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Seagrasses as indicators for coastal trace metal pollution: A global meta-analysis serving as a benchmark, and a Caribbean case study



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ABSTRACT

Seagrass beds are highly productive coastal ecosystems providing a large array of ecosystem services including fisheries and carbon sequestration. As seagrasses are known to be highly sensitive to anthropogenic forcing, we evaluated the use of trace metal concentrations in seagrasses as bioindicators for trace metal pollution of coastal regions at both global and local scale. We carried out a meta-analysis based on literature data to provide a global benchmark list for trace metal accumulation in seagrasses, which was lacking in literature. We subsequently carried out a case study at the Caribbean islands of Curaçao and Bonaire to test for local-scale differences in trace metal concentrations in seagrasses, and internal metal allocation. The benchmark and local study show that trace metal concentrations in seagrass leaves, regardless of the species, can vary over a 100–1000-fold range, and are related to the level of anthropogenic pressure, making seagrasses highly valuable indicators.

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1. Introduction

Billions of people live in coastal areas all over the world and it is expected that, in a couple of decades, even more than 50% of the expanding human population will be living within 150 km from the shore (Cohen, 2003; Cohen et al., 1997; Small and Nicholls, 2003). This leads to a steep increase in anthropogenic activities in coastal areas such as dredging, aquaculture, industrial activities and pollution, sewage discharge, and deforestation (Cohen, 2003; Mora, 2008). These activities severely threaten coastal ecosystems including coral reefs (Mumby et al., 2006, 2007), mangroves (Valiela et al., 2001) and seagrass beds (Waycott et al., 2009), which

not only provide a large suite of ecosystem services (Costanza et al., 1997), but are also strongly interconnected by fluxes of nutrients and dissolved organic matter, and by animal migration (Cowen et al., 2006; Nagelkerken, 2000).

To get insight into the extent and the spatial variation of anthropogenic pressure on coastal ecosystems and to locate sources of pollution, there is a strong need for good indicators. Bioindicators, including a variety of organisms such as clams, plants, copepods and microorganisms, can be used to identify anthropogenic disturbances and preferentially provide early warning signals for pollution or degradation (Linton and Warner, 2003). As they accumulate pollutants, these organisms also reflect low intensity, but chronic impacts, in contrast to physical or chemical parameters which often only present a snapshot of environmental conditions (Linton and Warner, 2003). Additionally, bioindicators can provide information on multiple spatial scales, as most ecosystems are heterogeneous, and are able to differentiate between natural variation and anthropogenic disturbance (Markert et al., 1999; Martínez-Crego et al., 2008).

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Seagrasses are known to be good bioindicators (Lee et al., 2004; Orth et al., 2006) as they are widespread and sensitive to environmental changes (Bhattacharya et al., 2003; Ferrat et al., 2003; Udy and Dennison, 1997; Walker and McComb, 1992), and are able to integrate ecological conditions and processes over various timescales from weeks to years (Gonzalez-Correa et al., 2005; Madden et al., 2009; Meehan and West, 2000). Seagrass bioindicators have predominantly been used in the Mediterranean and in Florida, where several complex indices have been developed based on seagrass characteristics ranging from the individual physiological level to the community level (Bennett et al., 2011; Lopez y Royo et al., 2011; Montefalcone, 2009; Moreno et al., 2001; Romero et al., 2007). However, most of the proposed indicator values are specific for areas and species, and can therefore not be used at a global scale.

Seagrasses have been shown to be indicative of trace metal pollution (Lafabrie et al., 2007, 2008; Sundelin and Eriksson, 2001), and as this type of pollution is becoming a major threat to coastal ecosystems in rapidly developing countries (Li et al., 2007), there is a strong need for reliable trace metal bioindicators. To use seagrasses as such, they need to be highly responsive in order to be able to detect differences at both local and global scale. As they are primary producers providing stock food to a large variety of coastal herbivores, seagrasses can also be expected to be indicative of trace metal concentrations at higher trophic levels. Their concentrations may therefore also be used to detect possible threats to ecosystem services such as fisheries. However, as of today, literature does not provide a complete reference overview of trace metal levels in seagrasses on a global scale. We therefore conducted a meta-analysis to compile this benchmark of global trace metal concentrations in seagrass leaves.

In addition, we studied potential trace metal pollution in seagrasses on a local scale to test whether it was possible to detect local-scale differences in trace metal concentrations in seagrasses. We focused on two Caribbean islands: Curaçao and Bonaire. The variation of anthropogenic pressure at an island scale makes these islands very suitable for this study. Moreover, the Caribbean represents a typical tropical area in which coastal ecosystems,

including seagrass beds, are suffering from anthropogenic impacts such as coastal development, tourism and growing industries and harbours (Phillips, 1992), oil drilling and accompanying spills, trace metal pollution (Thorhaug et al., 1985; Vera, 1992), and eutrophication (Burkholder et al., 2007; Short and Wyllie-Echeverria, 1996). However, the effects of pollution on seagrass meadows have been poorly studied in this area.

2. Material and methods

2.1. Global meta-analysis of trace metal levels in seagrass

We compiled a benchmark database (Table S1) for trace metal levels (Co, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Zn) in seagrass leaves ($\mu\text{g g}^{-1}$) using Web of Science (ISI; search: seagrass AND metals), data from grey literature, and additional unpublished data of the authors on *Zostera noltii* (Mauritania and The Netherlands), *Amphibolis antarctica* (Australia) and *Halodule uninervis* (Indonesia). Belowground and whole plant data were excluded. Data were derived from either tables or figures in the selected papers, and each unique location as stated in the paper was used. The meta-analysis included data from 47 different studies on seagrass beds all over the world (Fig. 1, Table S1). Replicate data points, including replicates from different seasons, were averaged per location, and data points were divided into polluted and unpolluted sites based on the description of the sites in the studies. Data included in our benchmark database spanned a 40-year period, from the 1970s up to 2011.

