Trace metal analysis in sea grasses from Mexican Caribbean Coast by particle induced X-ray emission (PIXE)

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The growing urban and tourist activity in the Mexican Caribbean coasts has resulted in an increase of chemical substances, metals in particular, discharged to the coastal waters. In order to reach an adequate management and conservation of these marine ecosystems it is necessary to perform an inventory of the actual conditions that reflect the vulnerability and the level of damage. Sea-grasses are considered good biological indicators of heavy metal contamination in marine systems. The goal of this preliminary work is to evaluate the concentrations of trace metals such as Cr, Mn, Fe, Co, Cu, Zn, and Pb in *Thalassia testudinum,* a very common sea-grass in the Mexican Caribbean Sea. Samples were collected from several locations in the coasts of the Yucatán Peninsula: Holbox, Blanquizal and Punta Allen, areas virtually uninfluenced by anthropogenic activities. Trace elements in different part plants were determined by particle induced X-ray emission (PIXE). This is a very suitable technique since it offers a fast, accurate and multi-element analysis. Also, the analysis by PIXE can be performed directly on powdered leaves without a laborious sample preparation. The trace metal concentration determined in sea-grasses growing in Caribbean generally fall in the range of the lowest valuables reported for sea grasses from the Gulf of Mexico. The results indicate that the studied areas do not present contamination by heavy metals.

Keywords: Trace metals; seagrass; biomonitors; PIXE.

El crecimiento de las actividades urbanas y turísticas en las costas del Caribe mexicano ha resultado en un incremento de las sustancias químicas, metales en particular y descargas en las aguas costeras. Con el objetivo de practicar un adecuado manejo y conservación de estos ecosistemas marinos es necesario levantar un inventario de las condiciones que refleje la vulnerabilidad y el nivel de daño. Los pastos marinos son considerados buenos indicadores biológicos de contaminación por metales pesados en sistemas marinos. El objetivo de este trabajo preliminar es evaluar la concentración de los metales trazas Cr, Mn, Fe, Co, Cu, Zn, y Pb en la *Thalassia testudinum,* un pasto marino muy común en el mar Caribe mexicano. Las muestras fueron colectadas en varias localidades de las costas de la Península de Yucatán: Holbox, Blanquizal y Punta Allen, áreas que están virtualmente influenciadas por actividad antropogénica. Se determinó la concentración de los elementos trazas estudiados en diferentes partes de la planta por la técnica de emisión de rayos X inducida por partículas (PIXE). Esta es una técnica muy ventajosa pues puede llevarse a cabo directamente en la muestra macerada sin una laboriosa preparación de la misma. Los valores de las concentraciones de los metales trazas determinadas en los pastos marinos que crecen en el Caribe son generalmente las menores reportadas para pastos marinos en el Golfo de México. Estos resultados indican que el área estudiada no presenta contaminación debida a metales pesados.

Descriptores: Elementos Traza; pastos marinos; monitores biológicos; PIXE.

PACS: 32.30.Rj; 07.88+I; 41.75.-i

1. Introduction

The increased anthropogenic activities in Mexican Caribbean Coasts represent a threat to coastal ecosystems [1]. At present it is necessary to find equilibrium between economical progress and environmental protection. In order to achieve this goal, political actions for a sustainable growth such as the use of renewable sources, recycling, waste water treatment, etc., have to be reinforced. The study of the ecosystems and their natural variations, or as a result of anthropogenic activities are substantially based in the analytical work, i.e., in the monitoring of the possible changes of the environment and in the development of new analytical techniques. In this work we applied the particle induced X-ray emission (PIXE) to evaluate the concentrations of trace metals such as Cr, Mn, Fe, Co, Cu, Zn and Pb in *Thalassia testudinum,* one of the most common sea-grasses in the Mexican Caribbean Sea (Fig. 1). The study was performed in several locations of the Yucatan peninsula considered non-contaminated areas, in order to establish the basal levels (in absence of pollution) that can serve as a basis for a later comparison of new levels and to detect any change indicating a possible new incorporation or increase of one or several heavy metals [2].

