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Iron, cadmium, and chromium in seagrass (*Thalassia testudinum*) from a coastal nature reserve in karstic Yucatán

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Abstract The management of protected areas in karstic regions is a challenge because flooded cave systems form there and provide underground hydrological conducts that may link different zones. As a consequence, affectations to the protected areas can possibly occur as a consequence of human activities in remote areas and may therefore pass undetected. Thus, the monitoring of possible contaminants in these regions is becoming imperative. In this work, we analyze the concentration of essential (iron) and non-essential metals (cadmium and chromium) in the seagrass *Thalassia testudinum* that grows in Yalahau Lagoon, located in a *near-to-pristine* protected area of the Yucatán Peninsula, close to the rapidly developing touristic belt of the Mexican Caribbean. Salinity and silicate patterns show that Yalahau is an evaporation lagoon, where groundwater discharge is important. High iron (>400 μg/g), cadmium (>4 μg/g), and chromium (≈1 μg/g) concentrations were found in the area of highest groundwater input of the lagoon. High levels (5.1 μg/g) were also found near the town dump. In the rest of the sampling sites, metal concentrations remained near to background levels as estimated from other works. Temporal changes of concentrations in the seagrass tissues show also a local input and an input from the groundwater that could provoke an environmental problem in the Yalahau Lagoon in the near future.

Keywords Biomonitors · Groundwater · Pollution · Seasonal · Trace metals · Yum Balam

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Introduction

The establishment of nature reserves is considered an effective action to protect ecosystems and their resources, particularly in the coastal zone, where interactions multiply. However, like most coastal areas, reserves are threatened by the increasing pollution of the regions surrounding them. Heavy metals are among the most dangerous pollutants. For more than 25 years, studies carried out have demonstrated increasing concentrations of some heavy metals in tropical lagoon and estuarine ecosystems in spite of the efforts to reduce environmental risks and safeguard the natural environment (Nienhuis 1986; Schlacher-Hoenlinger and Schlacher 1998; Ward 1989; Malea 1993; Ruelas-Inzunza and Páez-Osuna 2002, 2005; Frías-Espericueta et al. 2005; Whelan et al. 2005, 2011). Unlike pesticides, which mainly originate from human activities, trace metals are present naturally. In fact, some selected trace metals represent essential micronutrients, found in soils and seawater and as part of the rocks and marine sediments. Still, human activities increase the availability of heavy metals, which represent a potential problem, since some essential trace metals can be toxic at high concentrations (Ralph and Burchett 1998; Prange and Dennison 2000).

In many cases, to effectively protect nature reserves, the pollution of their watersheds has to be monitored and regulated. However, in karstic regions, where there is no surface runoff (Merino et al. 1990), the impact of human activities and disposals may remain undetected and the threat to protected areas, uncontrolled. This could be the case for the numerous reserves located in karstic regions, such as the Yucatán Peninsula, where it has been recently identified that groundwater pollution is an emergent menace (ArandaCirerol et al. 2006; Hernández-Terrones et al. 2011; Metcalfe et al. 2011). The complex structure of flooded cave systems and hydrological conducts in Yucatán is not well known yet (Smart et al. 2006), and it may link nature reserves and apparently unperturbed regions to areas of intensive urban development, such as Cancún and the "Riviera Maya" (Metcalfe et al. 2011). This may be the case of the Yum Balam Nature Reserve (recently protected, CONANP 1994) located near the city of Cancún in NE Yucatán, a protected area which comprises a rich variety of coastal ecosystems and communities, including mangroves, wetlands, seagrass meadows, coastal dunes, and coastal waters. Yum Balam was relatively undisturbed by human influence a decade ago (Tran et al. 2002), but it could be threatened by intensive tourist development and the emerging issue of groundwater pollution in the region (Hernández-Terrones et al. 2011; Metcalfe et al. 2011).

Seagrasses are among the most productive submerged communities (McRoy and McMillan 1977) and are crucial for many marine ecosystems since they provide habitat, sediment stability, nutrients, and food for many organisms (Klumpp et al. 1989). Moreover, it has been established that seagrasses capture trace metals from the marine environment via the leaves and the root-rhizomes. Metal concentrations in these tissues are frequently correlated with those in both the water column and the sediments (Lyngby and Brix 1982, 1983; Nienhuis 1986; Ward 1989), and for this reason, these plants can be used as "biological indicators" of metal contamination. Furthermore, being primary producers, seagrasses can be used as first-level indicators for monitoring trace metal levels in coastal marine environments.

Previous research on the interactions between metals and seagrasses has focused on the accumulation of metals in the plant (Nienhuis 1986; Ward 1989; Malea 1993; Schlacher-Hoenlinger and Schlacher 1998). For example, the seagrass *Posidonia oceanica* has been utilized as a biomarker of trace metal contamination, particularly on the Mediterranean coast (Maserti et al. 1988; Sanchiz et al. 1990; Costantini et al. 1991; Catsiki and Panayotidis 1993). Whelan et al. (2005, 2011) reported trace metal partitioning in *Thalassia testudinum* and sediments in the Lower Laguna Madre, Texas, USA, and in the Mexican Caribbean. They concluded that this seagrass is a good biomonitor but that care must be taken to analyze all the morphological units.

