Original Articles

Benthic foraminifera as bioindicators of potentially toxic element (PTE) pollution: Torrecillas lagoon (San Juan Bay Estuary), Puerto Rico

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ABSTRACT

Torrecillas Lagoon, on the north coast of Puerto Rico, has experienced extensive anthropogenic influence over the past 200 years. Elevated concentrations of Potentially Toxic Elements (PTEs) in bulk sediment (Cu, Zn, Pb, Ni, Cr, Li, V, Fe, As, Se, and Mn) have been reported in surficial sediments and have relatively uniform spatial distributions. Areas with higher concentrations are associated with a higher percentage of total organic carbon (TOC) and percent mud (mud), as well as anoxic conditions. *Ammonia beccarii*, *Quinqueloculina rhodiensis*, and *Triloculina oblonga* are the dominant foraminifers in the lagoon and are characteristic of stressed coastal environments. Bulk concentrations of Cu-Zn-Fe are negatively correlated with numerous foraminiferal taxa, absolute abundances, and diversity indices, though very few correlations with the bioavailable counterparts ($F2_{Tess}$-bioavailable) are observed. Similarly, relative abundances of *Quinqueloculina* and *Triloculina* positively correlate with bulk Cu-Zn-Fe but not with $F2_{Tess}$-bioavailable. The waters in Torrecillas lagoon show strong stratification, with hypoxic/anoxic (dissolved oxygen < 3 mg/L) and corrosive (pH < 7.4) conditions below 4 m depth. The presence of such strong gradients in very shallow water represents a dynamic chemical environment, with changes occurring on day-night cycles, tidal cycles, and especially with storm activity that induces mixing of otherwise highly stratified, very localized waters. Recognizing the potential for sequestered PTEs to be remobilized is an essential insight for coastal management agencies that must assess the risks of existing PTEs during coastal engineering activities (e.g., dredge and fill activities) and major storm events. Exchangeable and oxidizable fractions are likely more bioavailable than acid-soluble fractions in influencing the ecology of foraminifers under most circumstances.

1. Introduction

Estuaries are experiencing adverse changes that are influencing the natural variability of these unique ecosystems. Coastal lagoons commonly serve as sinks for a wide range of pollutants (Zaaboub et al., 2015). Rapid industrialization, overpopulation, surface-water alteration, and other factors are changing estuarine environments and biotic communities (e.g., Green-Ruíz et al., 2005; Hunter and Arbona, 1995). Estuaries receive approximately 80–90% of all waste released to marine environments (Gross, 1978) and therefore are particularly vulnerable to pollution by potentially toxic elements (PTEs). Such PTEs (see Martínez-Colón et al., 2009 for definition and further discussion) can have detrimental effects on water quality, aquatic ecosystems (i.e., reducing abundance and diversity, etc.), and human health (e.g., Laboy-Nieves, 2009).

In northern Puerto Rico, Torrecillas Lagoon (TL) has been extensively altered by anthropogenic activities (Figs. 1 and 2). From Pre-Colombian times to present, human settlements, agriculture, deforestation, landfills, dredging and urban development have affected this extensive lagoon system (Ellis, 1976; Seguinot-Barbosa, 1983). During the late 1800s, sugar cane was grown around TL (Wilson, 1899). At present, TL is influenced by a wide range of point and nonpoint sources of pollution (Table 1), coupled with localized anoxic conditions within dredged navigational channels.

Previous studies have reported that organic pollutants (PCBs, Dieldrin, DDT), as well as elevated concentrations of PTEs (As, Se, Cu, Hg, Cd Ni, Zn, and Pb) in sediments of TL are anthropogenic (Martínez-Colón and Hallock, 2010; San Juan Bay Estuary, 2000;
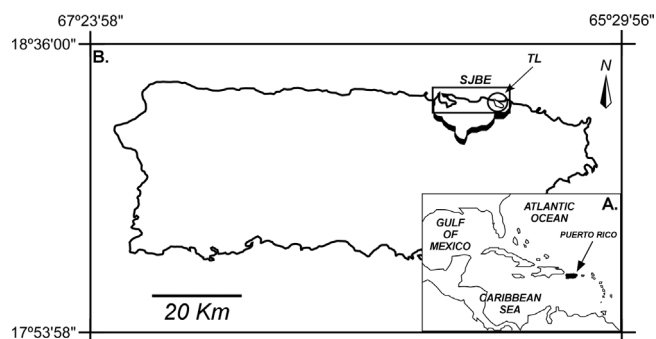
Fig. 1. A. Location of Puerto Rico. B. Map of Puerto Rico. Rectangle: San Juan Bay Estuary (SJBE). Circle: Torrecillas Lagoon (TL). Raised line: SJBE watershed.

Webb and Gómez-Gómez, 1998).

A variety of proxies has been implemented to identify pollutants in coastal waters, including macro- and micro-invertebrates, aquatic plants, and fish. Benthic foraminifers (shelled protists) were extensively used as bioindicators of pollution in coastal environments of Puerto Rico by Seiglie (1968, 1971, 1975c) and more recently by, for example, Oliver et al. (2014). Worldwide, foraminifers are known to respond to pollution through changes in assemblage makeup and abundance (e.g., Alve, 1995; Emrich et al., 2017; Yanko-Hombach et al., 2017), and to display a variety of morphological abnormalities that are considered a biological response to environmental stresses (e.g., Samir and El-Din, 2001; Yanko et al., 1998). Benthic foraminifers are likely sensitive to exposure to PTEs given the high surface-to-volume ratios of these

protists and their interrelationship with sediment type and physico-chemical parameters in bottom and pore waters. Benthic foraminifers also react to changes in sediment supply, dissolved oxygen (DO), food (organic matter), pH, and other external (abiotic) and internal (biotic) factors (Alve, 1995; Alve et al., 2016; Schönfeld et al., 2012), and are often among the last eukaryotic organisms to disappear completely from polluted sites, making them exceptional bioindicators (Alve, 1991; Schafer, 2000).

This research addresses the implementation of benthic foraminifers in TL as bioindicators of PTE pollution. The objectives were to determine the distribution and bioavailability of PTEs and their possible influence on the spatial distribution and morphological deformities of benthic foraminifers.

2. Study area

Puerto Rico is the easternmost island of the Caribbean Greater Antilles (Fig. 1A). The San Juan Bay Estuary (SJBE) system, the largest estuary (Fig. 1B) in Puerto Rico, was the first tropical island estuary in the National Estuarine Program, established in 1993, and comprises ~240 km² of land (drainage basin), of which 25 km² are inundated (Webb and Gómez-Gómez, 1998). Mean annual runoff is estimated to be 185 × 10⁶ m³ with suspended-sediment yields exceeding 15,000 mg/km²-yr (Webb and Gómez-Gómez, 1998). The SJBE system consists of semi-enclosed bays, lagoons (e.g., Torrecillas), and natural and dredged channels (e.g., Canal Suárez). Within the eastern subtidal portion of the SJBE system (Fig. 1B), TL (2.5 km²) has an average depth of 2.4 m and is probably the most complex system within the SJBE due to inflow-outflow sources of salt, fresh, and brackish waters (Gómez-
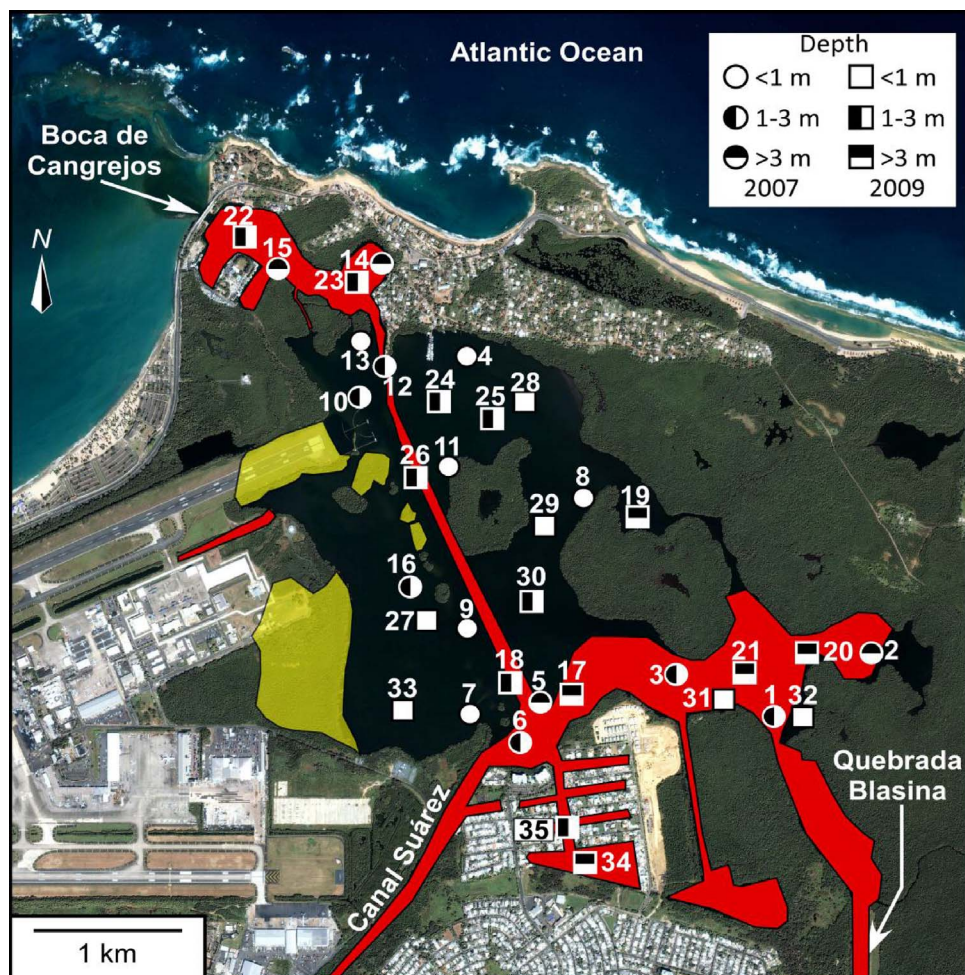