2.2. Local study

Samples were collected in January 2010 on the islands of Curaçao ($12^{\circ} 04' \text{ N}$, $68^{\circ} 51' \text{ W}$) and Bonaire ($12^{\circ} 15' \text{ N}$, $68^{\circ} 28' \text{ W}$) (Fig. 2). We sampled six different inland bays, which included 50% of all the islands' bays with seagrass and 90% of the total seagrass area, varying in their levels of anthropogenic disturbance (Table 1). The bays are dominated by mangrove (*Rhizophora mangle*) communities along the shores, and by subtidal seagrass beds of turtle grass (*Thalassia testudinum*) and manatee grass (*Syringodium filiforme*). On the island of Curaçao, we sampled in Piscadera Bay, Spanish Water Bay, Boka Ascension, Sint Anna Bay and Sint Joris Bay, and on Bonaire, Lac Bay (Fig. 2). Bays varied in size and morphology (Table 1), but they all experienced minimal wave stress and had a very limited tidal range of 30 cm (De Haan and Zaneveld, 1959). Sediment grain size of the bays ranged from coarse carbonate sediments on exposed sites (mean $D_{50} = 600 \mu\text{m}$) to finer grained, sandy sediments in the more sheltered areas (mean $D_{50} = 240 \mu\text{m}$) (Kuenen and Debrot, 1995). Boka Ascension was divided into inner and outer bay, as a large 10 cm deep shoal strongly limited water exchange between both parts.

2.3. Seagrass and porewater sampling

Although we sampled all seagrass species present in the bays, we mainly focused on *Thalassia testudinum* as this species and the very closely related *Thalassia*

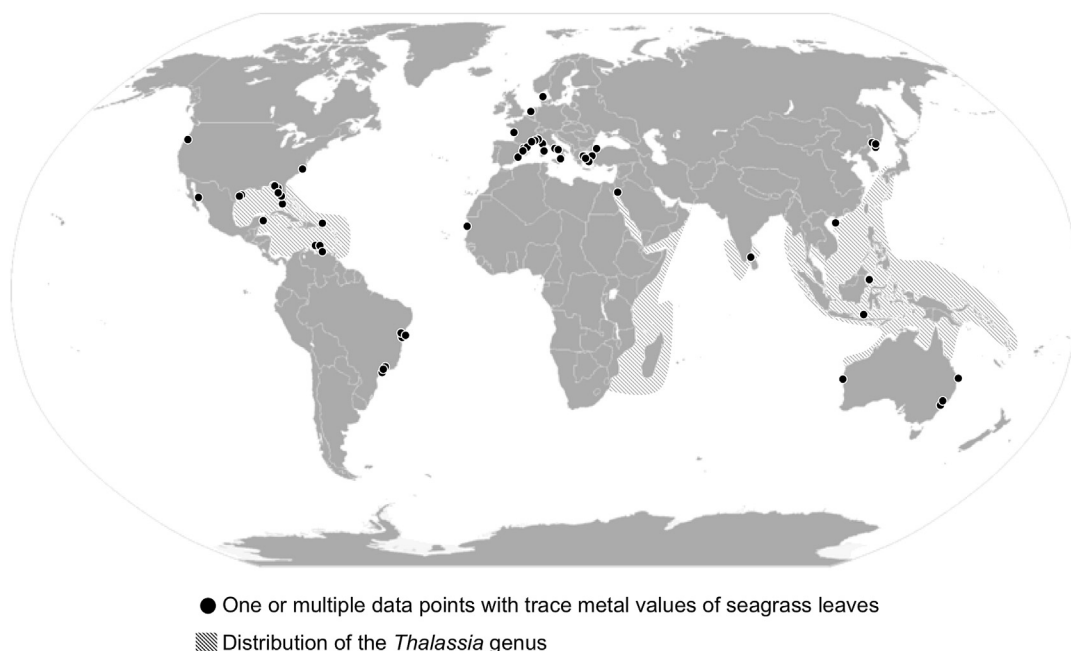


Fig. 1. Overview of all seagrass locations included in the meta-analysis (Table S1) on trace metals in seagrass leaves, and the distribution of the tropical seagrass genus *Thalassia* (adapted from Green and Short (2003)).

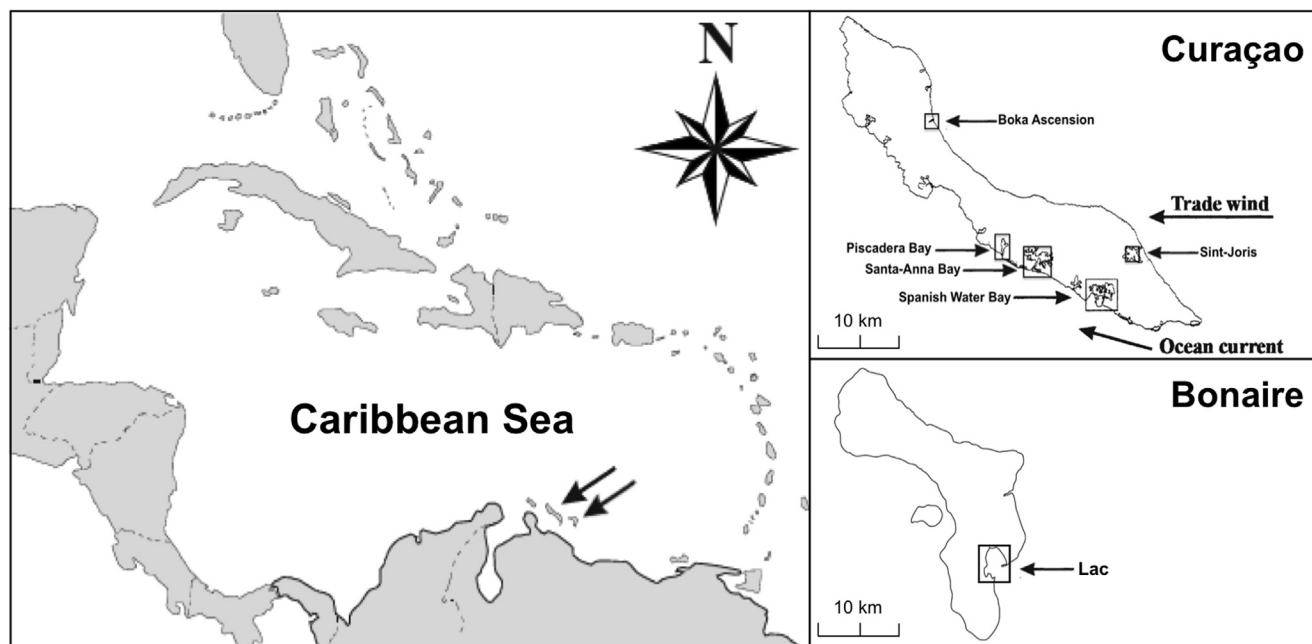


Fig. 2. Map showing the locations of Curaçao and Bonaire in the Caribbean Sea, and maps of both islands with the locations of the sampled bays.

hemprichii are widely distributed among the tropics (Fig. 1) (Green and Short, 2003; van Tussenbroek et al., 2006). Moreover, species of the genus *Thalassia* grow in shallow waters directly adjacent to coastal areas, are easy to recognize and are late successional species, which are able to accumulate trace metals (Fourqurean and Zieman, 2002).

