.; Gobbo, L.; Soares, A.M.V.M.; Argese, E. Biogeochemical dynamics and bioaccumulation processes in Manila clam: Implications for biodiversity and ecosystem services in the Ria de Aveiro Lagoon. *Estuar. Coast. Shelf Sci.* **2018**, *209*, 136–148. [CrossRef]

46. Sfriso, A.A.; Chiesa, S.; Sfriso, A.; Buosi, A.; Gobbo, L.; Boscolo Gnolo, A.; Argese, E. Spatial distribution, bioaccumulation profiles and risk for consumption of edible bivalves: A comparison among razor clam, Manila clam and cockles in the Venice Lagoon. *Sci. Total Environ.* **2018**, *643*, 579–591. [CrossRef]

47. Carvalho, S.; Moura, A.; Gaspar, M.B.; Pereira, P.; Cancela da Fonseca, L.; Falcão, M.; Drago, T.; Leitão, F.; Regala, J. Spatial and inter-annual variability of the macrobenthic communities within a coastal lagoon (Óbidos lagoon) and its relationship with environmental parameters. *Acta Oecologica* **2005**, *27*, 143–159. [CrossRef]

48. Carvalho, S.; Barata, M.; Pereira, F.; Gaspar, M.B.; Cancela da Fonseca, L.; Pousão-Ferreira, P. Distribution patterns of macrobenthic species in relation to organic enrichment within aquaculture earthen ponds. *Mar. Pollut. Bull.* **2006**, *52*, 1573–1584. [CrossRef]

49. Cecchi, R.; Ghermandi, G.; Zonta, R. Geochemical processes in Venice Lagoon by PIXE technique: An overview. *Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. Atoms* **1996**, *109–110*, 407–414. [CrossRef]

50. Hornberger, M.I.; Luoma, S.N.; van Geen, A.; Fuller, C.; Anima, R. Historical trends of metals in the sediments of San Francisco Bay, California. *Mar. Chem.* **1999**, *64*, 39–55. [CrossRef]

51. Zonta, R.; Zaggia, L.; Argese, E. Heavy metal and grain-size distributions in estuarine shallow water sediments of the Cona Marsh (Venice Lagoon, Italy). *Sci. Total Environ.* **1994**, *151*, 19–28. [CrossRef]

52. Casado-Martinez, M.C.; Smith, B.D.; DelValls, T.A.; Luoma, S.N.; Rainbow, P.S. Biodynamic modelling and the prediction of accumulated trace metal concentrations in the polychaete Arenicola marina. *Environ. Pollut.* **2009**, *157*, 2743–2750. [CrossRef]

53. Rainbow, P.S.; Luoma, S.N. Metal toxicity, uptake and bioaccumulation in aquatic invertebrates—Modelling zinc in crustaceans. *Aquat. Toxicol.* **2011**, *105*, 455–465. [CrossRef]

54. Molinaroli, E.; Guerzoni, S.; De Falco, G.; Sarretta, A.; Cucco, A.; Como, S.; Simeone, S.; Perilli, A.; Magni, P. Relationships between hydrodynamic parameters and grain size in two contrasting transitional environments: The Lagoons of Venice and Cabras, Italy. *Sediment. Geol.* **2009**, *219*, 196–207. [CrossRef]

55. Vasconcelos, M.T.S.D.; Leal, M.F.C. Antagonistic interactions of Pb and Cd on Cu uptake, growth inhibition and chelator release in the marine algae Emiliania huxleyi. *Mar. Chem.* **2001**, *75*, 123–139. [CrossRef]

56. Caliceti, M.; Argese, E.; Sfriso, A.; Pavoni, B. Heavy metal contamination in the seaweeds of the Venice lagoon. *Chemosphere* **2002**, *47*, 443–454. [CrossRef]

57. Fritioff, Å.; Kautsky, L.; Greger, M. Influence of temperature and salinity on heavy metal uptake by submersed plants. *Environ. Pollut.* **2005**, *133*, 265–274. [CrossRef] [PubMed]

58. Malea, P.; Haritonidis, S. Use of the green alga *Ulva rigida* C. Agardh as an indicator species to reassess metal pollution in the Thermaikos Gulf, Greece, after 13 years. *J. Appl. Phycol.* **2000**, *12*, 169–176. [CrossRef]

59. Villares, R.; Puente, X.; Carballeira, A. Seasonal variation and background levels of heavy metals in two green seaweeds. *Environ. Pollut.* **2002**, *119*, 79–90. [CrossRef]

60. Jarvis, T.A.; Bielmyer-Fraser, G.K. Accumulation and effects of metal mixtures in two seaweed species. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2015**, *171*, 28–33. [CrossRef]

61. Lee, W.Y.; Wang, W.X. Metal accumulation in the green macroalga *Ulva fasciata*: Effects of nitrate, ammonium and phosphate. *Sci. Total Environ.* **2001**, *278*, 11–22. [CrossRef]

62. Bibak, M.; Tahmasebi, S.; Sattari, M.; Kafaei, R.; Ramavandi, B. Empirical cumulative entropy as a new trace elements indicator to determine the relationship between algae-sediment pollution in the Persian Gulf, southern Iran. *Environ. Sci. Pollut. Res. Int.* **2021**, *28*, 4634–4644. [CrossRef]

63. Alahverdi, M.; Savabieasfahani, M. Metal pollution in seaweed and related sediment of the Persian Gulf, Iran. *Bull. Environ. Contam. Toxicol.* **2012**, *88*, 939–945. [CrossRef]

64. Argese, E.; Bettiol, C. Heavy metal partitioning in sediments from the lagoon of Venice (Italy). *Toxicol. Environ. Chem.* **2001**, *79*, 157–170. [CrossRef]