Fig. 2. Sampling sites in Torrecillas Lagoon: circles = 2007; squares = 2009. Areas influenced by dredge (red) and fill (faded yellow) are indicated (from Ellis, 1976). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Sources of Pollution.

Point Source	Type	Location	Reference
Sewage treatment plant	Raw sewage; PTEs	QuebradaBlasina	San Juan Bay Estuary (2000)
Collection system overflow	Raw sewage	South of TL	
Combined sewer outflows	Raw sewage; storm runoff	South of TL	
Marina sanitary discharges	Raw sewage	North/South of TL	
Dredging and filling	Sediment	All around TL	Ellis (1976)

Nonpoint Source	Type	Location	Reference
Illegal sanitary discharges	Raw sewage	All around TL	San Juan Bay Estuary (2000)
On-site septic tanks	Raw sewage	West-southwest margin of TL	
Storm runoff	PTEs, PCBs, etc.	All around TL	

Gómez et al., 1983).

Boca de Cangrejo is a dredged outlet connecting TL with the Atlantic Ocean (Fig. 2). The lagoons semidiurnal tidal range is approximately 0.60 m (Bunch et al., 2000), with longer residence times and increased volume of 110% attributed to dredging in the lagoon (Ellis, 1976). Net outflow into the Atlantic Ocean is approximately 230,000 m^3/day (Webb and Gómez-Gómez, 1998). Below 2 m water depth, anoxic conditions have been found and are attributed to disruption of circulation by dredging. Although denser seawater injected by flood tides can bring DO to the relatively deeper parts of the estuary, because neither wind nor tidal action is strong enough to mix the lagoon waters, they are typically stratified, impeding diffusion of oxygen and allowing slightly deeper areas to serve as nutrient traps (Ellis, 1976).

3. Methods

3.1. Field sampling

The methods implemented were prior to Schönfeld et al. (2012) FOBIMO protocols. Thirty-five samples were collected during two sampling trips in 2007 and 2009 (Fig. 2). A ponar grab sampler was used to collect most samples and the upper 2 cm of sediment were collected using a Teflon spoon. In some cases, sediment samples were collected via SCUBA diving. Each sample was placed into an acid-washed (10% HCl solution) plastic Nalgene© container and frozen for subsequent chemical and ecological analyses. A YSI-probe was used to determine in situ water column measurements at four stations for temperature (°C), pH, salinity, and DO during field sampling.

3.2. Laboratory sample preparation

3.2.1. Sediment samples

Sediment samples were freeze dried and sub-sampled for the following analyses: grain size, total organic carbon (TOC), percent carbonate (CO_3), PTE concentration in bulk sediment, and PTE $F2_{Tess}$-bioavailable concentration in mud fraction. For purposes of this study, what we reported as $F2_{Tess}$-Cu, Zn, and Fe was the $F2_{Tess}$-CO_3 second most "bioavailable" fraction as defined by Tessier et al. (1979) and as explained in Section 5.3.

For grain-size analysis, sub-samples were wet sieved over a 63 μm sieve and subsequently oven dried (50 °C) to determine the mud (silt and clay) fraction (<63 μm) by weight difference. The sand fractions (>63 μm) were then dry sieved. Each size fraction was weighed, and they were summed and converted into percentages; the median size fraction for each sample was expressed in phi (Φ) units. When used in conjunction with mud (%), Φ indicates sediment texture without listing all size fractions.

The TOC and CO_3 analyses followed the UIC Carbon Coulometer manufacturer protocols for titration and analysis. This procedure requires the conversion of inorganic and total carbon phases to carbon dioxide (CO_2). For TOC and CO_3, 0.5 g and 0.015–0.065 g of crushed sub-samples (100 mesh-size) were respectively combusted at 970 °C for 6 min (%Total Carbon) and acidified using 5 mL of 2N Perchloric acid for 5 min (%Total Inorganic Carbon).

For bulk PTE analyses, each dry sub-sample (0.5–1.2 g) was crushed into a fine powder (100 mesh-size) and sent to ACTLABS Laboratories Inc. (www.actlabs.com), for preparation and geochemical analysis of elements of concern (Cu, Zn, Pb, Ni, Cr, Li, V, Fe, As, Se, and Mn) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The analytical method implemented was Code Ultratrace 4 (ICP-MS). This method measures a suite of elements via "total" digestion with hydrofluoric, nitric and perchloric mixture, and hydrochloric acids at 260 °C.

The acid-soluble ($F2_{Tess}$: carbonate fraction) concentrations of copper (Cu), zinc (Zn), and iron (Fe) were determined to estimate bioavailability via Atomic Absorption Spectrometry (AAS) in the Geomicrobiology and Sedimentology Laboratory at Universidad Nacional Autónoma de México. Sediment sub-samples were dry sieved (<63 μm) using a polyethylene sieve and mesh. Sub-sample preparation of 0.25 g of sediment followed the protocols of Luoma and Bryan (1981), modified by Szefer et al. (1995) and Perez-Cid et al. (1998).

3.2.2. Foraminiferal samples

For foraminiferal assemblages, sediment sub-samples (2.8–23.4 g) were stained in a solution of Rose Bengal (1 g of dye in 1 L distilled water) for 12 h. Specimens whose interior exhibited an intense red stain were counted as alive at the time of collection, although necrotic cytoplasm may be stained. Some authors have noted shortcomings of this technique (Bernhard, 2000), but this is a standard approach widely used in benthic studies. Sub-samples (one per sampling station) were wet sieved through a 63 μm mesh to remove any mud particles and then dried (50 °C). Well preserved (stained/unstained) foraminifers were picked from the >63 μm under a dissecting microscope until, when possible, 200–300 individuals were counted. Specimens were identified using the generic taxonomy established by Loeblich and Tappan (1987) and to species level when possible (due to size <63 μm) following Poag (1981) and Seiglie (1971, 1975a,b,c). Relative abundances of *Ammonia* (ARA), *Quinqueloculina* (QRA), and *Triloculina* (TRA) are calculated as the number of individuals of the genus divided by the total number of foraminifers in the sample. Absolute abundances refer to number of individuals of a species in a sample.

3.3. Data analysis

The selection of diversity indexes and statistical tools are based upon applicability to the

proposed study, and general use in foraminiferal and ecological research (e.g., Hayek and Buzas, 1997). The parameters used in this study are described as follows:

(1) Species richness: S = number of species per sample.
(2) Foraminiferal Density: FD = number of tests/gram (total mass) in each sample.
(3) Shannon Index: $H(S) = -\Sigma p_i \times \ln(p_i)$ (Shannon, 1948).
(4) Equitability Index: $E = e^{H(S)}/n$ (Magurran, 1988).

Hierarchical cluster analysis was performed on all foraminiferal species, after adjustments for distribution and transformations to determine ecological assemblages. The first adjustment was the removal of any species that was not present in at least 5% of samples. Then data were standardized by calculating the ratio of each taxon to dry mass (g) in each sample (2.8–23.4 g). These data were fourth-root transformed (Parker and Arnold, 1999) using PRIMER$^{©}$ statistical software, thus creating a resemblance matrix that generated a group-average cluster dendrogram based on Bray-Curtis similarity.