T. testudinum is a climax species (Zieman 1982) and is an abundant seagrass in many tropical and subtropical environments throughout the Greater Caribbean, including the Yum Balam nature reserve. We have selected *T. testudinum* as an indicator seagrass species to study trace metal (iron, cadmium, and chromium) contamination in this reserve located in the karstic Yucatán Peninsula. The purpose of this study is to assess the concentrations of these trace metals in *T. testudinum* of this important protected area, in order to evaluate the present level of the pollution threats derived from the local activities and from the regional development through groundwater.

Methods

Geographic setting

Our area of interest, the Yum Balam reserve, is located in the northeast of Yucatán, Mexico. The reserve comprises an area of 154,000 ha, the majority of which corresponds to the Yalahau Lagoon (Fig. 1), which is separated from the open sea by the long dune barrier called Holbox Island. Deciduous tropical forest, flooded forests, mangroves, and wetlands crop the surroundings of the lagoon. It is also home to approximately 16,000 locals, most of them of Mayan origin, living in several townships located around the lagoon, like Chiquilá and Holbox. The Yum Balam reserve, and specifically the Yalahau Lagoon, was still found relatively undisturbed by human influence a decade ago (Tran et al. 2002), but it could be threatened both by disposal from the local communities and from the regional intensive urban and tourist development from Cancún and along the Riviera Maya.

The karstic nature of the Yucatán Peninsula allows a rapid infiltration of rainwater. It is now assumed that groundwater is the main source of continental water to the lagoons located along the northern coast of Yucatán (ArandaCirerol et al. 2006). However, in Yalahau, because of the turbidity of the water, the exact sites of sinkholes have not been identified so far. In terms of the possible sources of metals, in previous studies, it was assumed that the lack of surface runoff to coastal waters (Merino et al. 1990) determined that there were no significant iron inputs to the coastal environments, other than the atmospheric and local point sources (Duarte et al. 1995). Since there are no evident natural sources of chromium or cadmium in the region either, we assume that all the cadmium, chromium, and iron present in the lagoon should come from local input or polluted groundwater input.

Field methods

Our sampling sites in the Yalahau Lagoon are shown in Fig. 1 and Table 1. Monotypic meadows of *T. testudinum* were sampled along the main axis of the lagoon to assess spatial variation in metal concentrations. Sampling sites S1 and S2 were selected because they are close to the town dump of Holbox. Following the field methods previously reported by Whelan et al. (2005), leaves and root-rhizomes of *T. testudinum* were collected by hand. To collect the root-rhizomes, we removed the sediments by hand until it was possible to collect the sample. The leaf sampling was also made by hand, taking green leaves only. All samples

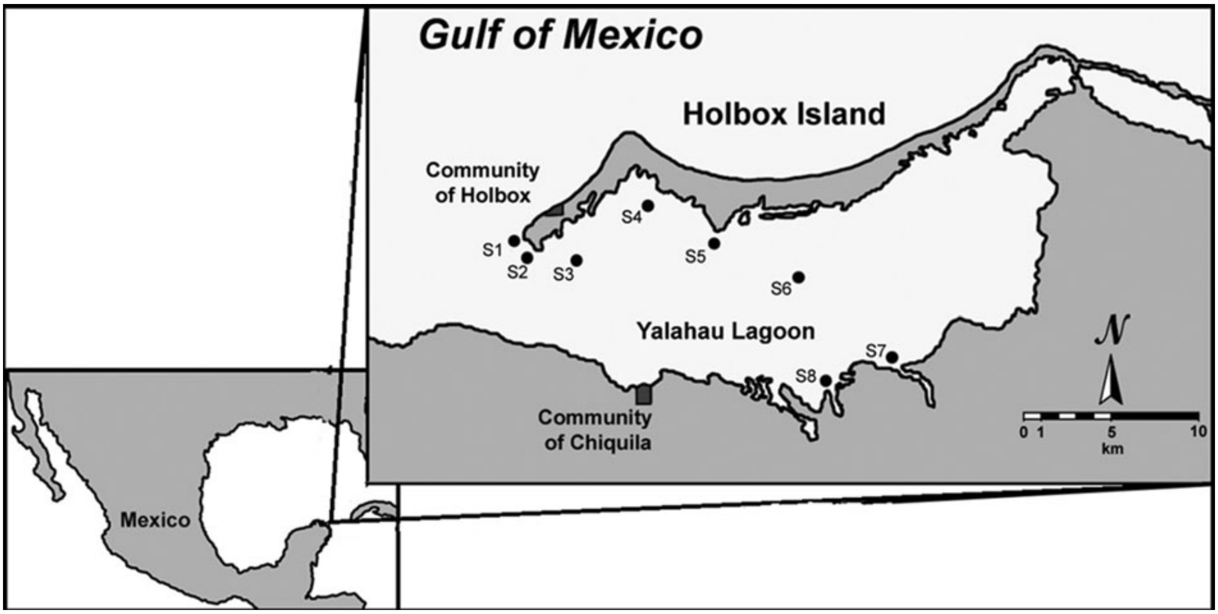

Fig. 1 Location of sampling sites in the Yalahau Lagoon, Yucatán peninsula, Mexico