2. Biological monitoring using sea-grasses

The use of living organisms as monitors of sea water pollution by heavy metals has generated a great interest among various scientific disciplines. Several aquatic organisms are frequently used as biomonitores: fish, mollusks, aquatic animals, sea plants and algae. Sea plants such as sea-grasses in particular, are interesting biomonitores, since they take up

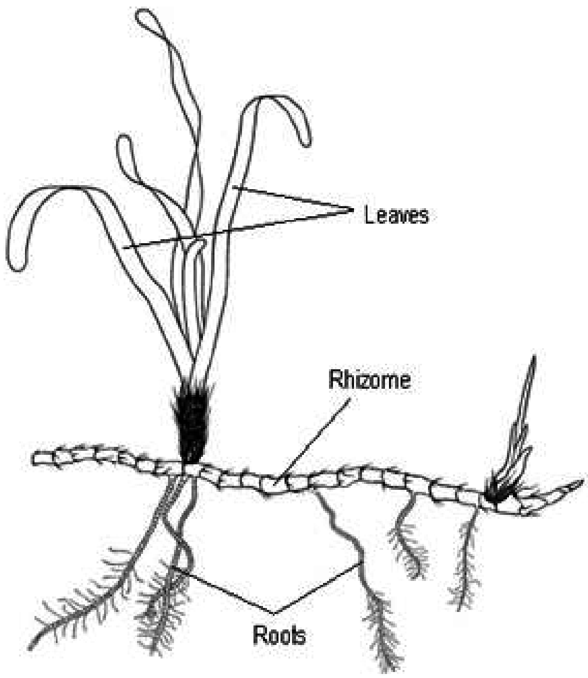

FIGURE 1. Morphological features of sea-grasses.

metals from water through their leaves and from sediment through their roots (Fig. 1) [3]. Therefore, any increase in the metal concentration of the surrounding water will be reflected in a higher uptake by the plant. Sea-grasses have been studied as biomarkers for trace metal contamination in several locations in the world; however there are few studies in tropical zones such as Mexican Caribbean coasts.

3. Particle induced X- Ray Emission

Since its discovery at the Lund Institute of Technology in 1970, PIXE has been used in a large number of environmental analyses [4]. It is nondestructive and because its multi-elemental characteristics is very useful in environmental samples. For PIXE analysis, samples are bombarded with particles (generally protons of 1 to 4 MeV) generated in small accelerators (Fig. 2). When particles interact with samples, inner shells of atoms from the sample are ejected as they acquire energy from the incident particle (ionization). Outer shell electrons then fill vacancies in the inner shell, emitting X-rays characteristic of the target elements emitting the X-rays. The PIXE spectra contain a series of X-ray peaks due to the K, L, M shell transitions. The X-ray production probability is very high for heavy elements and low for light elements. The concentration of the elements is determined from the intensity of X-ray emission peaks using spectral analysis programs. This technique is very suitable for sea-grass analysis; since no laborious preparation is needed and fast analysis can be performed (measurement takes 5 to 10 minutes). The detection limits (LOD) are of μg/g, with low variation from one element to another. Also the amount required for the analysis is low (less than 1 mg) and the technique has a good precision. Also, PIXE is a multi-elemental technique, allowing the detection of all elements from the periodic table with atomic number between 10 and 92 [4]. It is very sensitive to detect essential metals such as Zn, Cu, Ni, and Cr. Toxic metals such as Pb, Cd and Hg can also be detected by PIXE but generally the concentrations of these elements in seagrasses are under 10 μg/g (PIXE LOD). This may introduce high relative errors to the measurements.

4. Sample collection and preparation

Eight sea-grass species grow in Mexican Caribbean Sea being *Thalassia testudinum* the most common. These are flower producing plants that grow in extended areas in shallow waters. Samples were collected from three uncontaminated national reserves located in Yucatán Península in Mexico: Holbox form the reserve of "Yum Balam", and Blanquizal and Punta Allen from the reserve of "Sian Ka'an" (Fig. 3). Seagrasses from Holbox were collected from the Yalahau Lake (Fig. 3) in April, June and October 2005; samples from Blanquizal and Punta Allen were collected in April 2005. The rainy season is considered from June to October and the dry season from November to May. Samples were collected manually (approximately 50 g). Plants were growing at shallow depths (0.5m) and only occasionally were found at 2 to 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, pulverized and compressed into pellets.