Pearson correlation was performed to determine if any significant trends were found based on log-transformed data (Parker and Arnold, 1999). Pearson correlation analyses included mud, TOC, and CO_3, bulk PTE concentration, F2$_{Tess}$-bioavailable (acid-soluble) PTE concentration, S, FD, H(S), and E. This was done using PRIMER$^{©}$ (v. 6) statistical software (Clarke and Gorley, 2006).

Contour maps of mud, TOC, CO_3, bulk and F2$_T$-bioavailable PTEs, and foraminiferal data, were plotted using Arc GIS$^{©}$ (v.10) software (World Geodetic System84 datum) to illustrate potential hot spots of pollution, distribution of PTEs, and key foraminiferal genera.

4. Results

4.1. Sediment texture and composition

The dominant median grain size for all samples was mud ($\Phi > 4$; 51% of samples) followed by medium sand ($\Phi = 2$; 17% of samples) and gravel ($\Phi \leq -1$; 17% of samples) (Appendix A, Supplemental material). Percent mud (mud) decreased 200-fold (99.4–0.45%) from Quebrada Blasina on the SE towards the NW end of TL (connection to Atlantic Ocean) (Fig. 3a). Total organic carbon values ranged from 0.42–6%. Similar to mud, TOC decreased 20–fold from SE to NW (Fig. 3b). In contrast, CO_3 (0.32–66%) (Appendix A, Supplemental material) showed the opposite trend, increasing almost 200-fold from

SE to NW (Fig. 3c).

4.2. Potentially toxic element distribution

For bulk PTE concentrations, 11 PTEs were considered. Spatial distributions are shown in Fig. 4A–4N. Copper (0.9–118 mg/kg), Zn (4.8–237 mg/kg), Pb (2–38 mg/kg), Ni (6.9–33 mg/kg), Cr (6.64–65.39 mg/kg), Li (4.2–44.9 mg/kg), V (10–192 mg/kg) and Fe (11–134 mg/kg) generally increased from NW to SE. Concentrations of As (4.46–24.47 mg/kg), Se (0.3–1.7 mg/kg) and Mn (137–3020 mg/kg) were higher towards the middle of TL. Most PTEs varied over one order of magnitude except for Cu and Zn.

For bioavailability, only three PTEs (Cu, Zn, and Fe) were considered. Copper and zinc are well known PTEs that have been correlated to foraminiferal assemblage changes in field and culture studies. Iron, was selected due to its significance as a redox indicator. F2$_{Tess}$-bioavailable copper (F2$_{Tess}$-Cu) (5.87–67.73 mg/kg) had an almost uniform distribution except for two "hot spots" close to the Canal Suarez (east) and Quebrada Blasina (west); while F2$_{Tess}$-bioavailable zinc (F2$_{Tess}$-Zn) (13.02–72.63 mg/kg) showed an increasing NW–SE trend (Fig. 4D–E). The F2$_{Tess}$-bioavailable iron (F2$_{Tess}$-Fe) showed no discernible pattern (Fig. 4M). A factor of 11-fold in F2$_{Tess}$-Cu was observed and factor of 50-fold was observed for F2$_{Tess}$-Fe. The F2$_{Tess}$-Zn varied 6–fold in concentration. The sites at Canal Suarez, Lago Managua, and Quebrada Blasina, showed overall relatively high concentrations of both bulk and the bioavailable PTE counterparts (except F2$_{Tess}$-Cu).

A Pearson correlation matrix (Appendix B, Supplemental material) was calculated for the 11 elements of concern, as well as for bioavailable PTEs (F2$_{Tess}$-Cu, F2$_{Tess}$-Zn, F2$_{Tess}$-Fe), and TOC, CO_3 and mud. All PTEs positively correlated with mud, though for F2$_{Tess}$-Cu, F2$_{Tess}$-Zn, F2$_{Tess}$-Fe, As, and Se, the correlation was not significant at the 95% confidence level. Similarly, all except F2$_{Tess}$-Cu, F2$_{Tess}$-Zn, and F2$_{Tess}$-Fe significantly correlated with TOC. Thus, most PTE pairs were positively correlated (>0.34). However, As only correlated with Se and Mn. The F2$_{Tess}$-Cu and F2$_{Tess}$-Zn positively correlated with each other, as well as with Cu, Zn, Pb, Cr, and Li, and negatively with F2$_{Tess}$-Fe. None of the bioavailable PTEs significantly correlated with TOC or mud. Percent carbonate negatively correlated with most PTEs except for Se while correlations with F2$_{Tess}$-Cu and F2$_{Tess}$-Fe were not significant. Arsenic was the only PTE that correlated positively with CO_3.

4.3. Water column dissolved oxygen, pH, temperature and salinity

Dissolved oxygen concentrations in the water column generally decrease with depth. In all profiles except for Sampling Station #17, hypoxic conditions prevailed between 1.5–3.5 m, and anoxic conditions
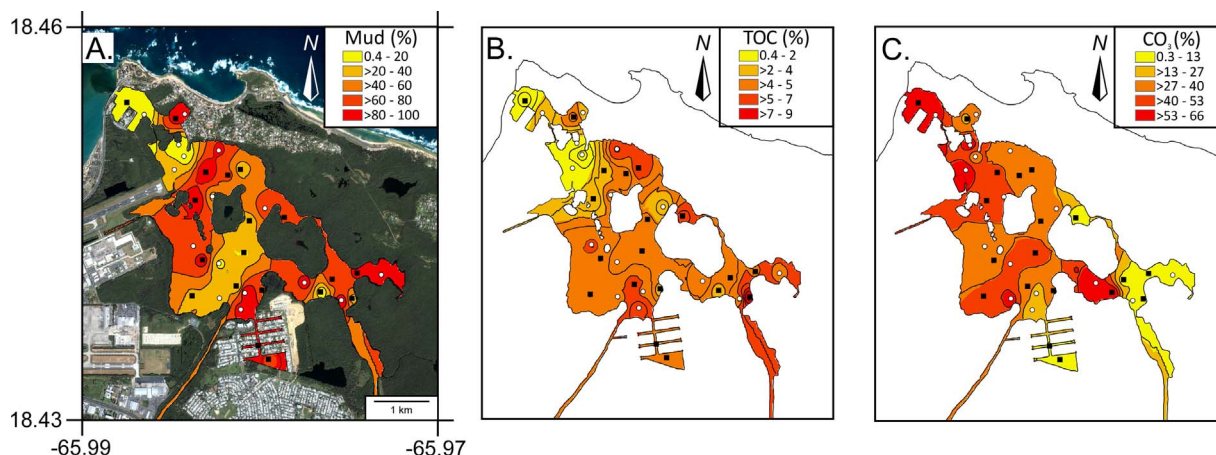