Sampling sites were 20 m from the shore at most, and could be reached directly or by boat. Piscadera Bay is the most anthropogenically-impacted bay where seagrass is still present, although it only grows in the shallowest parts of the murky waters. In contrast, Lac Bay represents the most pristine seagrass system, as it is a well-protected nature reserve. For each bay, samples were collected in gradients from the pollution source to the bay inlet (Table 1). At sites including seagrass, both porewater and seagrass samples were taken in the seagrass bed; at sites lacking seagrass, porewater samples were taken from the bare sediment.

On each sampling site, two porewater samples were collected within 1 m to include the natural biogeochemical heterogeneity of the sediment, and pooled. Samples were collected anaerobically, using 60 mL vacuumed syringes connected to ceramic soil moisture samplers (pore size 0.15 μm ; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) placed in the top 7 cm of the soil (Govers et al., 2014). Surface water samples were collected similarly in the upper 5 cm of the water column to filter samples prior to laboratory analyses. After measuring their salinity and pH values, samples were frozen on the day of sampling and stored until further analytical analysis. On each sampling site, a pooled sample of at least 10 seagrass shoots with their belowground biomass was manually collected for each species at water depths between 0.5 and 2.5 m, while snorkeling.

2.4. Sample analysis

Seagrass samples were split up into roots, rhizomes, sheaths and leaves, and all epiphytes were carefully removed using a scalpel. Subsequently, the samples

were dried at 60 °C for 48 h, weighed (g dry wt) and ground. Prior to elemental analyses, samples were digested in 5 mL pressure tubes, using 10 mg sample, 200 μL H_2O_2 and 700 μL HNO_3 in an autoclave for 30 min at 121 °C (ML autoclave, Tuttnauer, the Netherlands) and diluted with 9.1 water to 10 mL. Parallel analyses confirmed that the results of this method did not differ from those of another commonly used digestion method (Smolders et al., 2006) using 50 mg sample, 1 mL H_2O_2 and 2 mL HNO_3 in a digestion microwave (Ethos D, Milestone, Italy) diluted to 25 mL. Total concentrations of trace metals and other elements (Al, As, Ca, Cd, Co, Cr, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si, Sr, Zn) in seagrass tissue (leaves and rhizomes) were measured by inductively coupled plasma emission spectrometry (IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA, USA). Standard references (IPE-858, IPE-137; WEPAL, the Netherlands) were included in the analysis, and the average deviation amounted to 3.1%. Trace elements and trace metals in filtered, 3 times diluted porewater and surface water samples were measured using inductively coupled plasma emission spectrometry as described above.

2.5. Statistical analysis

Displayed values are means \pm standard error (SE). The number of replicates for each bay is displayed in Table 1. To compare the conditions among bays, we used a one-way ANOVA, with a homogeneity test prior to the analysis. If equal variances could not be assumed, data were log-transformed. We used a Tukey post-hoc test when the assumptions for the ANOVA were met, and a Games-Howell post-hoc test otherwise. Significant differences ($P < 0.05$) are indicated by different letters. When comparing two different means (distance to residential areas in Spanish Water Bay), we used an independent *t*-test (see appendix). Correlations were tested using Pearson's (parametric), or Spearman's (non-parametric) correlation coefficient. All statistical tests were performed in IBM SPSS Statistics 19.0 and R 2.13.

Table 1
General characteristics of the bays sampled. Bays varied in size and exposure to the open sea (related to the width of the bay mouth). The observed potential stressors are listed for each bay. The total number of sampling points refers to the number of replicates in each bay. Abbreviations of seagrass species: Tt = *Thalassia testudinum*, Sf = *Syringodium filiforme*, Hw = *Halodule wrightii* and Rm = *Ruppia maritima*. Only tissue of *Thalassia testudinum* was used for the trace metal analyses.

Bay	Island	Surface (km ²)	Width bay mouth (m)	Seagrass species	Local disturbance	Total # sampling points	# sampling points seagrass
Lac Bay	Bonaire	7.5	1600	Tt, Sf, Hw, Rm	Pristine, light recreation	11	11
Piscadera Bay	Curaçao	0.75	90	Tt, Sf	Sewage discharge, boating	9	6
Spanish Water Bay	Curaçao	3	90	Tt	Domestic sewage, boating	14	14
Boka Ascension	Curaçao	0.05	200				
Outer Bay				Tt, Sf, Hw	Pristine, turtle grazing	3	3
Inner Bay				None	Heavily polluted, waste dumping	3	0
Sint Anna Bay	Curaçao	4	230	None	Heavy industry; oil refinery	8	0
Sint Joris Bay	Curaçao	2.5	240	Tt	Pristine, some waste dumping	6	5

3. Results

3.1. Global benchmark study

To get insight into the trace metal concentration ranges at a global scale, as well as to put our own results into perspective, we plotted our data together with the median values of leaf metal values found in literature (Fig. 3) for locations all over the world (Fig. 1). Globally, trace metal concentrations in seagrass leaves ranged from $<0.03 \mu\text{g g}^{-1}$ dry wt for Cd to $>4000 \mu\text{g g}^{-1}$ dry wt for Fe (Fig. 3). For cadmium (Cd), cobalt (Co), copper (Cu) and lead (Pb), most of the levels measured in seagrass leaves of Curaçao and Bonaire were well below median benchmark values. For iron (Fe), nickel (Ni) and zinc (Zn), in contrast, values were mostly above median values of our global benchmark database, and chromium (Cr) values were well above median values (Fig. 3).