Table 1 Sampling sites in the Yalahau Lagoon

Station	Coordinates	Common name	Depth (m)
S1	N21°30.997′ W087°23.880′	Isla Pasión	2.5
S2	N21°30.255′ W087°23.525′	Basurero	1.8
S3	N21°29.890′ W087°22.208′	Boya de Recalada	1.2
S4	N21°31.044′ W087°19.248′	Isla Pájaros	1.4
S5	N21°60.427′ W087°17.576′	Punta Catalán	0.5
S6	N21°29.291′ W087°15.776′	Medio Laguna	1.1
S7	N21°26.351′ W087°11.174′	Yalikin	1.2
S8	N21°25.847′ W087°13.742′	Río Bomba	<0.5

were immediately washed with lagoon water to remove sediments, shells, and other debris. Epiphytes were hand-removed from the leaves surface. Leaves were separated from root-rhizomes and stored in separate plastic bags for their transport to the laboratory. At each site, three to five seagrass meadows were selected to cover the entire study site. From each seagrass meadow, approximately 10–15 plants were randomly collected in an area of around 4 m^2. Samples were mixed to obtain one composite sample for each site. Composite samples have been used previously (Lewis et al. 2007) and have the advantage of being representative of the shared seagrass conditions at each site. In order to better assess the main seasonal variation in metal concentrations, samples were collected during June of 2004 and January of 2005. Rainfall varied between 120 and 270 mm in the months of rainy season, while, in the dry season, the monthly average was approximately 40 mm (Fig. 2). The effects of rain in the aquatic plants are not instantaneous, considering that the maximum potential leaf age is 90 days (van Tussenbroek 1995). Thus, leaves collected in June began to grow in the three previous months, during the dry season (March to May; see Fig. 2), and leaves sampled in January indicate conditions experienced during the rainy season, as they began to grow in October or November. In the case of roots-rhizomes, which are much longer-lived than leaves (Gallegos et al. 1993), the tissues likely integrate the conditions experienced by the plant during longer periods. To assess the spatial and seasonal variations in water quality, surface-water salinity was measured with a multisonde YSI-85 at each sampling site. Surface water was also collected, filtered through a 0.45 μm Millipore membrane filter, and preserved at 4 °C for dissolved inorganic nutrient quantification in the laboratory.

Laboratory methods

In the laboratory, *T. testudinum* samples were oven-dried at 95 °C for 24 h. Approximately 0.2 g of dry leaves and roots-rhizomes were weighed and digested using concentrated nitric acid–hydrogen peroxide solution, following USEPA SW 846–3050 methodology. This treatment is not a complete digestion that oxidizes and dissolves all minerals including quartz and aluminosilicates. A complete digestion requires treatment with HF and $HClO_4$. The procedure that we used leaches and oxidizes the bioavailable metals (Whelan et al. 2005). From each 10-mL volumetric flask, three samples of 1 mL were transferred into tubes for the analysis of each metal. With this methodology, the analysis of each sample was done by triplicate for each metal. Fe was analyzed using a PE Analyst 800 flame atomic absorption instrument equipped with a high efficiency nebulizer. Cd and Cr were determined using a graphite furnace coupled to the same atomic absorption instrument. Samples were aspirated in triplicate, and, in the cases when the relative percent standard deviation was greater than 5 %, the samples were re-analyzed. Working standard solutions were made from dilutions of 1,000 ppm FLUKA standard solutions of each element. Quality assurance samples were analyzed in each analytical batch, or every ten samples. Table 2 reports the recoveries for the metals reported in this study compared with the results for the reference material (HS-Certified Reference Material, Orchard Leaves Solution). After digestion, samples were transferred and filtered into 10-mL volumetric flasks, brought to volume. Nutrients (ammonium, nitrate, nitrite, and silicate) were analyzed on the filtered water samples. Ammonium with the phenol–hypochlorite method, nitrites with the sulfanilamide method, nitrate as nitrites after reduction in cadmium–copper column, and reactive soluble silica through the blue-molybdenum method all are standard spectrophotometric techniques described in Parsons et al. (1984).

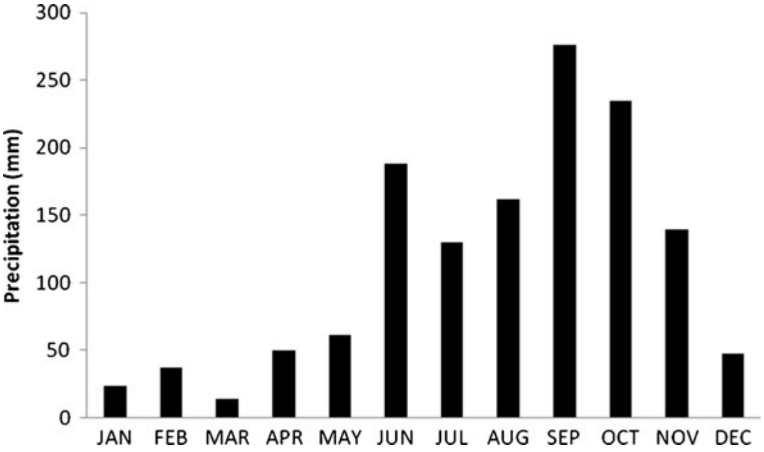

Fig. 2 Monthly rainfall (means for the 2000–2005 period) near Yalahau Lagoon at Solferino station, located 20 km from the studied area. Red de estaciones meteorológicas, SEMARNAT (Secretaría del Medio Ambiente y Recursos Naturales), CONAGUA (Comisión Nacional del Agua), and SMN (Servicio Meteorológico Nacional)

Statistical analysis

Statistical analysis was carried out using STATGRAPHICS Centurion XVI (Statpoint Tech). Analyses to compare metal concentration in samples of different sites were performed using one-way ANOVA ($p<0.05$) followed by Fisher's least-significant difference procedure for multiple comparisons. If the normality or the equal variance test failed, a Kruskal–Wallis test was used instead of one-way ANOVA. To analyze the differences among dry and rainy seasons, a *t* test was applied; when the differences in the standard deviation of the data differed significantly, a non-parametric Mann–Whitney *U* test was employed.