Fig. 3. Surface distribution of sediment characteristics. A: mud; B: Total organic carbon (TOC); and C: carbonate (CO_3).
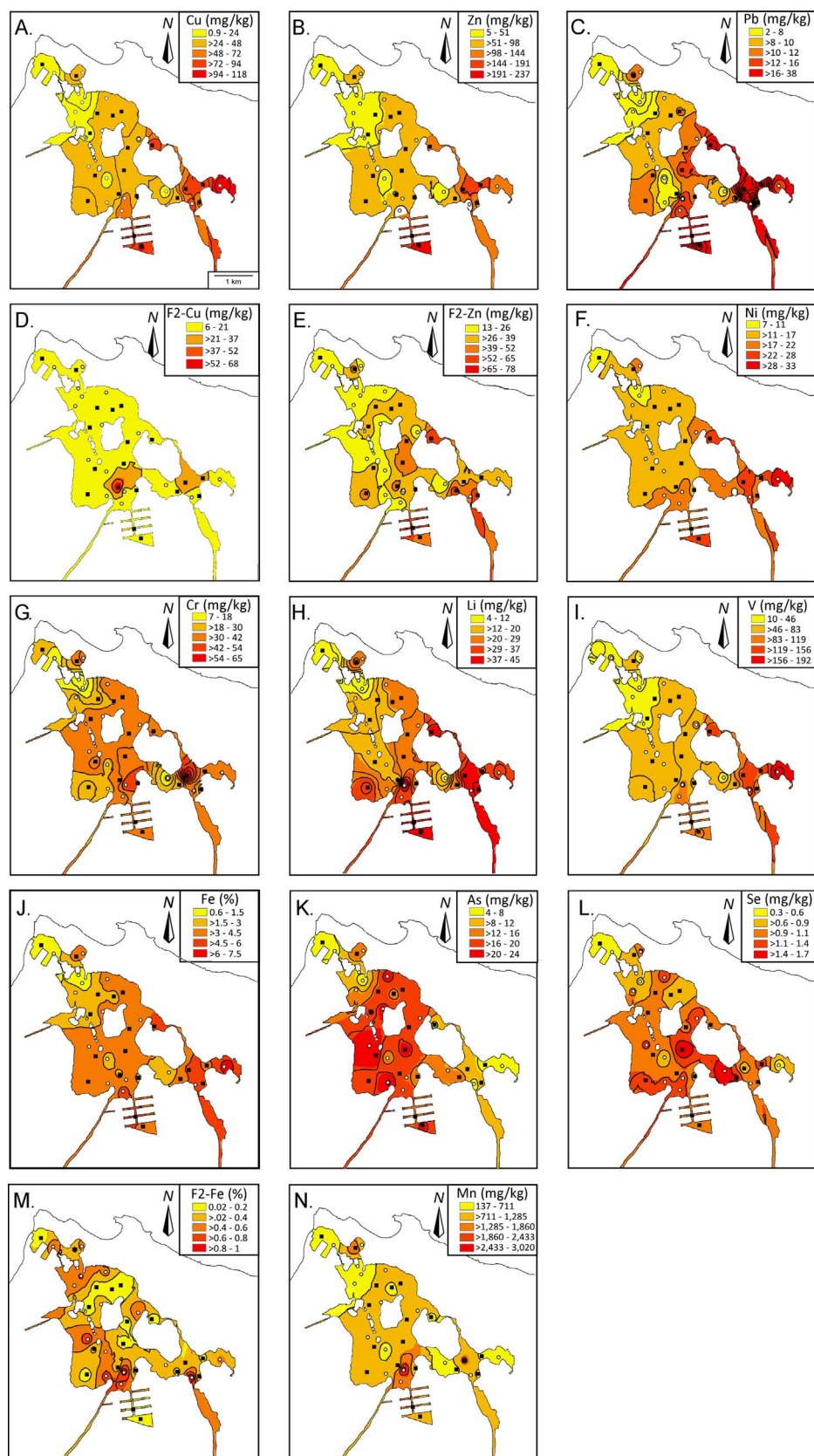
Fig. 4. PTE surface distribution. A: copper (Cu); B: zinc (Zn); C: lead (Pb); D: F2$_{Tess}$-Cu (acid-soluble copper); E: F2$_{Tess}$-Zn (acid-soluble zinc); F: nickel (Ni); G: chromium (Cr); H: lithium (Li), I: vanadium (V). All concentrations are in mg/kg (= ppm). White circles: 2007 sampling; and Black rectangles: 2009 sampling. J: iron (Fe); K: arsenic (As); L: selenium (Se); M: F2$_{Tess}$-Fe (acid-soluble iron); N: manganese (Mn). All concentrations are in mg/kg, except Fe (%). White circles: 2007 sampling; and Black rectangles: 2009 sampling.

were found at >3.5 m depth (Fig. 5A).

A pH < 8, which is below normal tropical seawater, was seen at 2–3 m depth in three of the profiles (Fig. 5B). Waters below 5 m were highly corrosive to CaCO$_3$ (pH < 7.4). In contrast, Site #17 showed

minimal variation in pH between surface and bottom waters (8.1).

A well-defined thermocline was observed between 0 and 6 m depth. Temperature remained constant at 26.3 °C at depths >6 m (Fig. 5C). A shallower site (#34) had a thermocline between 0 and 4 m, with
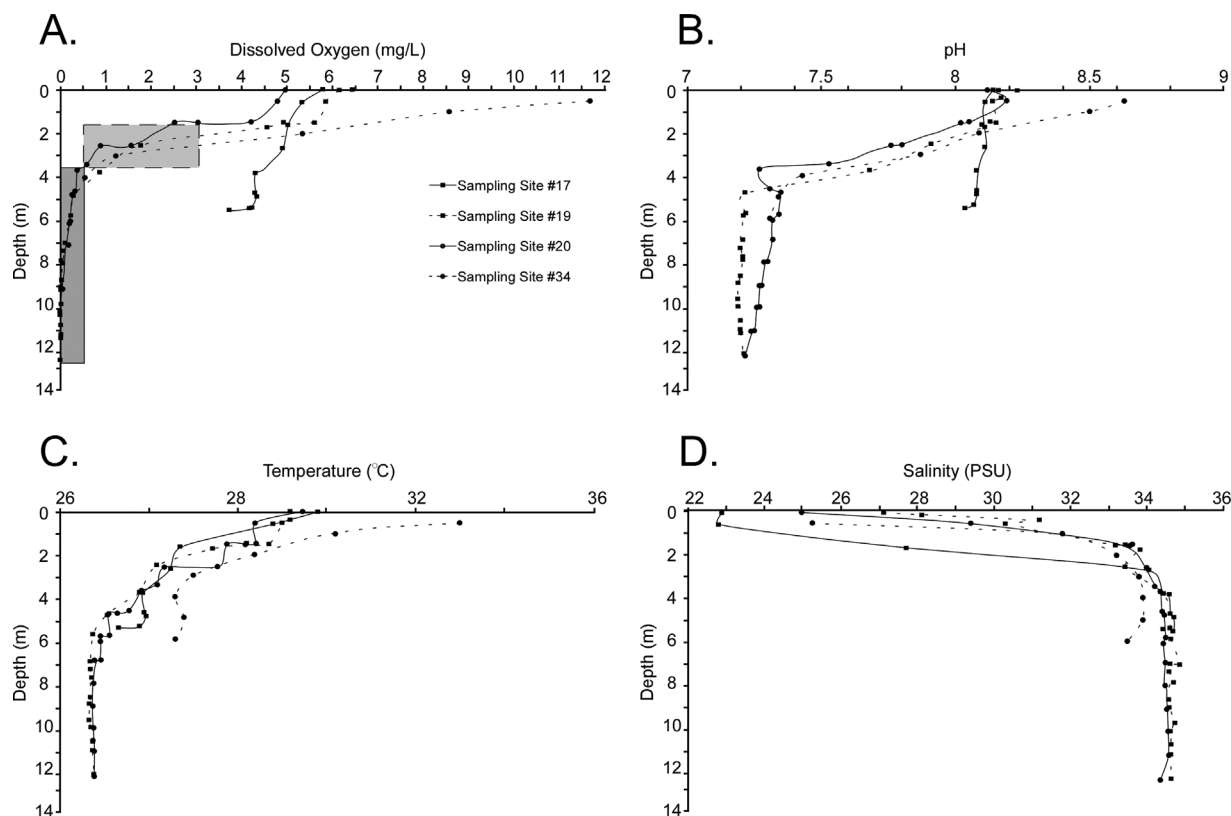
Fig. 5. Water column profiles. A: Dissolved oxygen. Light shaded area depicts hypoxic conditions (<3 mg/L) and dark shaded area depicts anoxia (<0.5 mg/L). B: pH. C: Temperature. D: Salinity.
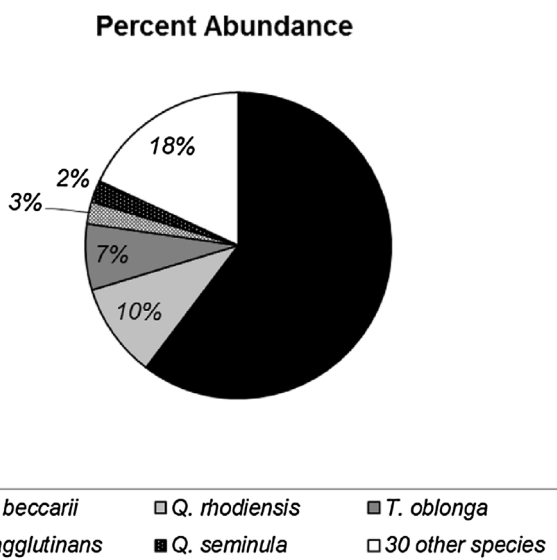

Fig. 6. Most dominant foraminifers.

temperature dropping from 30° to 27 °C. A halocline was observed between 1 and 2 m water depth with a salinity range between 25 and 35 (Fig. 5D). Site #19 was slightly less saline at the surface, with a value of 23.

4.4. Foraminiferal assemblages

From 35 sediment surface samples collected in TL, 6221 benthic foraminifers were picked, with 34 genera represented among which 50 species were identified (Appendix C, Supplemental material) (Fig. 6). Stained foraminifers were seldom encountered; therefore, all counts

represent total abundances. Of the 35 species present in at least 5% of the samples, *Ammonia beccarii* (3752 individuals), *Quinqueloculina rhodiensis* (621 individuals) and *Triloculina oblonga* (434 individuals) were the most abundant across all samples. *Ammobaculites agglutinans* (145 individuals) and *Quinqueloculina seminula* (144 individuals) were the fourth and fifth most abundant species. None of the other species accounted for more than 100 individuals across all samples.