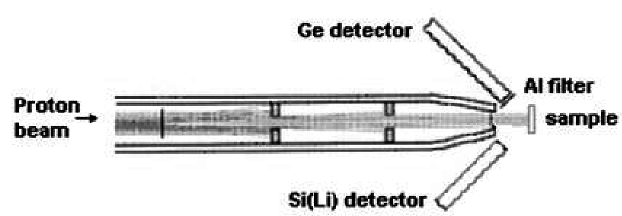

FIGURE 2. PIXE set-up for external analysis.

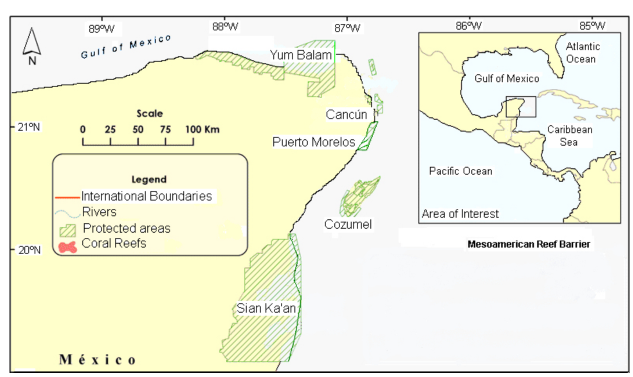

FIGURE 3. Yucatan Peninsula map showing the National Protected Areas of Yum Balam and Sian Kaan.

5. PIXE Analysis

Analysis of the pellets by PIXE was performed with an external beam setup at the 3 MV9SDH NEC Pelletron accelerator (Institute of Physics UNAM), with a 3 MeV proton beam for the primarily radiation [5]. A Canberra LEGe detector was used to measure heavy metal content. Calibration of the detection system was carried out with pellets of reference material Peach Leaves NIST 1547a. The computer code GUPIX was used to obtain quantitative results [6].

6. Results

Figure 4 shows the average content of 10 trace elements detected in different part plants of *T. testudinum* collected from Holbox, Blanquizal and Punta Allen in 2005. The highest ranked was for Br, Fe and Sr and Fe followed by Mn, Zn, Rb, and, Cu, Cr, Ni, Pb and Co. The concentration levels were similar for sea-grasses collected from the three study sites. There was not a clear difference between concentration levels in different plants parts. In general plants of *T. testudinum* collected from the three study sites showed very low total metal levels. When trace elements such as Mn, Ni, Cu and Zn are compared to corresponding data published by several authors (Table I)[7-11], average values obtained in this study are in the range of the lowest values published for *T. testudinum* from the Gulf of Mexico. Toxic metals such as Cr and Pb were detected in some replicates of the collected samples at levels similar to the highest levels reported for samples collected in the Gulf of Mexico (Table I). However, as stated in the PIXE analysis section, the limits of detection with PIXE for these elements are higher than or similar to the measured levels, introducing high errors in the measurements, and the shown results must be interpreted with caution.

TABLE I. Published showing of the concentration values (in μg/g) for some trace metals in Leaves (L) and Roots (R) of *T. testudinum*.

Site	Cr	Mn	Ni	Cu	Zn	Pb	Reference
Gulf of L				4.9	22		
Mexico R				4.3	29		Pulish, 1980
United							Montgomery
States L	8		4.5	25	7.0	6	& Price,1979
Gulf of L			11	13	18		
Mexico R			8	20	21		Botello 1996
Gulf of L			7.7	2.8	41.6	8.7	Noriega,
Mexico R			2.2	1.4	32.8	7.5	2001
Gulf of L		95-256		7-12.1	24.6	0.81-1.21	Whelan III *et*
Mexico R		21.0-45		5.0-8.0	24.1-23.4	0.75-1.2	*al.*, 2005

Total trace metal levels in leaves collected in April, June and October from Holbox are shown in Fig. 5. It is known that the rain effect in terms of nutrition is not observed immediately, since *T. testudinum* leaves grow in 3 to 4 months. Therefore we can consider that leaves collected in April grew during the rainy and dry season, leaves collected in June grew mostly during the dry season and leaves collected in October grew during the rainy season. In general trace metal levels are very similar, but except for Zn, a slight increase in the element content can be observed in leaves collected during October. These results may indicate a higher nutrient input by the rain.