Results and discussion

Hydrology

The average salinity of the lagoon was above 40 PSU, and salinity values rose from marine values near the inlet to hypersaline values in most of the sampling sites (Fig. 3a). This means that evaporation is greater than freshwater inputs in Yalahau, as found for other lagoons in the region (González et al. 1992; Herrera-Silveira 1994; Herrera-Silveira and Ramírez-Ramírez 1998). However, the seasonal differences observed in salinity are small, supporting that the relatively stable input of groundwater dominates in the lagoon water budget and overrides the variable fresh water discharges from rain and surface runoffs.

Table 2 Recovery of reference material (High Purity Standards Orchard Leaves)

	Fe	Cd	Cr
Certified	30.00	1.25	3.00
Measured	26.00±1.99	0.95±0.23	2.80±0.02
% Recovery	87	76	94

Concentrations in micrograms per gram dry weight

The importance of groundwater input to Yalahau Lagoon is also supported by the pattern found in silicate (Fig. 3b), which is a useful tracer of groundwater inputs in this region (Smith et al. 1999; Hernández-Terrones et al. 2011). Silicate concentrations increased significantly from surface marine values (<15 μM) at the lagoon inlet (S1) toward the inside of the lagoon, reaching a maximum of over 120 μM at the sampling site S5 and remaining high in sampling sites S6 and S7 (Fig. 3b) in both seasons. These concentrations fall within the range found for groundwater in sinkholes of NW Yucatán (ArandaCirerol et al. 2006; median=66.7 μM, range=4.8 to 439.4 μM) and are very similar to those of the groundwater in wells of NE Yucatán (Hernández-Terrones et al. 2011; mean=129.7 μM, SD=25.7). This supports that groundwater discharges are particularly important in the area around the sampling sites having the highest silicate concentrations in Yalahau Lagoon. The permanence of the same pattern during both the dry and rainy seasons' samplings supports the year-round prevalence of groundwater input at Yalahau.

Dissolved inorganic nitrogen (DIN) concentrations found in Yalahau Lagoon were similar to those reported for other coastal lagoons with groundwater discharge (i.e., Celestum and Dzilam de Bravo) in

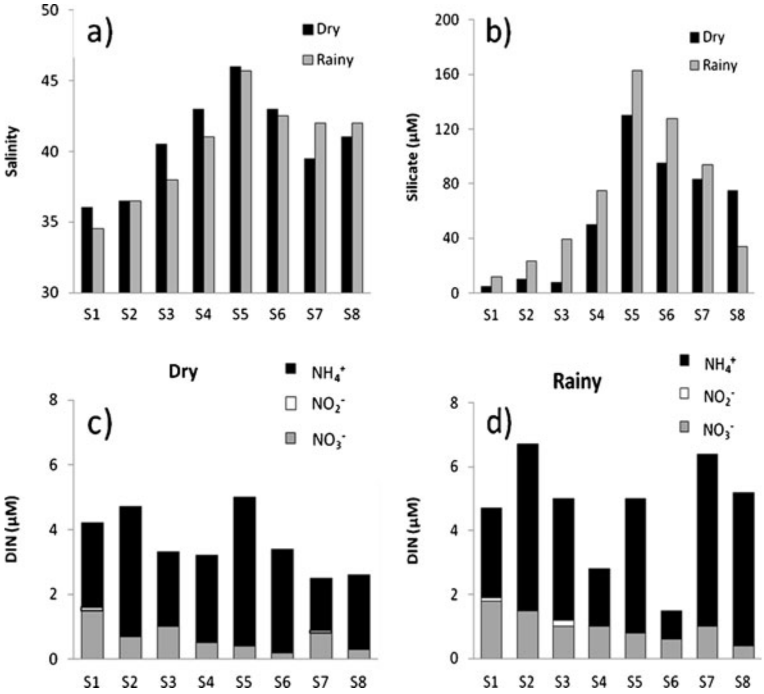

Fig. 3 Salinity (PSU) (**a**), silicate (**b**) and dissolved inorganic nitrogen (DIN) during the dry and rainy (**d**) seasons at the sampled stations. Detection limit: NO_3^- 0.05 μM, NO_2^- 0.01 μM, NH_4^+ 0.1 μM, silicate 0.1 μM

Northern Yucatán (ArandaCirerol et al. 2006), during both the dry and rainy seasons sampled (Fig. 3c and d). Surprisingly, a decreasing pattern of the DIN toward the lagoon inlet was not observed. In fact, the DIN concentrations found at sampling site S2 were among the highest both during the rainy and dry seasons and were dominated by ammonium. Since this nutrient is related to wastewaters (Newton and Mudge 2005), the high concentrations registered toward the lagoon inlet, and particularly at sampling site S2, could indicate local inputs.