Species richness (S) ranged from 5 to 22 species (Fig. 7A). The majority of the samples (71%) had relative low species richness (5–11). The two samples with high S values where found on opposite sides of the lagoon.

Foraminiferal densities (Fig. 7B) were also very low across TL. Variability ranged from 4 to 68 individuals/g and 54% of the samples had relative low densities (4–25). Similar to S, no apparent trend was observed except for areas of low density on the NW, central and SE sections of TL.

The Shannon Index [H(S)] (Fig. 7C) was used to evaluate the heterogeneity (i.e., diversity) of samples with respect to foraminiferal taxa. Index values ranged from 0.42–2.22. Only 40% of the samples had intermediate (1.03–1.62) H(S) values. Most samples with higher diversity were found towards the NW portion of TL.

The Equitability Index (E) measures the evenness of the foraminiferal distribution within the assemblage. The E values ranged from 0.20–0.87 (Fig. 7D). More than half of the samples (51%) had low equitability (0.20–0.42) values (i.e., high dominance), while 11% of the samples had higher (0.65–0.87) equitability values (i.e., low dominance).

Deformities of foraminiferal tests were found in 54% of the samples. All deformities were observed in miliolids except for one sample in which deformed *A. beccarii* tests were found. Percent deformities (FD) ranged from 2 to 18%.
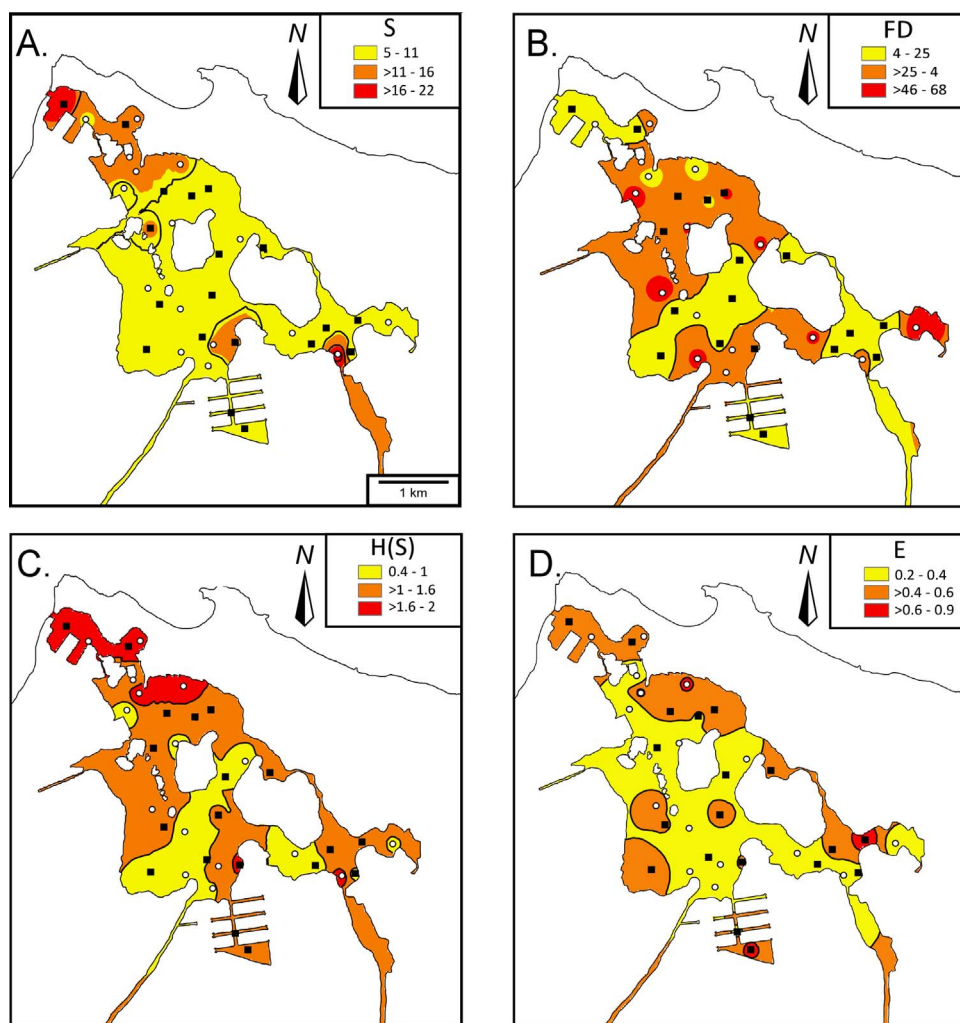
4.4.1. Foraminiferal cluster analysis and relative abundances

Three clusters were identified (Fig. 8). Cluster 1 was composed of *A. beccarii* (overwhelmingly dominant foraminifer in TL), *Q. rhodiensis*, and *T. oblonga*. Cluster 2 included other common nearshore and estuarine taxa. Cluster 3 included taxa more characteristic of open-marine shelf environments.

The relative abundances (RA) of *A. beccarii*, *Quinqueloculina* sp. and *Triloculina* sp. were plotted (Fig. 9). *Ammonia* RA (ARA) ranged from 0.7–91%. Five samples were classified as having low ARA (<30%), while 13 and 17 samples had medium (30–61%) or high abundances (>61%) respectively. Samples with high ARA values (53%) were mostly found towards the central–SW portion of the estuary. A very similar distribution to the pattern of low species richness was seen in Fig. 7A. *Quinqueloculina* RA (QRA) values ranged from 0 to 40% (Fig. 9B). Nearly all samples (80%) had a low QRA (<10%). Similarly, *Triloculina* RA (TRA) values ranged between 0 and 43% (Fig. 9C), with 85 of the samples having low TRA values.

A Pearson correlation matrix (Appendix D, Supplemental material) was calculated for the 11 PTEs of concern, including bioavailable elements (F2$_{Tess}$-Cu, F2$_{Tess}$-Zn F2$_{Tess}$-Fe), as well as TOC, CO$_3$, mud, foraminiferal taxa absolute abundances, ecological indices, relative abundances and percent deformities. Numerous genera negatively correlated with bulk PTEs, bioavailable PTEs, TOC and mud. From the dominant assemblage, *A. beccarii* absolute abundances negatively correlated with F2$_{Tess}$-Cu, F2$_{Tess}$-Zn and positively with F2$_{Tess}$-Fe while *Quinqueloculina rhodiensis* positively correlated with Cu, Cr, Fe, Mn, TOC and mud. *Triloculina oblonga* absolute abundances correlated

positively with Cu, Cr, Fe, V, and mud. Percent deformities correlated positively with F2$_{Tess}$-Cu and Cr and negatively with F2$_{Tess}$-Fe. Foraminiferal density correlated negatively with F2$_{Tess}$-Cu and F2$_{Tess}$-Zn and positively with F2$_{Tess}$-Fe. Species richness correlated negatively with Cu, Zn, Ni, Li, Se, Fe, V and TOC. The H(S) showed no correlation except negatively with Se; CO$_3$ correlated negatively with E. Relative abundances of *A. beccarii* showed no correlations, while QRA and TRA both positively correlated with Cu, Zn, Cr, Li, Fe, Mn, V, mud, and no correlation with bioavailable PTEs (F2$_{Tess}$-Cu, F2$_{Tess}$-Zn, F2$_{Tess}$-Fe). Lead correlated with TRA and TOC with QRA.