65. Sfriso, A.; Facca, C. Annual growth and environmental relationships of the invasive species *Sargassum muticum* and *Undaria pinnatifida* in the lagoon of Venice. *Estuar. Coast. Shelf Sci.* **2013**, *129*, 162–172. [CrossRef]

66. Escobar, J.A.; Rubio, M.A.; Lissi, E.A. SOD and catalase inactivation by singlet oxygen and peroxyl radicals. *Free Radic. Biol. Med.* **1996**, *20*, 285–290. [CrossRef]

67. Pereira, P.; Raimundo, J.; Araújo, O.; Canário, J.; Almeida, A.; Pacheco, M. Fish eyes and brain as primary targets for mercury accumulation—A new insight on environmental risk assessment. *Sci. Total Environ.* **2014**, *494–495*, 290–298. [CrossRef] [PubMed]

68. Kasiotis, K.M.; Emmanouil, C.; Anastasiadou, P.; Papadi-Psyllou, A.; Papadopoulos, A.; Okay, O.; Machera, K. Organic pollution and its effects in the marine mussel *Mytilus galloprovincialis* in Eastern Mediterranean coasts. *Chemosphere* **2015**, *119*, S145–S152. [CrossRef]

69. Emmanouil, C.; Green, R.M.; Willey, F.R.; Chipman, J.K. Oxidative damage in gill of *Mytilus edulis* from Merseyside, UK, and reversibility after depuration. *Environ. Pollut.* **2008**, *151*, 663–668. [CrossRef]

70. Vidal, M.-L.; Bassères, A.; Narbonne, J.-F. Seasonal variations of pollution biomarkers in two populations of *Corbicula fluminea* (Müller). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2002**, *131*, 133–151. [CrossRef]

71. Lin, H.; Sun, T.; Zhou, Y.; Zhang, X. Anti-oxidative feedback and biomarkers in the intertidal seagrass *Zostera japonica* induced by exposure to copper, lead and cadmium. *Mar. Pollut. Bull.* **2016**, *109*, 325–333. [CrossRef]

72. Marques, A.; Piló, D.; Carvalho, S.; Araújo, O.; Guilherme, S.; Santos, M.A.; Vale, C.; Pereira, F.; Pacheco, M.; Pereira, P. Metal bioaccumulation and oxidative stress profiles in *Ruditapes philippinarum*—insights towards its suitability as bioindicator of estuarine metal contamination. *Ecol. Indic.* **2018**, *95*, 1087–1099. [CrossRef]

73. Kournoutou, G.G.; Giannopoulou, P.C.; Sazakli, E.; Leotsinidis, M.; Kalpaxis, D.L.; Dinos, G.P. Oxidative Damage of Mussels Living in Seawater Enriched with Trace Metals, from the Viewpoint of Proteins Expression and Modification. *Toxics* **2020**, *8*, 89. [CrossRef]

74. Kournoutou, G.G.; Giannopoulou, P.C.; Sazakli, E.; Leotsinidis, M.; Kalpaxis, D.L. Oxidative damage of 18S and 5S ribosomal RNA in digestive gland of mussels exposed to trace metals. *Aquat. Toxicol.* **2017**, *192*, 136–147. [CrossRef]

75. Elbaz, A.; Wei, Y.Y.; Meng, Q.; Zheng, Q.; Yang, Z.M. Mercury-induced oxidative stress and impact on antioxidant enzymes in *Chlamydomonas reinhardtii*. *Ecotoxicology* **2010**, *19*, 1285–1293. [CrossRef]

76. Zhou, Z.S.; Wang, S.J.; Yang, Z.M. Biological detection and analysis of mercury toxicity to alfalfa (Medicago sativa) plants. *Chemosphere* **2008**, *70*, 1500–1509. [CrossRef]

77. Chen, J.; Shiyab, S.; Han, F.X.; Monts, D.L.; Waggoner, C.A.; Yang, Z.; Su, Y. Bioaccumulation and physiological effects of mercury in Pteris vittata and Nephrolepis exaltata. *Ecotoxicology* **2008**, *18*, 110. [CrossRef]

78. Ratte, H.T. Bioaccumulation and toxicity of silver compounds: A review. *Environ. Toxicol. Chem.* **1999**, *18*, 89–108. [CrossRef]

79. Bloom, N.S.; Lasorsa, B.K. Changes in mercury speciation and the release of methyl mercury as a result of marine sediment dredging activities. *Sci. Total Environ.* **1999**, *237–238*, 379–385. [CrossRef]

80. Bloom, N.S.; Moretto, L.M.; Ugo, P. A comparison of the speciation and fate of mercury in two contaminated coastal marine ecosystems: The Venice Lagoon (Italy) and Lavaca Bay (Texas). *Limnol. Oceanogr.* **2004**, *49*, 367–375. [CrossRef]

81. Favero, N.; Cattalini, F.; Bertaggia, D.; Albergoni, V. Metal accumulation in a biological indicator (*Ulva rigida*) from the lagoon of Venice (Italy). *Arch. Environ. Contam. Toxicol.* **1996**, *31*, 9–18. [CrossRef]

82. Sfriso, A.; Marcomini, A.; Pavoni, B. Annual nutrient exchanges between the central lagoon of Venice and the northern Adriatic Sea. *Sci. Total Environ.* **1994**, *156*, 77–92. [CrossRef]

83. Sfriso, A. Coexistence of *Ulva rigida* and *Ulva laetevirens* (Ulvales, Chlorophyta) in Venice Lagoon and other Italian transitional and marine environments. *Bot. Mar.* **2010**, *53*, 9–18. [CrossRef]