In our meta-analysis, we found significantly higher mean trace metal concentrations in seagrass leaves of polluted sites than in unpolluted sites for Cu, Hg, Ni, Pb, and Zn (Welch's *t*-test, $P < 0.05$ Table S2). Leaf trace metal concentrations of polluted sites were on average 2 times higher (81 vs. $45 \mu\text{g g}^{-1}$ dry wt for Zn, 15 vs. $8 \mu\text{g g}^{-1}$ dry wt for Ni, 15 vs. $9 \mu\text{g g}^{-1}$ dry wt for Cu), 3 times higher (15 vs. $5 \mu\text{g g}^{-1}$ dry wt for Pb) or 4 times higher (0.13 vs. $0.03 \mu\text{g g}^{-1}$ dry wt for Hg) on polluted sites compared to unpolluted sites. This implies that seagrass leaves can indeed be used as first-level bio-indicators for trace metal pollution.

3.2. Internal distribution of trace metals

To investigate the internal allocation of metals to the different plant parts, we measured trace metal concentrations in both leaves and rhizomes of *Thalassia testudinum*. All essential metals (Cu, Fe, Ni, Zn, Cr) displayed significant, positive correlations between leaves and rhizomes (linear regression, $R^2 > 0.15$, $P < 0.001$; Fig. 4). For the non-essential metals, however, we only found such relationship for Cd (linear regression, $R^2 = 0.17$, $P < 0.001$; Fig. 4). We found the strongest relationships (high R^2 values) for the essential metals that were present in the highest concentrations in the plants: Fe, Zn and Cu. Although data for most metals in the graph were near the 1:1 line (Fig. 4), some metals (e.g. Fe) were mainly accumulated in belowground parts, while others (e.g. Zn) were more concentrated in leaves.

3.3. Local trace metal concentrations in porewaters and leaves

In general, the polluted Piscadera Bay showed the highest leaf metal concentrations, while the protected Lac had the lowest levels for almost all measured metals (Table 2). In strong contrast, concentrations of trace metals in porewater and surface water samples (Table S3) did not show significant differences among the separate bays. In addition, we did not find any significant correlations between porewater and leaf concentrations of trace metals.

4. Discussion

There is a strong need for bioindicators that can be used to assess the actual status and health of coastal ecosystems globally in relation to anthropogenic pressure, and to metal pollution in particular (Linton and Warner, 2003; van Katwijk et al., 2011). We therefore compelled the first global literature overview for seagrass metal levels, which is important as a benchmark for seagrass research related to metal pollution. In addition, we studied the use of seagrasses as bioindicators for trace metal pollution in the poorly studied Caribbean seagrass beds of two islands where various

levels and sources of anthropogenic stressors were present. Based on our benchmark results, we could show that leaf concentrations of especially Cr, Fe, Ni and Zn were high at our case study sites.

4.1. Global-scale benchmark

We have here compiled a review of, to our best knowledge, all available literature on trace metal concentrations in seagrass leaves. Seagrasses are known metal accumulators, and in our literature study, we found a wide, 100–1000-fold, range in concentrations for all individual metals (Fig. 3), which underscores the suitability of seagrasses as sensitive bioindicators for the detection of trace metal pollution.

Literature data were, however, not equally divided among regions, and especially seagrass species occurring in the Mediterranean (e.g. *Posidonia oceanica*) have been very well studied. Some studies may show a bias for metal-polluted areas (Table S1, Table S2). Furthermore, for some metals such as Co and Hg, few data were available in the literature (Fig. 2). Data on trace metal pollution in tropical areas, where pollution and degradation of coastal ecosystems is increasing at an alarming rate, is mostly lacking. This data gap could rapidly be filled by using 'easy to pick' seagrasses in the areas that have not been covered yet, for example plants of the widespread *Thalassia* genus (Fig. 1).

Our global analysis of trace metal concentrations of polluted vs. unpolluted sites (Table S2) clearly shows that leaf trace metal values are significantly elevated ($2\text{--}4 \times$ higher) on polluted sites compared to unpolluted sites. This analysis proves that seagrass leaves can be used as first-level indicators for trace metal pollution of coastal areas.

Seagrass trace metal values are also known to vary seasonally, with lower trace metal values in the growing season than in the dormant season (Li and Huang, 2012; Schlacher-Hoenlinger and Schlacher, 1998). As most studies are conducted during the growing season (Table S1), our benchmark study may underestimate rather than overrate seagrass trace metal levels. Furthermore, seasonal differences were moderated in our data-set by averaging sample points from different seasons. It would however be very interesting to further investigate the importance of seasonality for the use of seagrasses as bioindicators for trace metal pollution.

4.2. Local study

We assessed trace metal concentrations in seagrasses as an indicator for their level of pollution in coastal areas and, more importantly, their biological availability. The bioavailability of metals and their mobility in the sediment is determined by their chemical speciation (Morillo et al., 2004), but seagrasses are also able to take up trace metals from the water column (Batley, 1987; Bond et al., 1985). Similar to nutrient measurements in abiotic compartments, measurements of trace metals in the water only provide a snapshot of trace metal loads as they are subjected to large variations in concentrations and fluxes (Ralph et al., 2006), while seagrass trace metal concentrations display a longer-term trace metal accumulation and related stress (Lafabrie et al., 2007, 2008; Sundelin and Eriksson, 2001).

Seagrass trace metal concentrations from the sampled bays indicated that Piscadera Bay, with a sewage outlet, was the most heavily polluted, whereas the conservation area Lac showed the lowest trace metal concentrations of all sampled bays, which is in accordance with our expectations. In contrast, porewater and surface water data did not provide this information, and were not correlated to plant concentrations, as expected.

