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Bioaccumulation and biomagnification of potential toxic elements (PTEs): An *Avicennia germinans–Uca rapax* trophic transfer story from Jobos Bay, Puerto Rico

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ABSTRACT

In southern Puerto Rico along the coastline bordering the Jobos Bay National Estuarine Research Reserve, environmental encroachment has exposed mangrove forest to different sources of pollution. Potentially toxic element concentrations from the $F1_{Tess}$ (exchangeable), $F4_{Tess}$ (oxidizable), mangrove leaf litter (MLL), and fiddler crab whole body soft tissue were analyzed to assess the fate and transport of pollutants from the environment and its transition into flora-fauna via trophic transfer. Geo-accumulation factor values suggest the bay has experienced limited to no pollution when combining the concentrations of potentially toxic elements extracted from the $F1_{Tess}$ and $F4_{Tess}$ sediment fractions. These geochemical sedimentary compartments are considered "bioavailable" to flora-fauna as evidenced by the bioaccumulated Cd-Ba-V-Cu-Zn-As-Se in the leaf litter of the black mangrove *Avicennia germinans* and in the fiddler crab *Uca rapax*. The biota-sediment accumulation factor ($F1_{Tess} + F4_{Tess}$) demonstrated that *Uca rapax* behave like a de-concentrator for most pollutants and as a macro-concentrator for Cu-As, while the bioconcentration factors identified only Cu-As-Se as being actively bioaccumulated in the fiddler crabs. Of all the potentially toxic elements studied, As is the only one to be biomagnified via sediment-*Avicennia germinans* leaf litter-*Uca rapax* food chain. An unexpected find of this study was that the excavated sediment "pellets" by *Uca rapax* contained up to 4x the concentrations of Cd-Ba-V-Cr-Co-Ni-Cu-Zn-As-Se when compared to the $F1_{Tess}$ sediment fraction from the surface, thus suggesting a variable redox boundary within the fiddler crab's burrow.

1. Introduction

Estuaries represent some of the most important and highly productive global ecosystems. These ecotones are recognized for their environmental, ecological and economic importance due to their ability to act as storm surge buffers, breeding sites and nurseries for a wide variety of organisms. In tropical to subtropical settings, the mangrove forests that make up a majority of these environments are comprehensively relied upon for timber, fuel and medicine (Alongi, 2002). Unfortunately, these coastal areas also represent some of the most contaminated environments due to land-based sources of pollution from agriculture, urban development, aquaculture, and mining. In the Caribbean for example, it has been found that mangrove leaves accumulate Potentially Toxic Elements (PTEs) in their leaves minimizing exportation to surrounding ecosystems and organisms (Mejías et al., 2013; Pryor and Wilking, 2011), as well as legacy organic contaminants (e.g., Shete et al., 2009; Alegría et al., 2016). Mangrove trees are not an exception to this phenomenon due to the high surface-to-volume ratio of their root system (Alongi, 2002). Previous research has shown the negative effects of heavy metals on mangrove ecosystems as evidenced by disturbances in population as well as biodiversity coupled with a reduction in biomass and ultimately plant mortality (Bayen, 2012). Estuarine pollution has been exacerbated due to the overexploitation of coastal areas and overpopulation, which results in an increase of pollutants (Alongi, 2002; Zitello et al., 2008).

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Among the major contaminants in estuarine environments, PTEs or heavy metals, pose a major threat to water quality, ecosystem functionality, biodiversity and food chain stability (e.g., Zitello et al., 2008; Martínez-Colón et al., 2009). The high affinity of PTEs towards organic matter and clay-sized particles, due to their high surface area and cation exchange capacity, allows these chemical pollutants to accumulate in areas with high organic matter (Apeti et al., 2010; De Paiva et al., 2008; Pait et al., 2012, 2008). Moreover, these pollutants can also potentially undergo remobilization through sediment resuspension (e.g., dredging, boating) and desorption (e.g, pH changes) that can increase their bioavailability and subsequent absorption and bioaccumulation/biomagnification by flora and/or fauna (Bryan, 1971; Albers and Camardese, 1993). Bioaccumulation relates to the fate and transport of PTEs and how it accumulates in tissues of living organisms whether is by active or passive absorption/ingestion (e.g., Martínez-Colón et al., 2009; National Research Council, 1981). Subsequently, these bioaccumulated contaminants can be biomagnified as it relates to their capacity to move up food chains and increase in concentration in relation to their surrounding media (e.g., water) or source (e.g., food).