Spatial variation of metals' concentration

Spatial variation of iron, cadmium, and chromium in leaves and root-rhizomes of *T. testudinum* was assessed using the January 2005 sampling, during which it was possible to collect samples in eight sites distributed throughout the Yalahau Lagoon (see Fig. 1). In most of the sites, the concentration of iron was near to 100 ppm (Figs. 4a and 5a), in agreement with previously reported values (Duarte et al. 1995). The exceptions were the concentrations at sites S5, S6, and S7, where most of the values were slightly higher than 400 ppm. At sites S5, S6, and S7, a high amount of cadmium (Figs. 4b and 5b) was also found. This matches with the increment of silicates (Fig. 3b) at these sites, which indicates high groundwater input there. Because there are no other significant sources of metal inputs to the coastal environments of NE Yucatán (Duarte et al. 1995), the increment of iron and cadmium in these sites is likely due to a result of groundwater input. For chromium, tissue samples exhibited homogenously small values (0.3–1.1 ppm, Figs. 4c and 5c), similar to the normal range generally for this element found in plants (0.5–1.5 μg/g, Felcman and Tristão-Bragança 1988). These results could indicate that, apparently, groundwater is not contaminated with chromium.

Metals and other contaminants could also arrive to the lagoon through local input from the town dump (S1 and S2 sites are close to the town dump). The highest cadmium concentration we found was observed on one of these sites (5.1 ppm, S1, Fig. 4b), supporting the possibility of important local inputs through runoff transports of metals in this area of Yalahau. In contrast, low levels of chromium indicate that it is not a contaminant in this area either. These results also support the utility of seagrass analyses as a way to detect pollution from local sources, like a small dump, because of the integrative nature of the seagrass.

For all metals, there were significant differences between sites (one-way ANOVA $p<0.05$, Figs. 4 and 5).

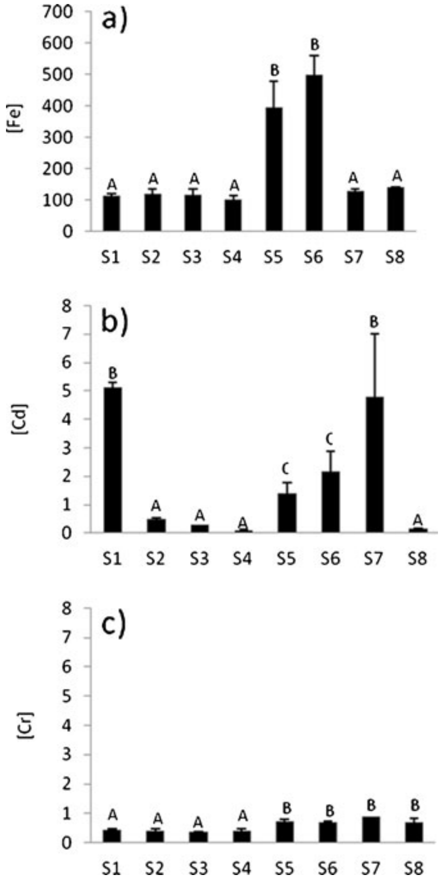

Fig. 4 **a** Iron, **b** cadmium, and **c** chromium content in leaves of *T. testudinum* at eight stations sampled during January 2005 at Yalahau Lagoon. Values in micrograms per gram dry weight ±SD. *Columns* with the *same label* (**a**, **b,** or **c**) do not differ significantly (p<0.05) according to the Fisher's multiple comparison tests. Detection limit: Fe 0.1 ppm, Cd 0.5 ppb, and Cr 0.4 ppb

For leaves, results are reported in Fig. 4. The sampling sites that do not differ significantly (Fisher's test, p< 0.05) were grouped. Group A corresponds to sites with the lowest metal concentration (i.e., with less anthropogenic influence). Group B represents those with the highest concentrations, which correspond to sites with high groundwater input or near local inputs. Only for cadmium was there a third group, Group C, with intermediate amounts of metals, composed also by groundwater-influenced sites. A, B, and C represent groups with significant differences among them. It is known that the metal content in seagrass leaves increases as a function of the metal concentration in the water (e.g., particularly for Cd, see Alvarez-Legorreta et al. 2008). However, the mechanisms of metal absorption by seagrass leaves are not well known, and this is a complex subject that deserves further detailed research (Slaveykova and Wilkinson 2005).

In terms of the metal concentrations in root-rhizomes (Fig. 5.), the sampling sites were separated only in two groups (A and B) in which metal concentration differ significantly: Group A, where the lowest metal concentration was found and B where the highest metal levels were grouped. Group B corresponds to all the groundwater-influenced sites (S5, S6, and S7), which exhibited significantly higher cadmium content than the rest of the sites. This suggests that cadmium has been reaching Yalahau through groundwater for a longer time than the lifespan of the leaves.