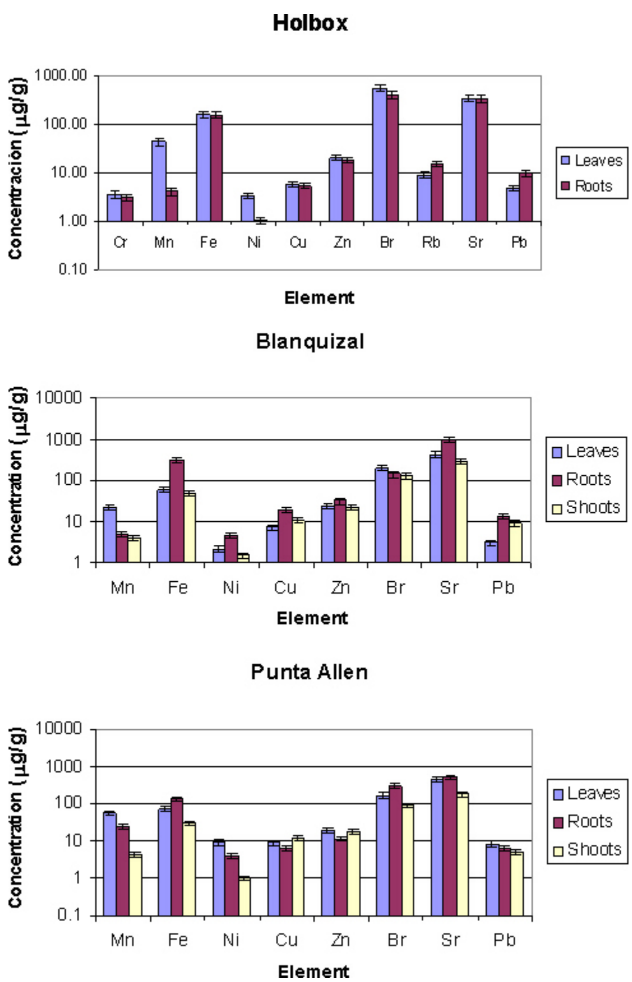

FIGURE 4. Top: Average trace metal concentration in leaves, roots and shoots of *Thalassia testudinum* collected in may 2005 en Holbox ($n = 3$), center: Blanquizal (Sian Kaan reserve, march 2005) and bottom: Punta Allen (march 2005).

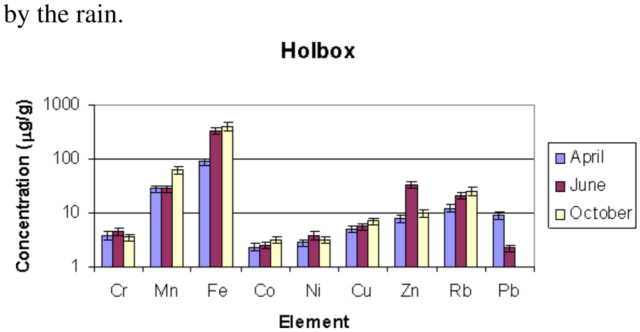

FIGURE 5. Average trace metal concentration in leaves collected in April, June and October 2005 in Holbox ($n = 3$).

7. Conclusions

Trace metal levels were low in most of the sampling sites from the Mexican Caribbean coasts. The metal concentrations generally fall in the range of the lowest valuables reported for *T. testudinum* collected from the Gulf of Mexico. Levels found for trace metals can be considered as useful background levels to which to refer in future research. The element absorption increases in leaves growing during the rainy season. The trace metal levels observed indicate that the studied Caribbean zones do not present contaminant levels. None of the metals analyzed are present at levels considered toxic. Being these ecosystems in good actual conditions in terms of heavy metals, it is convenient to take preventive actions in order to preserve these areas.

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