5. Discussion

5.1. Potentially toxic elements in sediments

The distributions of PTEs in TL are intimately related to sediment texture, TOC and water depth. The lagoon is a fairly restricted, low energy environment with surface sediments mostly dominated by mud (Fig. 3A). Coarsening of sediments towards the NW is related to higher energy environments; this also explains lower TOC values (Fig. 3B). Higher TOC values towards the SE are likely associated with local input, low wave energy, water stratification and water depth. Water depths vary from <1 m to 16 m (Fig. 2, Appendix A, Supplemental material), with some areas well mixed (e.g., Station #17) and others strongly stratified with respect to oxygen, pH, temperature and salinity (Fig. 5). Dredging operations until the mid-1970s (Fig. 2) created unconnected deeper "holes" (>3.5 m), where "oxiclines" develop (Fig. 5A) and
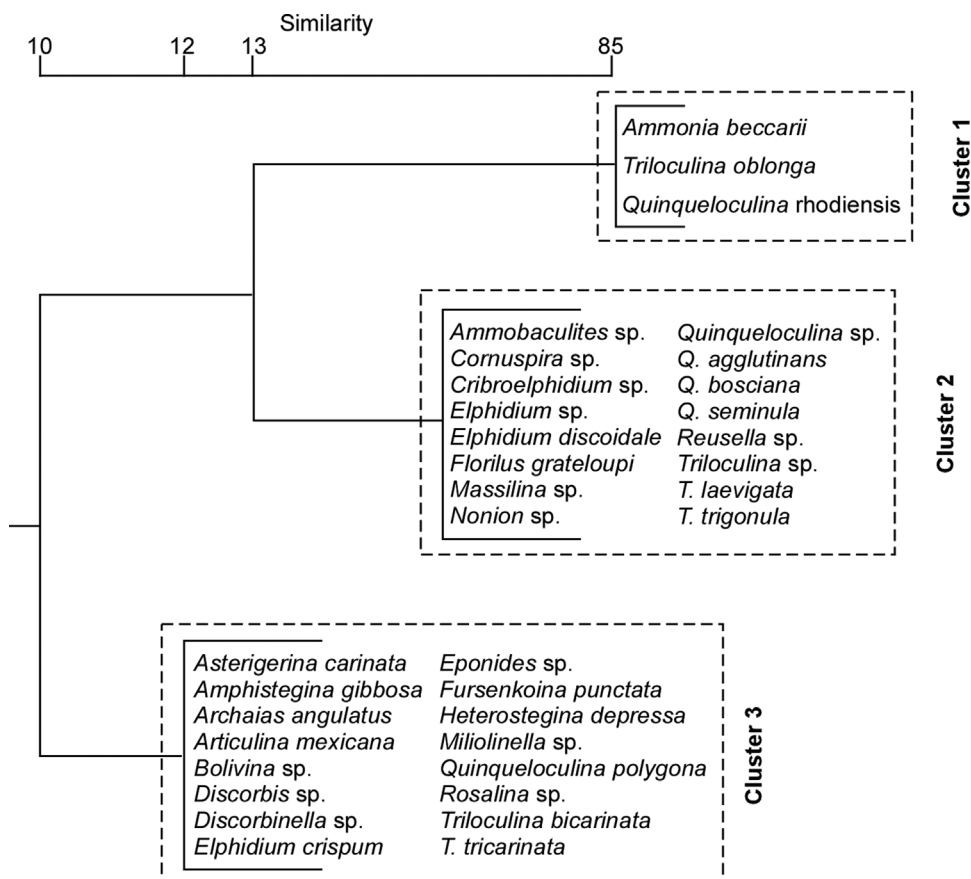
Fig. 8. Summary cluster diagram (Bray Curtis) for foraminiferal assemblages. Similarity not to scale.

allow for a preservation of organic matter. In addition, strong pH stratification, with values <7.4 at depths >5 m (Fig. 5B), is attributed to apparent oxygen utilization (low DO) in the SE section due to high mud and TOC (Fig. 3A–B).

Outflows from sewage treatment facilities, residential septic tanks, and "clandestine" solid waste dumps (e.g., Laguna Piñones) are sources of dissolved nutrients and organic matter that contribute to the TOC, which can complex PTEs. In addition, PTEs are also adsorbed by mud-size sediments (e.g., terrigenous input) from Quebrada Blasina and Canal Suarez. Under oxic conditions, PTEs (e.g., Cu, Pb, Zn) are sequestered by Fe/Mn oxides and, conversely, under anoxic environments, by sulfides (Davies et al., 2005; Larios et al., 2012; Zaaboub et al., 2015). Copper, Zn, Ni, Pb, and some other PTEs positively correlated with Fe/Mn (Appendix B, Supplemental material), indicative of oxic/anoxic boundary conditions. Moreover, framboidal pyrite was

commonly seen in the tests of foraminifers (Martínez-Colón and Hallock, 2010; Seiglie, 1973) and can be considered as a stress response to PTE pollution (Buzas-Stephens and Buzas, 2005) or post-mortem exposure to anoxia. In general, the highest PTE concentrations were found on the SE section (Cu, Zn, $F2_{Tess}$-Zn, Pb, Ni, Cr, Li, V, Fe and $F2_{Tess}$-Fe), nearest to anthropogenic sources, or revealed no discernable pattern ($F2_{Tess}$-Cu, As, Se and Mn) (Fig. 4).

5.2. Foraminiferal spatial distributions

Foraminiferal assemblages in TL are taxonomically characteristic of estuarine environments. Cluster analysis (Fig. 8) clearly separated the three dominant species (Cluster 1), from common nearshore and estuarine taxa that were much less prevalent. Cluster 3 included taxa such as *Amphistegina gibbosa* and *Heterostegina depressa* that are characteristic
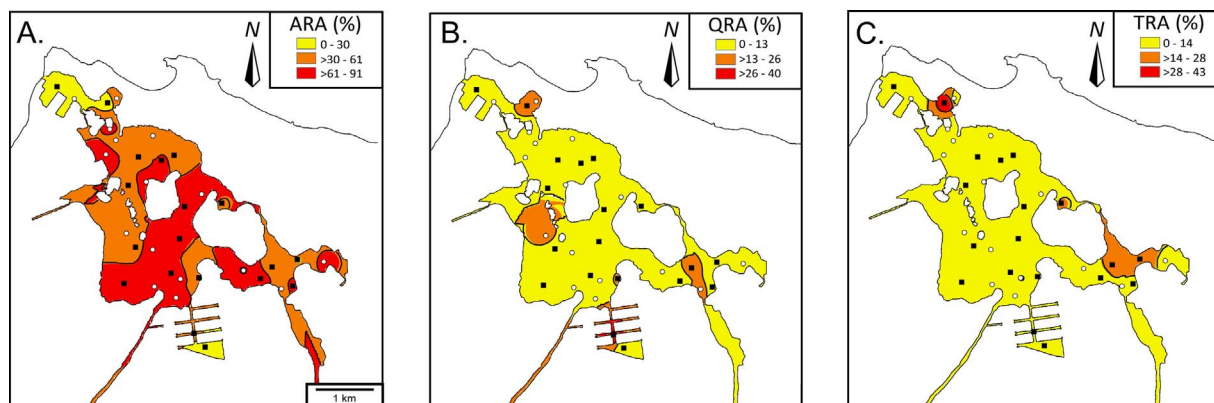

Fig. 9. Distribution of key foraminiferal genera. A: *Ammonia beccarii* relative abundance (ARA), B: *Quinqueloculina* sp. relative abundance (ARA), and C: *Triloculina* sp. relative abundance (TRA).

of open-marine shelf environments and were found primarily in samples directly influenced by connection to Atlantic waters.

Ammonia beccarii is well known to be stress-tolerant under anthropogenic as well as natural stressors. In general, fluctuations in salinity, food supply, temperature, oxygen, and pollutants, including PTEs (de Nooijer et al., 2007; Jorissen, 1999; among others), are some of the stressors that affect the distribution of this taxon. In other coastal areas in Puerto Rico, such as Jobos Bay and Guanyanilla Lagoon, *A. beccarii* dominate the assemblages, along with *Q. rhodiensis* in substrates impacted by organic pollution (Seiglie, 1968, 1975a, 1975b, 1975c). *Cribroelphidium excavatum* and some *Elphidium* spp. are also opportunistic, stress-tolerant taxa that can survive changes in oxygen concentrations (e.g., Dabbous and Scott, 2012; Debenay et al., 2009). Although found in the current study, these taxa were not common. However, a few other species recorded in TL, notably *Q. seminula*, are well known stress-tolerant taxa (e.g., Foster et al., 2012), especially in warm water environments.

Numerous studies involving the use of foraminifers as bioindicators of pollution have reported the impact of PTEs and other stressors on ecological indices (e.g., Alve et al., 2016; Bouchet et al., 2012; Schwing et al., 2016; Oliver et al., 2014). These indices, as calculated from foraminiferal assemblages in TL, are characteristic of an impacted estuarine environment. Species richness, FD, H(S) and E are very low compared to similar estuarine environments such as Biscayne Bay, Florida (e.g., Carnahan et al., 2009); Point Joinville, France (Debenay et al., 2001); and Cagliari, Sardinia (Schintu et al., 2016). Moreover, very few foraminifers were recorded as live in the samples collected from TL.