In the case of chromium, although there are also significant differences amongst the sites, all the

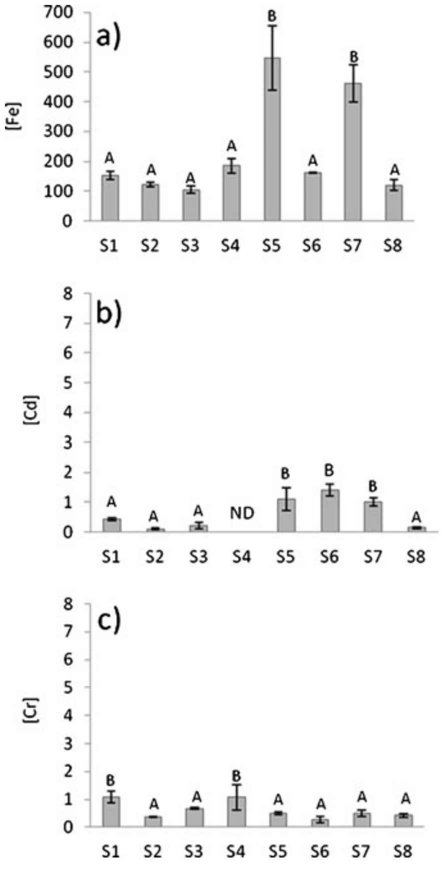

Fig. 5 **a** Iron, **b** cadmium, and **c** chromium content in root-rhizome systems of *T. testudinum* at eight stations sampled during January 2005 at Yalahau Lagoon. Values in micrograms per gram dry weight ±SD. *Columns* with the *same label* (**a**, **b**, or **c**) do not differ significantly (p<0.05) according to the Fisher's multiple comparison tests. *ND*: not detected with the applied method. Detection limit: Fe 0.1 ppm, Cd 0.5 ppb, and Cr 0.4 ppb

concentrations are relatively similar and low. We therefore do not attribute these differences to pollution but rather to natural variations and or measurement error.

Seasonal variation of metals' concentration

In order to analyze the seasonal variation of heavy metal concentration, a comparison of previous results with those from samples collected during June (dry season) was made. Due to the water conditions (turbidity), during the dry season, it was possible to collect samples only at three sites, which fortunately represent each of the three different groups: S2 influenced by the local input, S7 with groundwater influence, and S4 from the group with low anthropogenic influence. The overall seasonal variation is analyzed using the average values from these three sites (Table 3). The high positive changes of Table 3 for iron and cadmium indicate that their concentrations increased substantially in the rainy season. Such an increase is consistent with the two pollution sources here identified, since both dump runoff and groundwater input increase during the rainy season. In the case of chromium, although the small negative change suggests it, on the average, might have decreased during the rainy season, the stations showed opposites trends, so it is likely that natural variations and or measurement error do not allow the identification of a temporal trend.

The local seasonal variation was analyzed with the data reported in Figs. 6 and 7. Iron concentrations were significantly ($t$test, $p<0.05$) higher during the rainy season at all the sites in both leaves and root-rhizomes of *T. testudinum* (Figs. 6a and 7a). The leaf tissue had relatively consistent values, near to 60 ppm, during the dry season, and close to 110 ppm during the rainy season. Samples from S7 showed the highest iron concentration in the root-rhizome tissue but remained close to the average value in the leaf tissue. Duarte et al. (1995) reported the available data on iron concentration in seagrass tissues and showed that iron concentrations in *T. testudinum* growing on carbonate sediments in the Yucatán Peninsula are frequently below critical levels (<100 ppm iron) for angiosperms. Our data support that the seagrasses in this sediment are likely to experience iron deficiency only during the dry season.

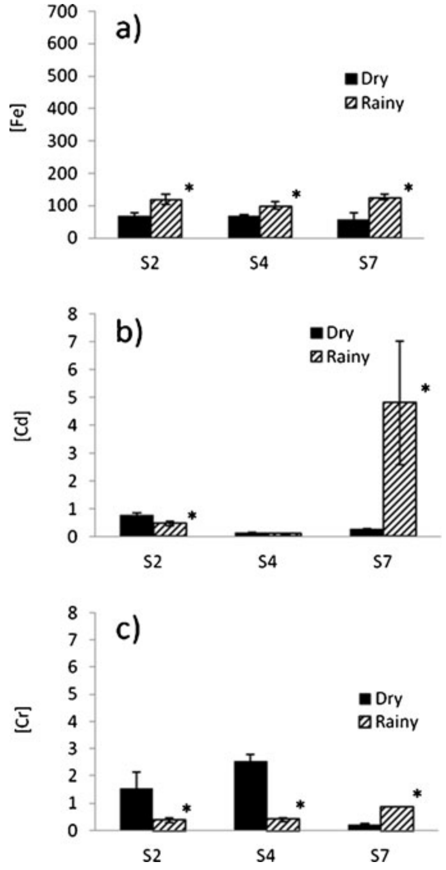

Fig. 6 **a** Iron, **b** cadmium, and **c** chromium content (micrograms per gram dry weight) in leaves of *T. testudinum* at three stations in Yalahau Lagoon. *DRY* season (June, 2004) and *RAINY* season (January, 2005). Values in micrograms per gram dry weight ±SD. *Asterisk* indicates significant differences between seasons ($p<0.05$). Detection limit: Fe 0.1 ppm, Cd 0.5 ppb, and Cr 0.4 ppb

Table 3 Mean seasonal variations in morphological units of *T. testudinum* in the Yalahau Lagoon, Holbox, Quintana Roo

	Dry season (June 2004)	Rainy season (January 2005)	% Change (January–June)
Leaf			
Fe	63.7	115.4	81
Cd	0.4	1.8	368
Cr	1.4	0.6	−62
Root/rhizome			
Fe	80.0	256.5	221
Cd	0.3	0.4	32
Cr	1.1	0.7	−38