The comparison of our own data to our worldwide benchmark list shows that levels of Cr appeared to be exceptionally high in all

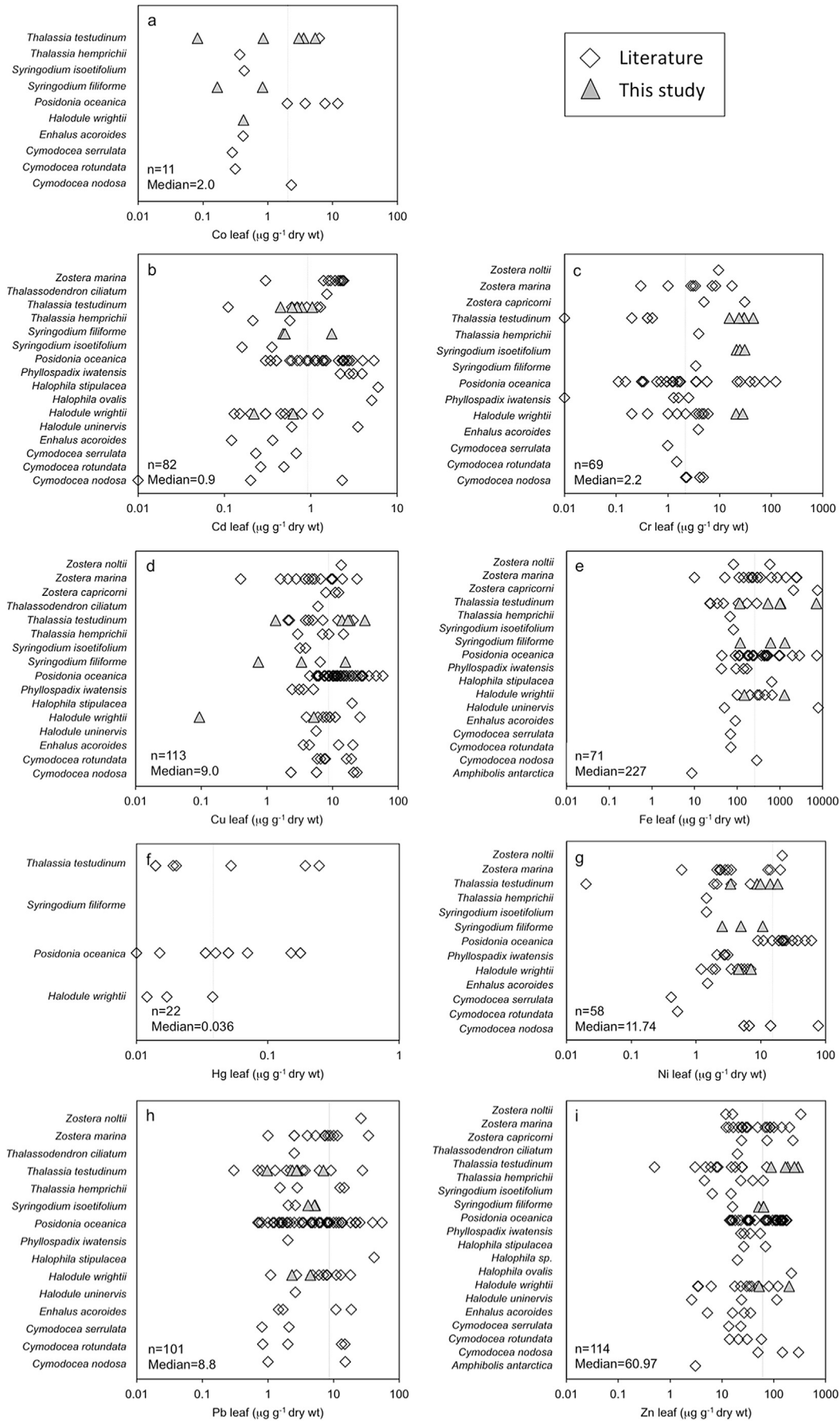


Fig. 3. An overview of trace metal (Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, Zn) concentrations in seagrass leaves from literature. The number of unique sample locations is indicated by *n*, displayed values are mean values as described in literature. The dotted line indicates the median value of all literature values. Grey triangles show mean trace metal concentrations measured in this study for 5 different bays (for number of replicates, see Table 1). Hg was not measured in our samples. Note that the logarithmic scales of the x-axis vary for the different metals.

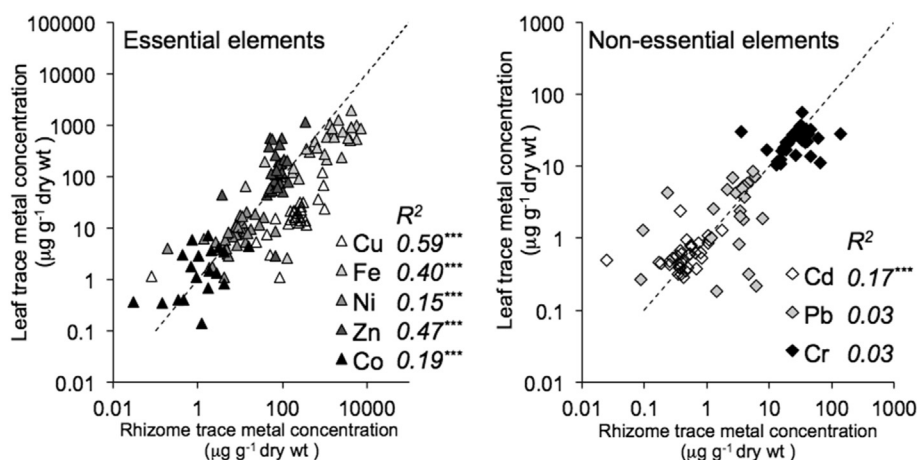


Fig. 4. Correlations between leaf and rhizome metal concentrations of essential (Cu, Fe, Ni, Zn, Co) and nonessential (Cd, Hg, Pb, Cr) metals. The dotted line represents the 1:1 line. Note the logarithmic scale of the x-axis. ns = not significant, * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

our study sites. Surprisingly, Cr levels appeared to be also high in the relatively undisturbed Lac, which suggests an external source of this metal. This is even more striking, because it is considered one of the least bioavailable metals in marine sediments (Morillo et al., 2004), which may imply that the Cr loads in our sampled bays are even higher than we might expect based on our results.

Although the concentrations of trace metals varied among sample points and between bays, it appeared to be very difficult to locate a point source for trace metals (Table S3 and Table S4) (Pergent-Martini and Pergent, 2000). Literature (Guzman and Garcia, 2002; Irvine and Birch, 1998) suggests that trace metal pollution in marine environments may originate from several processes and non-point-sources: runoff, flooding, mining, sewage, erosion, overuse of agrichemicals, industrial waste, atmospheric deposition, ports and refineries. We speculate that trace metals in the seagrass beds of Curacao originate from sewage (Piscadera Bay, Spanish Water Bay), ports and boating, but may also be of terrestrial origin, as high loads of terrigenous sediments are expected to have invaded the water due to deforestation and the construction of terrestrial drainage areas (Kuenen and Debrot, 1995). Furthermore, Curaçao is home to a large oil refinery and two of its bays (Caracas Bay and Bullen Bay, not sampled) have been subjected to frequent oil spills and accompanying pollution in the past (Nagelkerken and Debrot, 1995). This oil pollution may also have reached other, nearby bays.