The highest S values were found towards the NW and SE sections of the lagoon (Fig. 7A). The data show no correlation with either mud or CO_3. However, the significant negative correlation with TOC was likely related to redox changes (suboxic to oxic conditions) (Fig. 5A). Ellis and Gómez-Gómez (1976) previously recorded anaerobic conditions below 2 m water depth and DO levels between 1 and 3.4 mg/L, consistent with the current conditions of the lagoon (Fig. 5A). Anoxia associated with abundant organic matter and lack of mixing is highly detrimental to most foraminiferal species (Jorissen, 1999; Schönfeld et al., 2012). Species richness also negatively correlated with several PTE bulk concentrations in TL, which is in agreement with similar studies elsewhere (Alve, 1991; Armynot du Châtelet et al., 2004; Martins et al., 2016; Salvi et al., 2015).

The FD has lower values towards the NW and SE of the lagoon (Fig. 7B). Interestingly, FD shows no correlation with any parameter except negatively with $F2_{Tess}$-Cu and $F2_{Tess}$-Zn and positively with $F2_{Tess}$-Fe. Romano et al. (2008) and Frontalini and Coccioni (2008) found similar low densities in anthropogenically impacted environments. In some coastal environments, low FD has been reported to correlate to low TOC (< 2%) (Armynot du Châtelet et al., 2009). Although no correlation was found with TOC, pH and anoxia are more likely controlling mechanisms. Debenay and Fernandez (2009) and Martins et al. (2011, 2013) also found that bioavailable PTEs negatively correlated with foraminiferal density. In addition, dispersal by tidal currents (e.g., Arslan et al., 2016b) into the highly corrosive conditions in deeper zones of TL are likely to dissolve the foraminiferal tests, which would contribute to low FD values, and to changes in distribution and dominance. Dormancy is another factor to consider since reports have shown that propagules remain in stasis until environmental conditions are more favorable (Alve and Goldstein, 2003; Ross and Hallock, 2016) which could also be the case in the deeper portions of TL.

Similar to S and FD, diversity indices are known to be low in areas of PTE pollution (e.g., Bergin et al., 2006; Foster et al., 2012; Kfouri et al., 2005; Martínez-Colón and Hallock, 2010; Schintu et al., 2016). Both H(S) and E show a similar spatial pattern, with lower values towards the center of the lagoon (Fig. 7C–D). The low diversities and abundances of foraminiferal tests are indicative of stressed environments, but PTEs do

not appear to be the primary environmental factor stressing the assemblages in the lagoon given few significant correlations with PTEs. The same conclusion was reached, based upon a short-sediment core from TL in which the temporal variability of H(S), E and FD reflected the carbonate preservation potential (pH) and organic matter content of the lagoon (Martínez-Colón and Hallock, 2010).

The relative abundances of *Q. rhodiensis* and TRA were generally quite low, while ARA was generally more dominant towards the center of the lagoon (Fig. 9), which is dissected by dredged channels (Fig. 2). The fact that ARA shows no correlations further demonstrates that this species is nearly ubiquitous in the lagoon. Moreover, the high-Mg calcite miliolid tests are more prone to dissolution in deeper, corrosive areas of the lagoon. Dissolution could also help explain the low foraminiferal densities and indices overall in TL; the dominant species showing dissolution scars support this hypothesis (see Plate I in Martínez-Colón et al., 2009). Dias et al. (2010) noted lower number of foraminifers in assemblages dominated by agglutinated forms at pH < 7.6. In TL, profiles show pH as low as 7.2 at depths > 3.5 m (Fig. 5B), although agglutinated specimens were rarely found. The QRA and TRA positively correlated with several bulk PTEs and not with the bioavailable counterparts ($F2_{Tess}$-Cu, $F2_{Tess}$-Zn, $F2_{Tess}$-Fe). This observation is not consistent with studies that have concluded that bulk PTE concentrations are foraminiferal stressors (e.g., Fontanier et al., 2012; Schintu et al., 2016). The significance of this finding is that conclusions should not be based on bulk PTE concentrations but instead must consider chemical fractionations as well as enrichment (see Section 5.3 for discussion). In addition, the positive correlation between absolute abundances of *Q. rhodiensis* and QRA with TOC and mud, and *T. oblonga* and TRA with mud, suggests that sediment texture and organic matter (as reflected by anoxic conditions) could be the main controlling factors and PTEs are secondary.

Numerous studies have debated the importance of test deformities as a proxy of pollution (see Table 4 in Martínez-Colón et al., 2009). Deformed foraminifers were found in 54% of the samples from TL, with 18% deformed foraminifers recorded in one sample. The foraminifers exhibiting deformed tests were mostly miliolids, especially *Q. rhodiensis,* as previously observed by Martínez-Colón and Hallock (2010). Although deformities correlated positively with $F2_{Tess}$-Cu and Cr, and negatively with $F2_{Tess}$-Fe, Seiglie (1975c) noted that abnormal morphologies of *Q. rhodiensis* could be influenced by eutrophication.

5.3. Bulk vs bioavailable potentially toxic elements

In environmental micropaleontological studies, the standard methodology has been to assess total PTE concentrations in bulk sediments (e.g., Armynot du Châtelet et al., 2004; Arslan et al., 2016a; Eichler et al., 2012; Salvi et al., 2015), while far fewer studies have considered the bioavailability of PTEs (Martins et al., 2011, 2013, 2015; Schintu et al., 2016). Tessier et al. (1979) noted that PTEs could be found in various sedimentary components ("fractions"). Fractionation can be influenced by sediment type, organic matter content, pH, alkalinity, Eh, salinity, and other environmental factors. The mobility of PTEs during sequential extraction procedures has been related to the degree of operational bioavailability (Bacon and Davidson, 2008; Zimmerman and Weindorf, 2010). These interpretations in fractionation resulted in five different sediment chemical fractions following Tessier et al. (1979) nomenclature: exchangeable (PTEs adsorbed to clays: $F1_{Tess}$ are most bioavailable and are readily released by complexation and minor pH changes), acid-soluble (PTEs bound to carbonates: $F2_{Tess}$-CO_3 can be released during dissolution), reducible (PTEs bound to oxidized minerals: $F3_{Tess}$-Fe/Mn can be released in reducing conditions), oxidizable (PTEs bound to organic matter: $F4_{Tess}$-organic matter), and residual (PTEs found in the crystalline structure of silicate minerals: $F5_{Tess}$-lithics are the least bioavailable and will not be released to the environment).

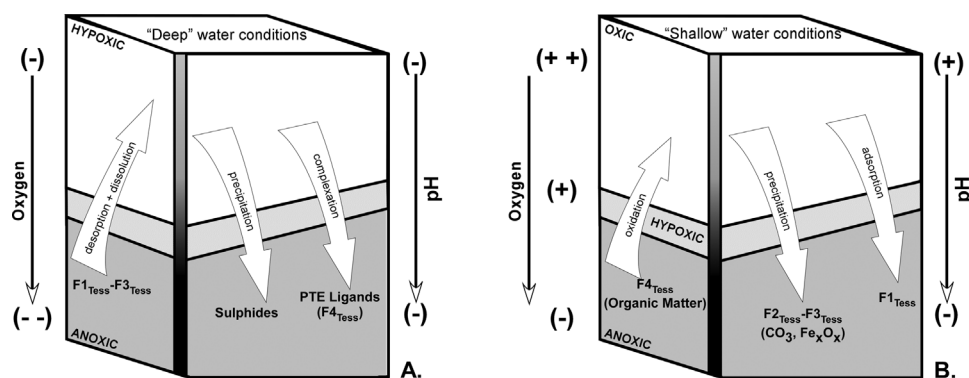The physico-chemical conditions of TL serve as a good example of

Fig. 10. Simplified model of the fate and trasport of PTEs. A: low oxygen and pH conditions. B: high oxygen and pH conditions. Positive/negative signs: pH/oxygen increase or decrease. Modified from Martínez-Colón et al. (2009).

the behavior and distribution of PTEs among the different sediment chemical fractions. Fig. 10 illustrates the fate and transport of PTEs between sediment chemical fractions (e.g., $F1_{Tess}$–$F4_{Tess}$) in the lagoon based on pH and DO characteristics with depth. In the deeper areas of the lagoon (> 3.5 m) low pH and anoxic conditions prevail (Fig. 5A–B). Acidic conditions allow for PTEs to desorb and dissolve from clays ($F1_{Tess}$) and carbonates ($F2_{Tess}$) respectively, allowing the pollutants to exist in solution (free ions) (Fig. 10A). PTEs in the reducible fraction ($F3_{Tess}$) are released by dissolution of oxide minerals under hypoxic and anoxic conditions. The fates of these PTEs in solution are controlled by low oxygen conditions and low Eh that promote their sequestration by sulfide precipitation (e.g., pyrite- FeS_2) or by becoming complexed with organic matter and other ligands (e.g., Cl^-, SO_4^{-2}) (e.g., Martínez-Colón et al., 2009). PTEs in organic matter ($F4_{Tess}$) can be released under highly oxygenated conditions and they can be bound by sulfide precipitation, complexation or adsorption by clays (Fig. 10B). Conditions in TL promoting oxidation of organic matter can be related to strong mixing events (e.g., tropical storms) or to shallow and well-oxygenated areas. Under oxygenated and normal-marine pH conditions (Fig. 10B), PTE remobilization is limited to adsorption ($F1_{Tess}$), $CaCO_3$ precipitation ($F2_{Tess}$) and oxide mineral formation ($F3_{Tess}$). Based on these observations, PTEs in solution (e.g., free-ions) or bound to organic matter should be more readily bioavailable during calcification and feeding (e.g., detritus-feeding protists such as benthic foraminifera).

