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Trace metals in the seagrass *Thalassia testudinum* from the Mexican Caribbean coast†

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The impact of human activities on Mexican Caribbean zones is reflected in the partial or total destruction of these habitats and the increasing settlement of urban and tourist resorts. As a consequence, waste water discharged to sea increases the levels of chemical elements and foreign substances in the marine environment. In order to accomplish an adequate management and conservation of the coastal ecosystems, it is necessary to make an adequate diagnosis of the situation through factors that may monitor the vulnerability as well as the level of damage of the aquatic communities. In this work we present results on the use of seagrass *Thalassia testudinum*, as a potential bio-indicator of metal pollution in Caribbean seawater. Seagrass was collected in Holbox and Puerto Morelos (considered as mildly influenced by human activities) in the State of Quintana Roo. Trace metals were determined by particle induced x-ray emission (PIXE) and atomic absorption (AA). Results are reported for metals such as Fe, Mn, Cu, Zn, Pb, and Cd. The metal distribution on the different parts of the plant, the differences between two sites, and the effect of season are discussed. Copyright © 2008 John Wiley & Sons, Ltd.

INTRODUCTION

The Mexican Caribbean coast represents an important amount and diversity of natural resources.[1] In order to accomplish an adequate management and conservation of these coastal ecosystems, it is necessary to make an adequate diagnosis of the actual situation through factors that may monitor their vulnerability with regard to an increased settlement of urban and tourist resorts. In this work we present results on the use of *Thalassia testudinum*, the most common seagrass in the Mexican Caribbean Sea, as a potential bio-indicator of the actual levels of trace metals in coastal water. Seagrasses take up metals from both water through their leaves and from sediment through their roots. Any increase in the metal concentration may be reflected in a higher uptake.[2]

Particle induced x-ray emission (PIXE) was used to determine background levels of total trace metals in Mexican Caribbean seagrasses. By its multielemental characteristics PIXE has the advantage of monitoring essential and toxic trace metals as a function of external conditions such as rain, geographical location, wastewater discharge, etc. PIXE also offers reasonably good detection limits for most trace metals and precise as well as fast analyses, which allow the management of a large number of samples. Trace elements detected include Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Br, Rb, Sr, Cd, and Pb. From the detected trace metals, we focused our attention on some metals determined in previous studies, such as Fe, Mn, Cu, Zn, Pb, and Cd. The first four elements are considered essential elements for life while the last two (Pb and Cd) are toxic metals.

Data about levels of metals in seagrasses from tropical coasts are scarce. Therefore since no previous data are available from the Mexican Caribbean coast, the trace metal levels obtained in this study are compared with those published for samples of *T. testudinum* collected from the coastal waters of the Gulf of Mexico.[3–5] The metal distribution on the different parts of the plant as well as the effect of season was followed for metals such as Fe, Mn, Cu, Zn, Pb, and Cd. We present here the differences between two sites and the effect of season as well as comparison with published data.

MATERIAL AND METHODS

Sampling sites

T. testudinum is distributed from Florida in the Gulf of Mexico through the Caribbean, the Bermudas, Central America, and Venezuela.[1] It is the most common seagrass among eight species growing in the estuaries and reefs from the Caribbean Sea. Plants were collected from two towns dedicated to fishing located in the state of Quintana Roo in the Yucatan Peninsula: Holbox and Puerto Morelos (Fig. 1). Holbox (N 21° 29.291′W 087°15.776′) is a small island (1800 inhabitants) located in the limits of the Gulf of Mexico and Caribbean Sea. It belongs to the park *Yum Balam*, a natural protected area since 1994. Puerto Morelos (N 20°50′50.5″ W 86°52′30.6″), with 1500 residents, is located 18 km south of Cancun, in *Parque Nacional de la región Arrecife de Puerto Morelos*. This area belongs to the Mesoamerican