Jobos Bay (JB) in southern Puerto Rico, part of NOAA's National Estuarine Research Reserve System (Fig. 1a-b), is a groundwater–fed estuary. Since the 1800′s its shorelines and ecosystems have been drastically altered by anthropogenic activities such as agriculture and urban/industrial development coupled more recently with an oil refinery, coal-fired plant, pharmaceutical facilities, solid waste landfill and a thermoelectric power plant (Berman-Santana, 1996; Zitello et al., 2008; Aldarondo-Torres et al., 2010). The sediments from JB have previously been categorized as low-moderately polluted with respect to PTEs such as Cu-Pb-Zn-Cr-Cd-Hg (e.g., Aldarondo-Torres et al., 2010; Apeti et al., 2010). A handful of environmental studies conducted to date have addressed only the presence/absence and quantification of PTEs and other pollutants in organisms (including mussels, amphibians, and corals) (e.g., Aldarondo-Torres et al., 2010; Apeti et al., 2010; Pait et al., 2008, 2012; Zanders and Rojas, 1996; Zitello et al., 2008). However, no studies in JB have assessed the bioaccumulation, biomagnification and uptake pathways of PTEs associated with herbivore-plant interactions in a mangrove forest dominated by the black mangrove *Avicennia germinans*.

The ability to monitor coastal ecosystems is crucial to assess anthropogenic impacts and subsequent rehabilitation. The presence of PTEs in JB has raised the important question of whether these contaminants biomagnify up food chains while adversely affecting flora/fauna. The species abundance, diversity and richness of organisms such as protists (Martínez-Colón et al., 2018) and bacteria (Aylagas et al., 2017), among others have been traditionally used as bioindicators to monitor ecosystem impact and health. Of these, fiddler crabs are a promising bioindicator for studying PTE impacts (e.g. Bergey and Weis, 2007; Alvaro et al., 2016). Fiddler crabs are found along coastal (intertidal), lagoon and mangrove environments in sand, mud or silt sediments (Thurman et al., 2010). The species of the genus *Uca* are burrowers and foragers that feed on microalgae, bacteria (e.g., Meziane and Tsuchiya, 2002; Demopoulos et al., 2007; Bartolini et al., 2009; Bartolini et al., 2011) and on mangrove detritus (France, 1998; Demopoulos et al., 2007) as they are considered important bioturbating animals in mangrove forests (Nielsen et al., 2003). The bioturbation generated by the fiddler crabs during burrowing not only provides an escape route against predators and shelter during high tide (Jones, 1984; Genomi, 1991), but also provides a bioengineering mechanism for sediment irrigation (i.e., salt dilution), aeriation, and increased water uptake by mangrove trees (Bartolini et al., 2011). Most importantly, fiddler crabs are keystone species and are heavily involved in the supply and transfer of energy in mangrove systems and in mangrove ecosystem functioning (e.g., nutrient turnover) (Smith et al., 1991; Bergey and Weis, 2007; Kristensen, 2008). Although several publications have documented the presence of PTEs in fiddler crabs (e.g., Bretler et al., 1981; Zanders and Rojas, 1996; Bergey and Weis, 2007; Pinheiro et al., 2012), none has studied the potential uptake via trophic transfer mechanisms.

Suggested sources of pollutants to fiddler crabs have been identified mainly as water and mangrove detritus (Zanders and Rojas, 1996). Since *U. rapax* also interacts intimately with sediments during feeding, another potential pathway is the interaction of fiddler crabs with polluted sediments. It has been noted that through the process of creating their burrows, the crabs tend to alter the biogeochemistry of sediments by modifying sediment texture (sand, mud), redox reactions, organic matter and drainage (Genomi, 1991; Smith et al., 1991; Zanders and Rojas, 1996; Bartolini et al., 2011). These parameters, in turn, will influence the remobilization, transport and potential bioavailability of PTEs. The fiddler crab, *Uca rapax* in JB, therefore, represents an ideal test species since they are found within the *A. germinans* mangrove zonation.