Values in micrograms per gram dry weight. These results are average values of three locations (S2, S4, and S7). The percent change is calculated from the difference between January and June values divided by the June values times 100

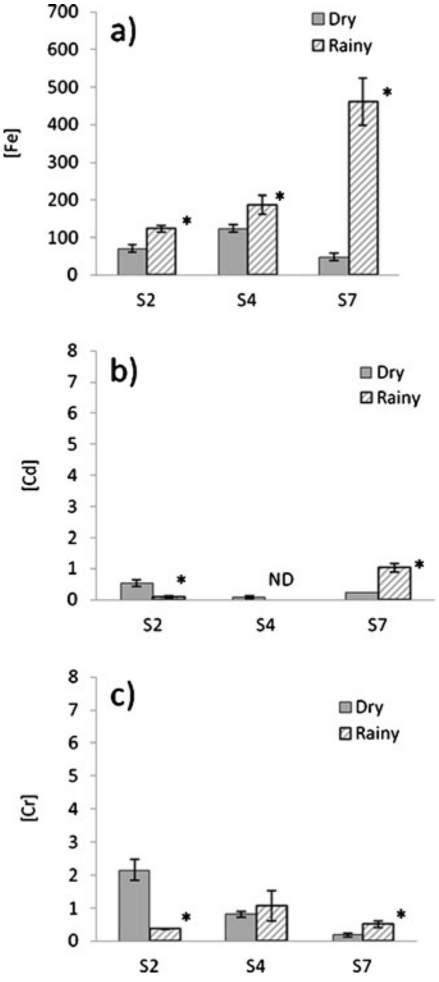

Fig. 7 **a** Iron, **b** cadmium, and **c** chromium content (micrograms per gram dry weight) in root-rhizome system of *T. testudinum* at three stations in Yalahau Lagoon. *DRY* season (June 2004) and *RAINY* season (January 2005) Values in micrograms per gram dry weight ±SD. *Asterisk* indicates significant differences between seasons ($p<0.05$). *ND*: not detected with applied method. Detection limit: Fe 0.1 ppm, Cd 0.5 ppb, and Cr 0.4 ppb

The local seasonal variations were also significantly (*t*test, $p<0.05$) different for the concentration of cadmium in leaves and root-rhizomes, except for tissues form site S4 where the concentration is very small (Figs. 6b and 7b). The results for cadmium in leaves and root-rhizomes of *T. testudinum* indicated that the concentration of this metal decreased in the rainy season, except at S7 where it increased dramatically (Mann–Whitney *U* test, $p<0.05$). As was discussed previously, groundwater input is likely responsible for the increment of the amount of cadmium in this sampling site. If this is so, groundwater could be the principal source of cadmium to the Yalahau Lagoon. Concerning other sites, S2 is close to the local input that comes from the town dump. Batteries are present in the town dump, and the runoff probably transports the metals into the seawater. This is likely the reason why the concentration at S2 is higher than at S4.

For chromium, although the differences between seasons were also significant at most sites (Figs. 6c and 7c), in some samples (leaves from S2 and S4), the concentration decreased during the rainy season, while it showed an increment in samples from other sites (leaves, S7; root-rhizomes, S4 and S7). These results agree with the idea that there is no extra chromium in this region, and only natural variations are observed.

Comparison with other polluted areas

There are scant results in the literature concerning the concentration of iron, cadmium, and chromium in *T. testudinum*. For iron, Duarte et al. (1995) reported values from the Caribbean and the Gulf of Mexico, and for the Lower Laguna Madre, Texas, USA, and the Mexican Caribbean, values were reported by Whelan et al. (2005, 2011, respectively). Table 4 provides a comparison between the results of this work and others previously reported. In this table, Group A represents the average of metal level of the group of sites with the lowest concentration in the Yalahau Lagoon, Group B the mean of the group of sites with the highest metal concentration, and only for cadmium was there a third (significantly different from the other two) group of sites with intermediate values. The sites within each group were not significantly different from each other (Fig. 4, Fisher's test $p<0.05$). Groups A, B, and C differ significantly between them.

In the case of iron, our low values (Group A with lower groundwater input) are relatively consistent with those reported for karstic locations (e.g., Duarte et al. 1995; Whelan et al. 2011). In contrast, at the sampling sites of Group B, where silicate evidences a direct influence of groundwater, the iron concentrations we found are above most of those summarized in Table 4 and similar to the highest values found even in terrigenous environment. This is a surprising result for a karstic environment, where there are no known natural sources of iron (Duarte et al. 1995). If, as our results suggest, the iron is reaching Yalahau Lagoon through groundwater input, the source of this metal could be far away from the lagoon, and likely outside the reserve. We know that station 2 is very close to a local point

Table 4 Heavy metal concentrations (values in micrograms per gram dry weight) in *T. testudinum*