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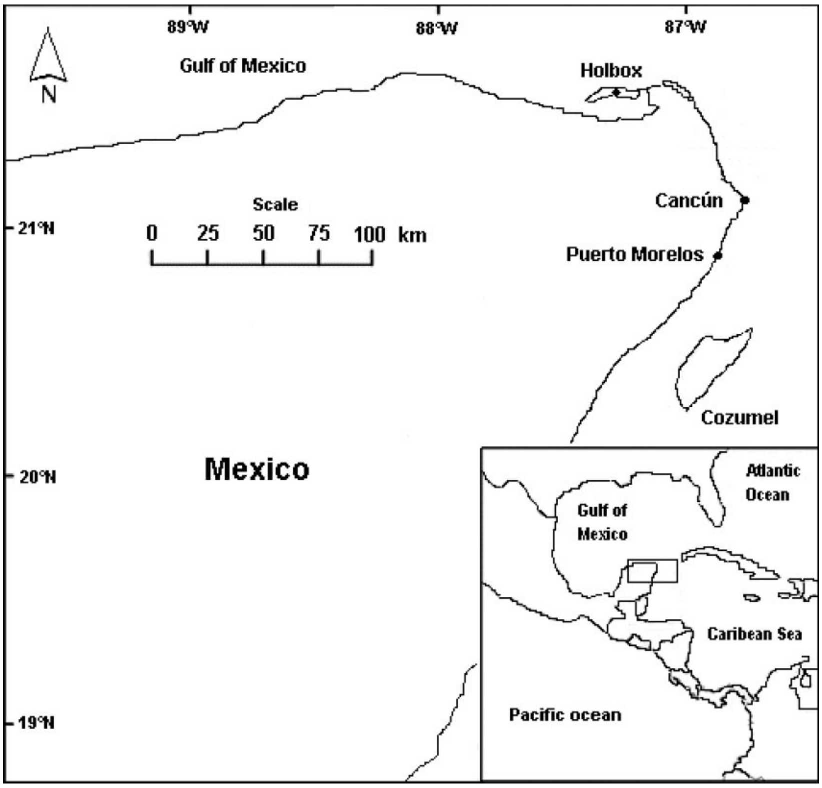

Figure 1. Sampling sites on the Mexican Caribbean coast: Holbox and Puerto Morelos.

Table 1. Trace metal concentration ranges (in µg/g dry weight) in *T. testudinum* leaves collected from Holbox and P. Morelos in July 2004

Element	Holbox	Puerto Morelos
Ti	11–13	1–9
V	3–7	1–2
Cr[a]	Nd–4.5	Nd
Mn	25–30	7–20
Fe	89–500	195–380
Ni	3–4	3–13
Cu	4–7	11–25
Zn	31–34	13–22
As	Nd–3	Nd–3
Br	300–600	330–600
Rb	18–29	4–13
Sr	200–600	220–720
Cd[a]	Nd–1	Nd -0.5

[a] Determined by AA. Nd, not detected.

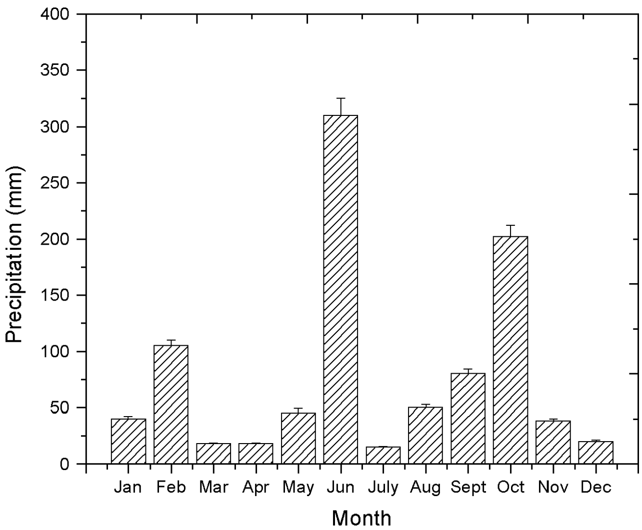

Figure 2. Rain pattern in Puerto Morelos during 2004.

Barrier Reef in the Caribbean Sea. No industrial activities that could strongly affect the environment are carried out in both areas. Domestic and groundwater discharges are the main continental sources of metals to the coastal waters.[6]

Collection was performed several times throughout the year 2004. Seagrasses from Yalahau Lagoon in Holbox were collected in January, April, July, and October; and those from Puerto Morelos were collected in January and July. Samples (approximately 50 g) were collected manually. The samples grew at shallow depths (0.5 m) and only occasionally were found at 2–3 m depth. Plants were kept in plastic bags and washed thoroughly with tap water and rinsed with deionized water at the laboratory (removing the epiphytes attached to the surface). Plant parts were separated, dried in a stove, ground to a fine powder, and pressed into pellets.