4.3. Internal distribution

We compared metal concentrations in the different plant parts and found significant correlations between leaf and rhizome metal concentrations of the essential elements (Fig. 3). As plants need these elements either directly as micronutrients (Cu, Fe, Ni, Zn) or as an essential element (Co) for the nitrogenase enzymes fuelling

N₂-fixation by associated microorganisms (Welsh, 2000), they possess mechanisms for active allocation for most of these metals (Adriano, 2001; Marschner, 1995). As the concentrations represent redistribution in addition to uptake, we cannot draw conclusions about specific uptake ratios for leaves and rhizomes. In addition, all metals, including the non-essential elements showed fairly similar metal concentrations for both the leaves and rhizomes (close to the 1:1 line), which underlines the use of seagrass leaf material as indicator for trace metals.

4.4. Seagrasses as indicators for bioaccumulation

Although we mainly focused on the use of trace metal concentrations in seagrasses as indicator for pollution, the accumulation of metals may also affect seagrass health. Many trace metals are naturally abundant in seagrass beds (Batley, 1987; Prange and Dennison, 2000), but high concentrations can become toxic to seagrass and also be indicative of toxicity to other coastal organisms (Macinnis-Ng and Ralph, 2002; Prange and Dennison, 2000; Ralph and Burchett, 1998). Even sublethal levels of trace metals may have large effects on seagrass dominated ecosystems, as they are persistent and may accumulate in the food web, with toxic effects at higher trophic levels (Ikem and Egiebor, 2005; Schüürmann and Markert, 1998). In addition, trace metal accumulation in plants is often associated with changes in photosynthetic rates (Conroy et al., 1991; Macfarlane and Burchett, 2001; Prange and Dennison, 2000) and inhibited metabolic activity (Ralph and Burchett, 1998). This may lead to decreased growth rates or even result in plant die-off (Clijsters and Van Assche, 1985).

Trace metal accumulation in seagrasses can be used as a first level measurement to assess the contamination of the specific marine environment (Prange and Dennison, 2000). Moreover, as accumulation magnifies in the food web for some trace elements,

Table 2

Trace metal concentrations in seagrass leaves (in g g^{-1} , mean values per bay, for number of replicates see Table 1) of *Thalassia testudinum* in all studied bays. Significant differences ($P < 0.05$) are indicated by different letters. Ranking is the mean pollution ranking of all 8 metals.

Bay	Cd	Co	Cr	Cu	Fe	Hg	Ni	Pb	Zn	Ranking
Boka Ascension	0.93 ^a	3.10 ^{ab}	32.01 ^{ab}	10.27 ^{ab}	3725.63 ^{ac}	NA	9.49 ^{abc}	5.93 ^a	74.97 ^a	2
Lac Bay	0.44 ^a	0.08 ^a	29.50 ^a	1.36 ^a	115.48 ^b	NA	3.41 ^a	2.37 ^b	184.67 ^a	5
Piscadera Bay	0.79 ^a	3.57 ^{ab}	30.34 ^a	13.83 ^{ab}	1044.48 ^a	NA	18.13 ^c	2.83 ^{ab}	295.52 ^a	1
Sint Joris Bay	0.61 ^a	3.02 ^b	23.98 ^{ab}	17.21 ^{ab}	1003.03 ^a	NA	9.89 ^b	0.97 ^b	248.58 ^a	3
Spanish Water Bay	0.69 ^a	0.85 ^{ab}	15.57 ^b	31.02 ^b	523.94 ^c	NA	8.81 ^b	2.71 ^b	168.53 ^a	4

high trace metal levels in primary producers such as seagrasses may indicate serious trace metal pollution in the whole food web.

5. Conclusions and recommendations

Summarizing, we here present a global list for trace metal concentrations in seagrass leaves, which can be used in further studies to relate local trace metal pollution to a benchmark list. Moreover, we showed that seagrasses in general, and *Thalassia* spp. in particular, can be used as an easy to sample and widespread bioindicator species for trace metal pollution of especially Cu, Hg, Ni, Pb, and Zn. As little information is available on the effects of trace metal pollution on seagrass physiology and on the bio-accumulative effects of trace metals in seagrass-based foodwebs, we strongly recommend further research on these topics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2014.08.028>.