Location	Geologic setting	Fe	Cd	Cr	Reference
T. testudinum leaves					
Southern Gulf of Mexico	Terrigenous	71–533	–	–	Duarte et al. 1995
Laguna Madre, Texas	Terrigenous	169–287	–	–	Whelan et al. 2005
Guayanilla Bay, Puerto Rico	Terrigenous/karstic	106	1.3	–	Schroeder and Thorhaug 1980
Mexican Caribbean	Karstic	62.5–80.6	–	–	Duarte et al. 1995
Mexican Caribbean	Karstic	22.7–47.5	–	0.4–0.5	Whelan et al. 2011
Florida, USA	Karstic	–	0.1–1.2	5	Lewis et al. 2007
Yalahau Lagoon	Karstic				
	Group A	119.0	0.2	0.4	This study
	Group B	445.7	5.0	0.7	
	Group C		1.8		
T. testudinum root-rhizomes					
Laguna Madre, Texas	Terrigenous	113–418	–	–	Whelan et al. 2005
Guayanilla Bay, Puerto Rico	Terrigenous /karstic	132.7–622.5	0.8–1.8	–	Schroeder and Thorhaug 1980
Mexican Caribbean	Karstic	20.3–40.8	–	0.4–0.7	Whelan et al. 2011
Florida, USA	Karstic	–	0.1–1.3	5	Lewis et al. 2007
Yalahau Lagoon	Karstic				
	Group A	141.4	0.2	0.5	This study
	Group B	504.3	1.2	1.1	

Group A: average of the group of sites with lowest concentrations, Group B: average of the group of sites with highest concentrations, Group C: average of the group of sites with intermediate (but significantly different from the other two groups) concentrations. Letters A, B, and C depict groups of stations significantly different. Other results reported before are included for comparison

discharge of wastewater from Holbox town, and therefore, organic pollution is expected. At the same time, water exchange is high at station 2 because it is near to the sea inlet, and therefore, pollutants dilution and flushing are probably high there. At the same time, Fe might not necessarily be high in wastewater from the local towns in Holbox because there are no automobiles (nor any industries that might produce important iron wastes) on the island. In any case, our data show Fe concentrations at station 2 were not elevated at least at the moment we sampled. We trust our Fe results because they are consistent with the levels previously found in seagrasses under Fe limitation in the area (i.e., Duarte et al. 1995) and higher in the area with groundwater discharge. Fe in groundwater is expected to be higher, either because it is derived from the same natural source that provides the highs Si levels or from regional groundwater pollution from the junkyards of bigger cities in the continental Quintana Roo. It is important that research is conducted to identify this source and its nature. Although iron is an essential element (Whelan et al. 2005) and its high concentrations do not represent a pollution problem itself, it is important to identify how the iron is reaching the groundwater, because other, more threatening pollutants could also be arriving through the same route.

Although the complex structure of flooded cave systems and hydrological conducts in Yucatán is not well known yet (Smart et al. 2006), there are indications of the regional groundwater flow patterns (Perry et al. 2002; Escolero-Fuentes 2007; Bauer-Gottwein et al. 2011). In the northeast coast of Yucatán, many submarine springs are found in the back reef lagoons, and a general west–east groundwater flow toward the coast is likely important. However, a complex of fractures (the Holbox Fracture System) runs in a south–north direction and is thought to channel groundwater in this direction, toward the Yalahau Lagoon (Perry et al. 2002). Our results support this possibility and outline the need of detailed studies on groundwater flow to identify the source of the high Fe and Cd concentrations we found.

For cadmium and chromium (Table 4), the levels are relatively consistent with those reported previously by other authors. In leaves, our values of cadmium in sampling sites of groups B and C (that represent pollution from the groundwater and the local input) range from 1.4 to 5.1 μg/g. The comparison with previous reports indicates that these values are higher than those found in Puerto Rico (Schroeder and Thorhaug 1980) and Florida (Lewis et al. 2007). The very high concentration of cadmium that was found at S7 (5.1 μg/g) during 2005 (Fig. 6b) is a remarkable result that merits further studies, since it indicates that groundwater input could be the source of this contaminant.

Coastal marine pollution could be a potential problem in the Yalahau Lagoon. The seasonal and spatial patterns found in this work appear to be more influenced by environmental events in a regional scale, such as discharges to the coast due to the polluted groundwater that cause an increment in metals' concentration in sites near to a discharge area. Other factors that have a less impact in the seasonal and spatial variations are local inputs of pollution such as the municipal dump.

Conclusions

1. The silicate concentration pattern found supports that groundwater discharges are important in Yalahau lagoon, and particularly in the area around sites S5, S6, and S7, which exhibit the highest silicate concentrations. The year-round dominance of groundwater input over rainfall and surface runoffs at Yalahau is supported by the permanence of this pattern during both the dry and rainy seasons.
2. Iron concentrations found in *T. testudinum* growing in most of Yalahau Lagoon were relatively low (~100 μg/g), as found for the region and other karstic areas. In contrast, significantly higher iron concentrations (>400 μg/g), as high as in terrigenous settings, were observed in the area of greatest groundwater input. It is important to determine the nature of source for this iron.
3. The high cadmium concentrations (up to 5 μg/g) found in *T. testudinum* of Yalahau lagoon suggest pollution from the small dump of the town of Holbox and also from the groundwater input. Chromium concentrations (~1 μg/g) remained near background levels and apparently do not indicate pollution by this metal yet.
4. Our results support the utility of *T. testudinum* as a biomonitor for trace metals in contaminated sites. They also show metal pollution in a protected area, where local population and development are small. Most of the pollution seems to come through groundwater, supporting that reserves in karstic areas can be threatened by development in distant areas.

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