PIXE analysis

Total trace metals of pellets were determined using PIXE. Three replicates were prepared and analyzed from each sample. Analysis by PIXE was performed with an external beam setup at the 3 MV9SDH NEC Pelletron accelerator (IF-UNAM), with a 3-MeV proton beam for the primarily radiation.[7] A Canberra LEGe detector was used to measure heavy metal content. Calibration of the detection system was

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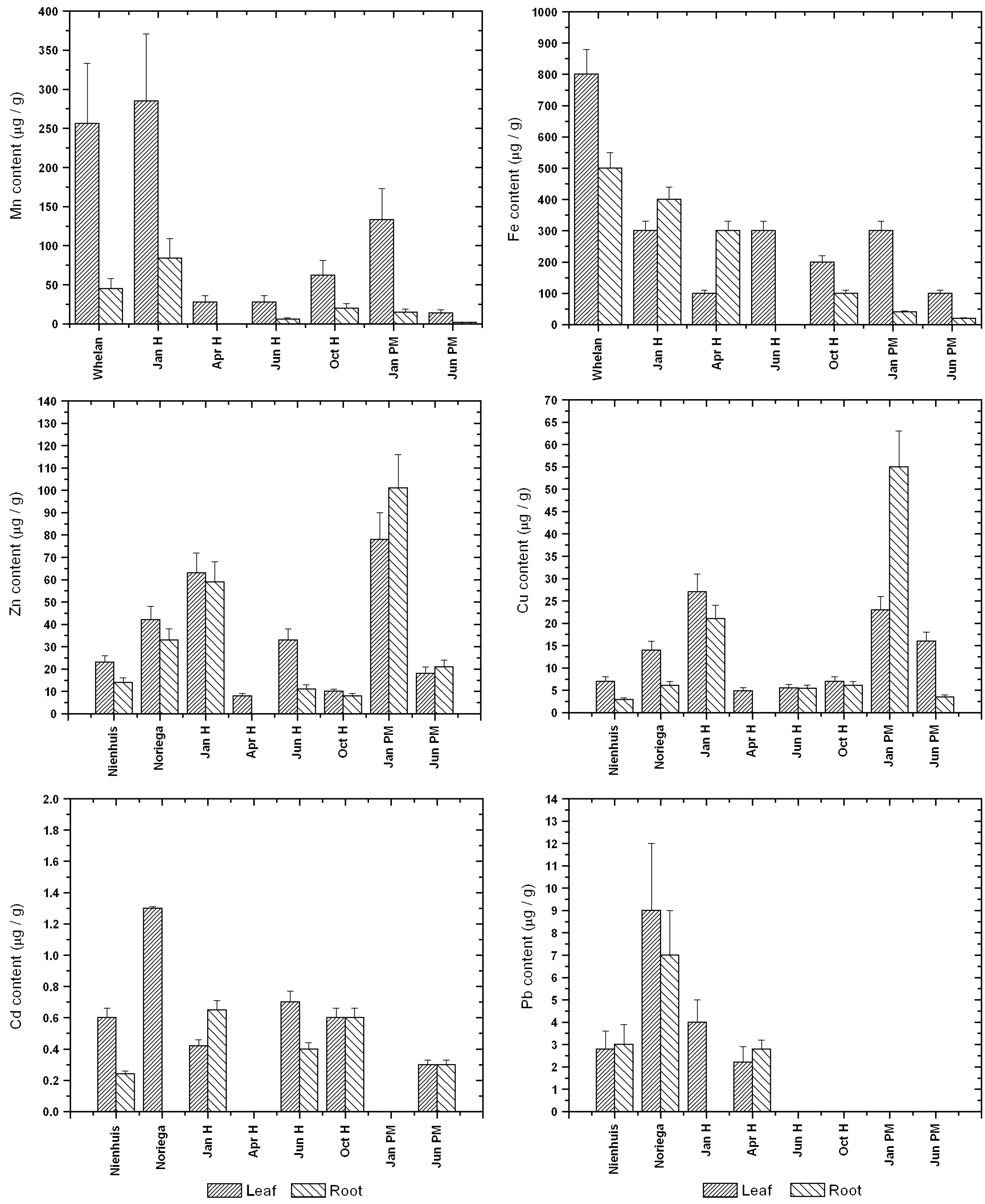

Figure 3. Concentration of metals (μg/g dry weight) in leaves and roots of *Thalassia testudinum*. Seagrasses from Holbox were collected in January, April, July, and October 2004; and those from Puerto Morelos were collected in January and July 2004. Cadmium and Lead were determined by AA.

carried out with pellets of reference material Peach Leaves NIST 1547a. The computer code GUPIX was used to obtain quantitative results.[8] PIXE analyses of trace metals were complemented by atomic absorption (AA) analysis (using a graphite furnace and flame) for those elements close to or under PIXE limit of detection (LOD). AA was performed with a Perkin-Elmer Model 31 10 equipment.