References

- Adriano, D.C., 2001. Trace Elements in Terrestrial Environments – Biogeochemistry, Bioavailability, and Risks of Metals, second ed. Sheridan Books Inc., Ann Arbor, USA.
- Batley, G.E., 1987. Heavy metal speciation in water, sediments and biota from Lake Macquarie, New South Wales. *Aust. J. Mar. Freshw. Res.* 38, 591–606.
- Bennett, S., Roca, G., Romero, J., Alcoverro, T., 2011. Ecological status of seagrass ecosystems: an uncertainty analysis of the meadow classification based on the *Posidonia oceanica* multivariate index (POMI). *Mar. Pollut. Bull.* 62, 1616–1621.
- Bhattacharya, B., Sarkar, S.K., Das, R., 2003. Seasonal variations and inherent variability of selenium in marine biota of a tropical wetland ecosystem: implications for bioindicator species. *Ecol. Indic.* 2, 367–375.
- Bond, A.M., Bradbury, J.R., Hudson, A., Garnham, J.S., Hanna, P.J., Strother, S., 1985. Kinetic studies of lead (II) uptake by the seagrass *Zostera muelleri* in water by radiotracing, atomic absorption spectrometry and electrochemical techniques. *Mar. Chem.* 16, 1–9.
- Burkholder, J., Tomasko, D., Touchette, B., 2007. Seagrasses and eutrophication. *J. Exp. Mar. Biol. Ecol.* 350, 46–72.
- Clijsters, H., Van Assche, F., 1985. Inhibition of photosynthesis by heavy metals. *Photosynth. Res.* 7, 31–40.
- Cohen, J.E., 2003. Human population: the next half century. *Science* 302, 1172–1175.
- Cohen, J.E., Small, C., Mellinger, A., Gallup, J., Sachs, J., 1997. Estimates of coastal populations. *Science* 278, 1211–1212.
- Conroy, B.A., Lake, P., Buchhorn, N., McDouall-Hill, J., Hughes, L., 1991. Studies on the effects of heavy metals on seagrasses in Lake Macquarie. In: Whitehead, J.H., Kidd, R.W., Bridgeman, H.A. (Eds.), *Lake Macquarie: an Environmental Reappraisal*. University of Newcastle, Newcastle.
- Costanza, R., d'Arge, R., deGroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., Oneill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., vandenBelt, M., 1997. The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260.
- Cowen, R.K., Paris, C.B., Srinivasan, A., 2006. Scaling of connectivity in marine populations. *Science* 311, 522–527.
- De Haan, D., Zaneveld, J.S., 1959. Some notes on tides in Annabaai harbour, Curaçao, Netherlands Antilles. *Bull. Mar. Sci.* 9, 224–236.
- Ferrat, L., Pergent-Martini, C., Roméo, M., 2003. Assessment of the use of bio-markers in aquatic plants for the evaluation of environmental quality: application to seagrasses. *Aquat. Toxicol.* 65, 187–204.
- Fourqurean, J.W., Zieman, J.C., 2002. Nutrient content of the seagrass *Thalassia testudinum* reveals regional patterns of relative availability of nitrogen and phosphorus in the Florida keys USA. *Biogeochemistry* 61, 229–245.
- Gonzalez-Correa, J.M., Bayle, J.T., Sanchez-Lizasa, J.L., Valle, C., Sanchez-Jerez, P., Ruiz, J.M., 2005. Recovery of deep *Posidonia oceanica* meadows degraded by trawling. *J. Exp. Mar. Biol. Ecol.* 320.
- Govers, L.L., Pieck, T., Bouma, T.J., Suykerbuyk, W., Smolders, A., van Katwijk, M.M., 2014. Seagrasses are negatively affected by organic matter loading and *Arenicola marina* activity in a laboratory experiment. *Oecologia* 175, 677–685.
- Green, E.P., Short, F.T., 2003. *World Atlas of Seagrasses*. University of California Press, Berkeley, California.
- Guzman, H.M., Garcia, E.M., 2002. Mercury levels in coral reefs along the Caribbean coast of Central America. *Mar. Pollut. Bull.* 44, 1420–1451.
- Ikem, A., Egiebor, N.O., 2005. Assessment of trace elements in canned fishes marketed in Georgia and Alabama. *J. Food Compos.* 18, 771–787.
- Irvine, I., Birch, G.F., 1998. Distribution of Heavy Metals in Surficial Sediments of Port Jackson, vol. 45. New South Wales. *Australian Journal of Earth Sciences*, Sydney, pp. 297–304.
- Kuenen, M., Debrot, A.O., 1995. A quantitative study of the seagrass and algal meadows of the Spaanse Water, Curacao, the Netherlands Antilles. *Aquat. Bot.* 51, 291–310.
- Lafabrie, C., Pergent, G., Kantin, R., Pergent-Martini, C., Gonzalez, J.L., 2007. Trace metals assessment in water, sediment, mussel and seagrass species – validation of the use of *Posidonia oceanica* as a metal biomonitor. *Chemosphere* 68, 2033–2039.
- Lafabrie, C., Pergent-Martini, C., Pergent, G., 2008. Metal contamination of *Posidonia oceanica* meadows along the corsican coastline (Mediterranean). *Environ. Pollut.* 151, 262–268.
- Lee, K.-S., Short, F.T., Burdick, D.M., 2004. Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries. *Aquat. Bot.* 78, 197–216.
- Li, L., Huang, X., 2012. Three tropical seagrasses as potential bio-indicators to trace metals in Xincun Bay, Hainan Island, South China. *Chin. J. Oceanol. Limnol.* 30, 212–224.
- Li, Q., Yu, Z., Chu, B., Zhang, N., Cai, S., Fang, J., 2007. Heavy metals in coastal wetland sediments of the Pearl River Estuary, China. *Environ. Pollut.* 149, 158–164.
- Linton, D.M., Warner, G.F., 2003. Biological indicators in the Caribbean coastal zone and their role in integrated coastal management. *Ocean. Coast. Manag.* 46, 261–276.
- Lopez y Royo, C., Pergent, G., Alcoverro, T., Buia, M.C., Casazza, G., Martínez-Crego, B., Pérez, M., Silvestre, F., Romero, J., 2011. The seagrass *Posidonia oceanica* as indicator of coastal water quality: experimental intercalibration of classification systems. *Ecol. Indic.* 11, 557–563.
- Macfarlane, G.R., Burchett, M.D., 2001. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Mar. Pollut. Bull.* 42, 233–240.
- Macinnis-Ng, C.M.O., Ralph, P.J., 2002. Towards a more ecologically relevant assessment of the impact of heavy metals on the photosynthesis of the seagrass, *Zostera capricorni*. *Mar. Pollut. Bull.* 45, 100–106.
- Madden, C.J., Rudnick, D.T., McDonald, A.A., Cuniff, K.M., Fourqurean, J.W., 2009. Ecological indicators for assessing and communicating seagrass status and trends in Florida Bay. *Ecol. Indic.* 9, S68–S82.
- Markert, B., Wappelhorst, O., Weckert, V., Herpin, U., Siewers, U., Frieke, K., Breulmann, G., 1999. The use of bioindicators for monitoring the heavy-metal status of the environment. *J. Radioanal. Nucl. Chem.* 240, 425–429.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*, second ed. Academic Press, London.
- Martínez-Crego, B., Vergés, A., Alcoverro, T., Romero, J., 2008. Selection of multiple seagrass indicators for environmental biomonitoring. *Mar. Ecol. Prog. Ser.* 361, 93–109.
- Meehan, A.J., West, R.J., 2000. Recovery times for a damaged *Posidonia australis* bed in south eastern Australia. *Aquat. Bot.* 67, 161–167.
- Montefalcone, M., 2009. Ecosystem health assessment using the Mediterranean seagrass *Posidonia oceanica*: a review. *Ecol. Indic.* 9, 595–604.
- Mora, C., 2008. A clear human footprint in the coral reefs of the Caribbean. *Proc. Biol. Sci.* 275, 767–773.
- Moreno, D., Aguilera, P.A., Castro, H., 2001. Assessment of the conservation status of seagrass (*Posidonia oceanica*) meadows: implications for monitoring strategy and the decision-making process. *Biol. Conserv.* 102, 325–332.
- Morillo, J., Usero, J., Gracia, I., 2004. Heavy metal distribution in marine sediments from the southwest coast of Spain. *Chemosphere* 55, 431–442.
- Mumby, P.J., Dahlgren, C.P., Harborne, A.R., Kappel, C.V., Micheli, F., Brumbaugh, D.R., Holmes, K.E., Mendes, J.M., Broad, K., Sanchirico, J.N., Buch, K., Box, S., Stoffle, R.W., Gill, A.B., 2006. Fishing, trophic cascades, and the process of grazing on coral reefs. *Science* 311, 98–101.
- Mumby, P.J., Hastings, A., Edwards, H.J., 2007. Thresholds and the resilience of Caribbean coral reefs. *Nature* 450, 98–101.
- Nagelkerken, I., 2000. Importance of mangroves, seagrass beds and the shallow coral reef as a nursery for important coral reef fishes, using a visual census technique. *Estuarine. Coast. Shelf Sci.* 51, 31–44.
- Nagelkerken, I., Debrot, A.O., 1995. Mollusc communities of tropical rubble shores of Curaçao: long-term (7+ years) impacts of oil pollution. *Mar. Pollut. Bull.* 9, 592–598.
- Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L.J., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. *Bioscience* 56, 987–996.