The main goal of this research was to assess the incorporation of PTEs and to study the uptake mechanisms by *U. rapax*, in JB. The objectives were to assess bioaccumulation and biomagnification by quantifying PTE concentrations in sediments, *A. germinans* leaf litter, and in *U. rapax*.

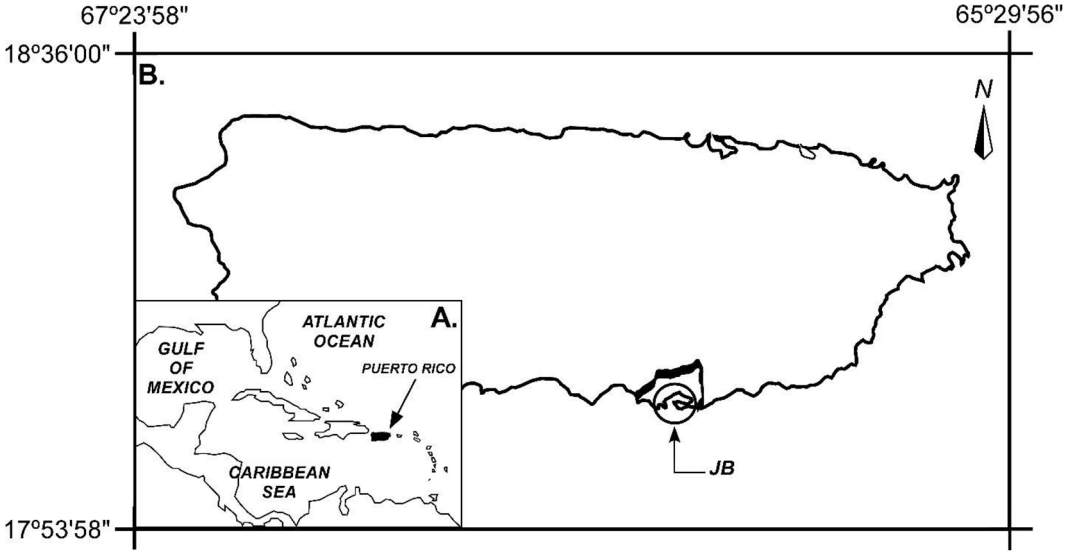

Fig. 1. A. Location of Puerto Rico. B. Map of Puerto Rico. Circle: Jobos Bay (JB). Raised line: JB watershed.

2. Study area

Jobos Bay is the second largest estuary in Puerto Rico. Its dimensions are 6 km east- west and 2 km north–south (Seiglie, 1971) and encompasses a variety of habitats in which mangrove forests, seagrass beds, and coral reefs are the three main sensitive coastal habitats in the reserve (Aldarondo-Torres et al., 2010; Alegría et al., 2016). In addition, the reserve includes a chain of 17 mangrove islets towards the southwest side of the bay (Berman-Santana, 1996). The swamp and marsh sediments of JB are largely muck and peat with a sandy to silty component. Mangroves, comprising most of the superficial area of the estuary, overlay these deposits (Whitall et al., 2011). Given the multiple sources of point and non-point sources of pollution (Table 1), PTEs used in agrochemicals for example are transported via groundwater and surface runoff into JB (Zitello et al., 2008; Pait et al., 2012; Alvaro et al., 2016).

3. Methods

3.1. Field sampling

Three matrices (sediment, mangrove leaf litter, and *U. rapax*) were collected from nine sites (Fig. 2). At each site, ~30 g of *A. germinans* leaf litter was collected (MLL matrix) followed by ~40 g of sediment from the top ~4 cm ($F1_{Tess}$ and $F4_{Tess}$ matrices). For *U. rapax*, a total of 40 male specimens were collected at each sampling site (360 total individuals). Adult specimens were collected by hand and selection was based on propodus size (1.9–2.2 cm). A soil pH meter was used to assess the soils acidity. At each site, the meter was inserted at a depth of 4 cm and this was done three times (average pH reported).

All sample matrices were placed into acid-washed Nalgene containers, frozen and subsequently freeze-dried (sediment and mangrove litter) and oven dried after dissection (*U. rapax*) for further analysis.