RESULTS AND DISCUSSION

In the analysis of seagrasses, up to 13 trace elements were typically determined. From them, 11 elements were detected by PIXE in a short time. This fact shows the capabilities of this technique for multielemental determination in environmental samples without extensive sample preparation. Table 1

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shows the concentration ranges of trace elements observed in the leaves of *T. testudinum* collected from Holbox and Puerto Morelos in July 2004. These results show that the highest accumulation was for Br, Fe, Sr, Mn, Zn, Rb, and Ti followed by V, Cu, Cr, Ni, As, and Cd. Toxic metals such as As, Pb, and Cd were only occasionally found. For the above elements, six elements (four essentials and two toxic elements) were chosen in order to study the temporal and spatial variation.

The Yucatan Peninsula has scarce nutrient sources since this is a calcareous zone. Temperature varies a few degrees (2–3 °C) throughout the year. Seagrass growth depends mainly on the intake of nutrients from rain and rain filtration through the karstic type of soil, since there are very few rivers in the area.[6] Climate can be described in terms of three seasons: the rainy season from June to October, Nortes (Northern winds) from November to February, and the dry season from March to May. Hurricanes occur from August to September.[9]

Figure 2 shows the annual pattern of rain in Puerto Morelos for 2004 (kindly provided by Dr Jorge Herrera-Silveira). The rain effect on the growth of seagrasses in terms of nutrition is not observed immediately, since the leaves of *T. testudinum* grow in 3–4 months. Therefore for example, leaves collected in October would have grown from May or June (rainy season) while leaves collected in July correspond to the dry season. Figure 3 shows the metal concentrations of the selected essential elements (Mn, Fe, Cu, and Zn) and the toxic elements (Cd and Pb) in the leaves and roots of *T. testudinum* from Holbox and Puerto Morelos. For comparison purposes, the graphs include published results for *T. testudinum* collected in different sites from the Gulf of Mexico.[3–5] Whelan *et al*.[5] concluded that their study area (Laguna Madre in Texas) is a nonpolluted zone and reported that *T. testudinum* absorbs heavy metals, depending on the season, metal type, temperature, and light exposure.

Figure 3 shows that in general, the metals studied accumulate in seagrasses in a similar way in both sites. Essential metals show the highest levels in seagrasses in January. These observations apply to leaves and roots as well. These results reflect the higher nutrient input in the rain at the end of the previous year.

Toxic metals Cd and Pb were under or close to PIXE LOD, respectively; therefore analyses of these elements were performed by AA. With these techniques, these elements were not detected in all samples. When measured, Cd and Pb levels were similar in leaves and roots, showing that Pb is absorbed in leaves as well as in roots as previously reported.[10]

When the six elements are compared to published data, some differences appear: Mn is lower in seagrasses from the Caribbean compared to published data for the Gulf. It is more concentrated in leaves than in roots indicating that this metal is absorbed by leaves more than by roots, in agreement with previous reports.[5] Iron levels are lower in Caribbean seagrasses compared to published data for those from the Gulf of Mexico. This is not surprising if we consider that the Yucatan peninsula is a calcareous region, where sediments are low in Fe compared to the Gulf of Mexico.[1] Average Zn levels are similar to those published for seagrasses from unpolluted zones in the Gulf of Mexico. Seagrasses collected in January show high Cu levels compared to those published for samples collected from unpolluted zones in the Gulf of Mexico, but the samples from the other months show similar values. Average levels of Cd and Pb are similar to those published for unpolluted zones.

CONCLUSIONS

Plants of *T. testudinum* growing in the coastal areas of Holbox and Puerto Morelos showed very low total metal levels. Average values are in the range of data published for *T. testudinum* from unpolluted areas from the Gulf of Mexico. It can be concluded that the population activities have not resulted in an increase of the metal levels in the area. Toxic metals such as Cd and Pb, were detected only in some of the samples, at low levels. Since these ecosystems are actually in good conditions in terms of heavy metals, it is possible to take preventive actions in order to preserve these areas. PIXE showed to be an adequate technique to study temporal and spatial variations of essential metals with no chemical treatment of samples. However, the toxic elements analyzed are present in very low concentrations, which are below or close to the LOD. In future studies of unpolluted zones, it is desirable to reduce the LOD by combining PIXE analysis with chemical treatment to increase the sensitivity.

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