- Pergent-Martini, C., Pergent, G., 2000. Are marine phanerogams a valuable tool in the evaluation of marine trace metal contamination: example of the Mediterranean sea? *Int. J. Environ. Pollut.* 13, 126–147.
- Phillips, R.C., 1992. The seagrass ecosystem and resources in Latin America. In: Seeliger, U. (Ed.), *Coastal Plant Communities of Latin America*. Academic Press, San Diego, California.
- Prange, J.A., Dennison, W.C., 2000. Physiological responses of five seagrass species to trace metals. *Mar. Pollut. Bull.* 41, 327–336.
- Ralph, P.J., Burchett, M.D., 1998. Photosynthetic response of *Halophila ovalis* to heavy metal stress. *Environ. Pollut.* 103, 91–101.
- Ralph, P.J., Tomasko, D., Moore, K., Seddon, S., Macinnis, Ng, Catriona, M.O., 2006. Human impacts on seagrasses: eutrophication, sedimentation and contamination. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), *Seagrasses: Biology, Ecology and Conservation*. Springer, The Netherlands.
- Romero, J., Martinez-Crego, B., Alcoverro, T., Perez, M., 2007. A multivariate index based on the seagrass *Posidonia oceanica* (POMI) to assess ecological status of coastal waters under the water framework directive (WFD). *Mar. Pollut. Bull.* 55, 196–204.
- Schlacher-Hoenlinger, M.A., Schlacher, T.A., 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.
- Schüürmann, G., Markert, B., 1998. *Ecotoxicology: Ecological Fundamentals, Chemical Exposure, and Biological Effects*. Wiley and Spektrum Akademischer Verlag, New York and Heidelberg.
- Short, F.T., Wyllie-Echeverria, S., 1996. Natural and human-induced disturbances of seagrasses. *Environ. Conserv.* 23, 17–27.
- Small, C., Nicholls, R.J., 2003. Analysis of human settlement in coastal zones. *J. Coast. Res.* 19, 584–599.
- Smolders, A.J.P., Moonen, M., Zwaga, K., Lucassen, E., Lamers, L.P.M., Roelofs, J.G.M., 2006. Changes in pore water chemistry of desiccating freshwater sediments with different sulphur contents. *Geoderma* 132, 372–383.
- Sundelin, B., Eriksson, A., 2001. Mobility and bioavailability of trace metals in sulfidic coastal sediments. *Environ. Toxicol. Chem.* 20, 748–756.
- Thorhaug, A., Miller, B., Jupp, B., Booker, F., 1985. Effects of a variety of impacts on seagrass restoration in Jamaica. *Mar. Pollut. Bull.* 16, 355–360.
- Udy, J.W., Dennison, W.C., 1997. Growth and physiological responses of three seagrass species to elevated nutrients in Moreton Bay, Australia. *J. Exp. Mar. Biol. Ecol.* 217, 253–277.
- Valiela, I., Bowen, J.L., York, J.K., 2001. Mangrove forests: one of the world's threatened major tropical environments. *Bioscience* 51, 807–815.
- van Katwijk, M.M., van der Welle, M.E.W., Lucassen, E.C.H.E.T., Vonk, J.A., Christianen, M.J.A., Kiswara, W., Hakim, I.I., al, Arifin, A., Bouma, T.J., Roelofs, J.G.M., Lamers, L.P.M., 2011. Early warning indicators for river nutrient and sediment loads in tropical seagrass beds: a benchmark from a near-pristine archipelago in Indonesia. *Mar. Pollut. Bull.* 62, 1512–1520.
- van Tussenbroek, B.I., Vonk, J.A., Stapel, J., Erftemeijer, P.L.A., Middelburg, J.J., 2006. The biology of *Thalassia*: paradigms and recent advances in research. In: Larkum, A.W.D., Orth, R.J., Duarte, A.C. (Eds.), *Seagrasses: Biology, Ecology and Conservation*. Springer, Dordrecht, the Netherlands.
- Vera, B., 1992. Seagrasses of the Venezuelan coast: distribution and community components. In: Seeliger, U. (Ed.), *Coastal Plant Communities of Latin America*. Academic Press, San Diego, California, pp. 135–140.
- Walker, D.I., McComb, A.J., 1992. Seagrass degradation in Australian coastal waters. *Aquat. Bot.* 25, 191–195.
- Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proc. Natl. Acad. Sci.* 106, 12377–12381.
- Welsh, D.T., 2000. Nitrogen fixation in seagrass meadows: regulation, plant-bacteria interactions and significance to primary productivity. *Ecol. Lett.* 3, 58–72.