3.2. Laboratory sample preparation

3.2.1. Sediment samples

Sediment sub-samples were analyzed for total organic carbon (TOC), $F1_{Tess}$ (exchangeable PTEs) and $F4_{Tess}$ (organic matter-bound PTEs). For purposes of this study, what we reported as $F1_{Tess}$ and $F4_{Tess}$ are the "bioavailable" fractions as defined by Tessier et al. (1979). The choice of the $F4_{Tess}$ fraction is based on the close interaction between organic matter as a food/nutrient source to both *A. germinans* and *U. rapax*. Similarly, the $F1_{Tess}$ fraction is based on how readily PTE's can desorb into surrounding water (e.g., soil water, porewater) within the rhizosphere or at the surface with tidal-induced salinity and pH changes which will affect the PTEs' potential bioavailability (Martínez-Colón et al., 2009, 2018) to both black mangroves and fiddler crabs.

Table 1
Sources of pollution in Jobos Bay.

Source	Type	Location	Reference
Landfill	Solid waste	Salinas municipality	NOAA-CZM (2018)
Recycling facility	Tires	JB visitor center	
Thermoelectric plant	Air pollution	Proximal to JB	
Quarry	Rock, dust	Proximal to JB	
Superfund site	Halogenated solvents	Guayama municipality	
Petrochemical	Benzene	West of JB	
Coal power plant	Ash	Guayama municipality	
Agriculture	Pesticides	North of JB	Berman-Santana (1996)
Pharmaceutical	Chemicals	East-northeast of JB	
Illegal dump sites	Transformers	JB wetlands	
Manufacturing plant	Plastic	1/4-mile North of JB	
Mining	Salt	West of JB	
Thermoelectric Plant	Chlorinated waters	Discharge into JB	
Sewage	Raw	Adjacent to JB	Whitall et al. (2011)
Surface runoff	Oil, pesticides, etc.	Flowing towards JB	
Local tourism	Oil, plastic, etc.	Nearby Municipalities	
Toxic landfill	N/A	Northeast of JB	

The sediment TOC values were determined via loss-on-ignition (LOI) following the protocols of van Dijk et al. (2005). One-gram sub-samples were placed in ceramic crucibles then heated in an oven at 105 °C for one hour to ensure complete dryness and subsequently heated at 550 °C for four hours using a muffle furnace. The result of the dry weight (DW) differences was expressed as percent of organic matter using Heiri et al. (2001) LOI equation:

$$LOI = ([DW_{105} - DW_{550}]/DW_{105}) \times 100 (1)$$

Another one-gram sediment sub-sample from each site was homogenized using an agate mortar and pestle. Subsequently the sub-samples were processed for PTE concentrations through a sequential extraction procedure following the published methodologies of Tessier et al. (1979). In summary, of the five chemical fractions described by Tessier et al. (1979), only the exchangeable PTEs in the $F1_{Tess}$ fraction were extracted using sodium acetate while the organic-bound PTEs in the $F4_{Tess}$ fraction were extracted using concentrated nitric acid (HNO_3) and hydrogen peroxide.

3.2.2. Avicennia germinans mangrove leaf litter (MLL) and Uca rapax samples

For the MLL, two-gram sub-samples were ashed for 12 h at 450 °C following the published protocol of Ramos e Silva et al. (2006). In summary, the ashes were extracted in concentrated HNO_3 (5 mL) while heated at 80 °C until dryness followed by a concentrated hydrochloric acid (HCl) (10 mL) digestion.

All *U. rapax* specimens (~10 per sample) were dissected while partially frozen using a pair of stainless-steel scissors to remove the whole soft-tissue and fluids. After drying (40 °C) the samples were homogenized via agate mortar and pestle to make a composite sample per site. A one-gram sub-sample was digested using 1.5 mL of concentrated HNO_3 (Zanders and Rojas, 1996).