For example, absolute abundances of *A. beccarii* and *Triloculina* coupled with FD have no correlation with bulk Cu, Zn and Fe, but are negatively correlated with $F2_{Tess}$-Cu, $F2_{Tess}$-Zn, and positively with $F2_{Tess}$-Fe (except *Triloculina)* (Appendix D, Supplemental material). However, these PTEs may have limited bioavailability to the foraminifers. Fraction $F2_{Tess}$-Cu, as well for Zn and Fe, translates to Cu ions locked in the crystalline structure of carbonate minerals. The only scenario in which PTEs found in the $F2_{Tess}$ fraction would become bioavailable is when dissolution of the carbonate minerals occurs and the PTEs are remobilized in solution (e.g., in low pH conditions).

Numerous authors have found correlations between bulk Cu and key foraminiferal taxa (e.g., Alve, 1991; Debenay and Fernandez, 2009; Martins et al., 2011). In TL for example, QRA, and TRA are positively correlated to bulk Cu, Zn, and Fe (and these with TOC and mud) but not to $F2_{Tess}$-Cu, $F2_{Tess}$-Zn, and $F2_{Tess}$-Fe (Appendix D- Supplemental material). Similarly, absolute abundances of *Q. rhodiensis* positively correlated with bulk Cu and Fe but not with their bioavailable counterparts. These correlations indicate that the cumulative nature of bulk concentrations may explain some stress responses, but not all, and those inconsistencies in interpretations can occur when chemical speciation and fractionation are not considered. Our study suggests that the $F2_{Tess}$ acid-soluble fraction is not a satisfactory assessment choice due to its very limited impact on foraminifers, as they do not have digestive systems, and environments that are sufficiently acidic to dissolve $CaCO_3$ are not amenable to calcareous foraminifers.

Martins et al. (2011, 2013) and Schintu et al. (2016) conducted PTE

fractionation following the European Community Bureau of Reference protocols (BCR). For example, the $F1_{BCR}$ fraction combines the exchangeable ($F1_{Tess}$) and acid-soluble ($F2_{Tess}$) fractions of Tessier et al. (1979) and the $F2_{BCR}$ and $F3_{BCR}$ are the reducible and oxidizable fractions respectively. They found significant correlations between $F1_{BCR}$ and $F2_{BCR}$ with assemblages (*Ammonia tepida* and *Quinqueloculina seminulum*) and deformities. This observation is also inconclusive because it is challenging to determine which PTEs in the $F1_{BCR}$ fraction (exchangeable or acid-soluble) are affecting the foraminifers. In addition, these studies have found relative high accumulation of PTEs in $F2_{BCR}$ (Fe/Mn oxides) and have described this fraction as highly mobile and bioavailable.

Caution also must be exercised when interpreting the statistical correlation between ecological parameters and Fe/Mn oxide fraction. For example, in TL the positive correlation of Cu with Fe/Mn suggests that this PTE could be in the $F3_{Tess}$ fraction (Appendix B, Supplemental material). Furthermore, the positive correlation of QRA and TRA with Fe/Mn (Appendix D, Supplemental material) could be interpreted as the $F3_{Tess}$ being a bioavailable stressor to foraminifers. However, PTEs potentially found in this $F3_{Tess}$ fraction are not directly bioavailable because these contaminants will be found within the crystalline structure of Fe_xO_x/Mn_xO_x minerals, but can only be released under reducing conditions. Larios et al. (2012) found that As^{+5} bound to ferric hydroxides was later released as As^{+3} under reducing conditions.

When examining the influence of PTEs on foraminiferal assemblages, fractionation of each PTE of interest, including the chemical conditions under which they might become bioavailable, should be considered. Sequential extractions are required to elucidate which potential bioavailable fraction will have an impact. Of the five fractions, the $F1_{Tess}$ exchangeable phase is considered the most bioavailable and the $F5_{Tess}$ residual phase to be the least bioavailable (e.g., Tessier et al., 1979). The question is: What chemical fraction is considered bioavailable to the foraminifers? Benthic foraminifers are detritivores, PTEs found in $F4_{Tess}$ fraction should be considered highly bioavailable since this is a likely pathway of PTE bioaccumulation (Martínez-Colón et al., 2009). However, studies have concluded that PTEs in the $F3_{BCR}$ fraction to be less toxic and bioavailable than those found in the $F2_{BCR}$ fraction in coastal environments in Portugal (Martins et al., 2013) and Sardinia (Schintu et al., 2016). Fraction $F1_{Tess}$ could also be considered bioavailable, since PTEs could be readily desorbed into free ions by minor changes in pH, Eh and salinity. This scenario could happen at the sediment water interface or during calcification in which foraminifers reduce their internal pH during vacuolization. This is consistent with the findings of Zaaboub et al. (2015), in which they concluded that PTEs in the exchangeable portion of the $F1_{Tess}$ fraction is the most mobile and that remobilization is controlled largely by pH and redox conditions.

Moreover, the environmental conditions in the studied areas must also be considered. For example, in TL, the presence of well oxygenated, hypoxic and anoxic conditions, as well as normal marine pH to low pH

(7.2) gradients, within a few meters of each other in very shallow water, represents a potentially very dynamic chemical environment, with some changes in chemical environment occurring on day-night cycles, tidal cycles, and especially with storm activity that induces mixing of otherwise highly stratified, very localized waters. Thus, the bioavailability of individual elements can change spatially on scales ranging from centimeters to kilometers, and temporally on scales ranging from hours to season or possibly years (e.g., major storm events).

6. Conclusion

Several areas in Torrecillas Lagoon show strong stratification with hypoxic/anoxic (DO < 3 mg/L) and corrosive (pH < 7.4) conditions below 3.5 m depth. These settings likely impact both PTE fractionation and foraminiferal assemblage distributions. Foraminiferal assemblages dominated by low densities of *A. beccarii*, *Q. rhodiensis*, and *T. oblonga*, and coupled with low ecological index values [H(S), E, FD] and deformed foraminifers (2–18%), are indicative of stressed conditions. The spatial variability of PTEs in bulk concentrations (concentration gradient: Zn > V > Cu > Cr > Li > Ni > As > Pb > Fe > Mn > Se) suggests the influence of numerous point and non-point sources of pollution. Bulk PTE concentrations are mostly negatively correlated with foraminiferal taxa and several ecological indices, but very minimal correlation with the acid-soluble "bioavailable" PTE fraction ($F2_{Tess}$) (concentration gradient: $F2_{Tess}$-Cu > $F2_{Tess}$-Zn > $F2_{Tess}$-Fe) was found.

Interpretations based on bulk concentrations are not recommended since there is no direct way of determining which chemical fraction is bioavailable and is having an impact on foraminifers. In retrospect, the assessment of $F2_{Tess}$ as a bioavailable fraction impacting foraminifers is also not recommended. In this study, PTEs correlated positively with mud and TOC, and are inferred to be adsorbed or complexed in the $F1_{Tess}$ (exchangeable) and $F4_{Tess}$ (oxidizable) fractions. These two fractions are likely of greater importance and bioavailability than the $F2_{Tess}$ (acid-soluble), $F3_{Tess}$ (reducible), and $F5_{Tess}$ (residual) fractions.

The discrepancies observed between bulk and $F2_{Tess}$-bioavailable PTEs with foraminifers strongly support the idea of implementing sequential extraction protocols instead of the total digestion to better elucidate the actual impacts on foraminifers. Caution is recommended when not considering the bioavailability of PTEs in pollution studies due to the potential for overestimation. Moreover, the recognition of environmental conditions, the dynamic nature of the environment, and the potential for sequestered and complexed PTEs to be mobilized can provide essential insight for coastal management agencies that must assess the risks of existing PTEs and future pollution sources, especially during coastal engineering activities (e.g., dredge and fill activities) and major storm events.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ecolind.2017.10.045.

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