All the extracts from all three matrices were diluted to 30 mL with 3% HNO_3 for trace metal analysis (Cd, Ba, Hg, Pb, V, Cr, Co, Ni, Cu, Zn, As, and Se) and the instrumental analysis was conducted on an Agilent 7700 Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The 7700 ICP-MS was configured with the standard sample introduction system consisting of a Micromist glass concentric nebulizer, quartz spray chamber, and quartz torch with 2.5 mm id injector. The interface consisted of a nickel-plated copper sampling cone and a nickel skimmer cone. A 5-level external calibration (1–100 ppb) was performed for each element prior to analysis of the samples. Calibration solutions were prepared using Environmental Calibration Standard (Agilent Technologies) for Cd, Ba, Pb, V, Cr, Co, Ni, Cu, Zn, As, Se and Multielement Calibration Standard (Agilent Technologies) for Hg. The calibration curves with $R^2 > 0.9990$ were accepted for concentration calculation, otherwise the instrument was tuned again. All analytes were acquired in helium (He) collision mode to reliably reduce/eliminate all common polyatomic interferences using kinetic energy discrimination (KED). The operating conditions of the instrument were as follows: Integration time 0.1 s, sampling period 0.31 s, acquisition time 22.76 s, RF power 1550 W, RF matching 1.78 V, carrier gas 0.9 L min^{-1}, make up gas 0.1 L min^{-1}, nebulizer pump 0.1 rps, He gas flow 4.5 mL min^{-1}. Readings were taken as three replicates.

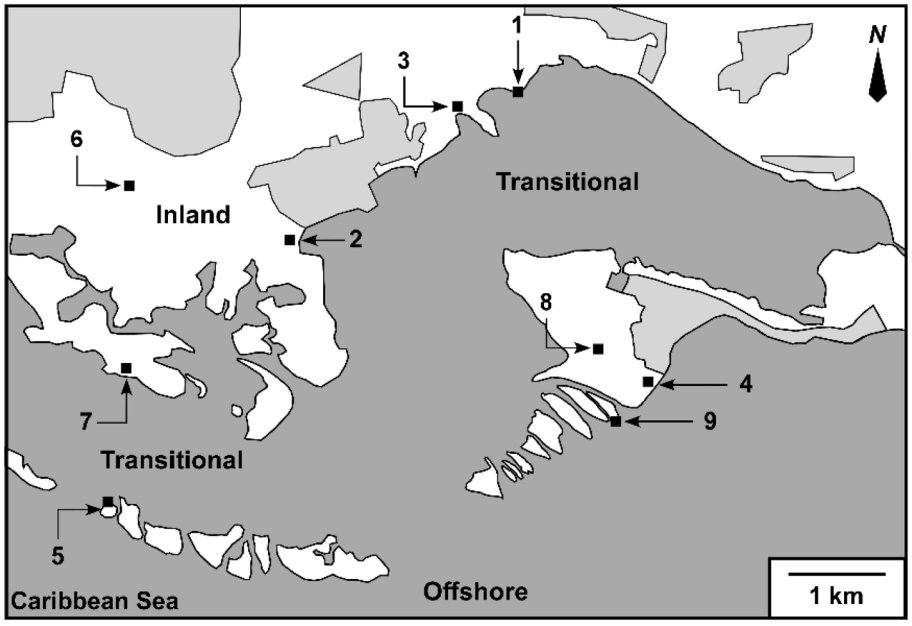

Fig. 2. Sampling stations in Jobos Bay. Gray: seawater. Light gray: anthropogenic development (e.g., housing, agricultural, industrial). Black squares: sampling site.

3.3. Data analysis

The sediment geoaccumulation index (I_{geo}) values were used to evaluate the extent of contamination in all sampling sites. The I_{geo} was calculated using the Muller (1979) equation with regional background crustal (shale) values from Krauskopf and Bird (1995):

$I_{geo} = Log_2$ ([PTE concentration in sediment]/[1.5*PTE concentration in shale])(2)

The factor 1.5 is used to minimize any variations in the background crustal values of shale. The class values of this index are categorized as uncontaminated ($I_{geo} < 0$), uncontaminated to moderately ($0 \leq I_{geo} \leq 1$), moderately ($1 \leq I_{geo} \leq 2$), moderately to strongly ($2 \leq I_{geo} \leq 3$), strongly ($3 \leq I_{geo} \leq 4$), strong to extremely ($4 \leq I_{geo} \leq 5$), or extremely ($I_{geo} \geq 5$) contaminated. This index takes into account the total or bulk PTE concentration in the sediments (e.g., Martínez-Colón et al., 2009). Since we only determined the PTEs in two sediment chemical fractions ($F1_{Tess}$ and $F4_{Tess}$), these were added in order to have a semi-total concentration before calculating the index.

The Biota-Sediment Accumulation Factor (BSAF) was calculated to determine the accumulation of PTEs in *U. rapax* from sediment sources. This index also provides information on how readily bioaccumulated PTEs are in reference to the organism (Judd et al., 2013). In addition, BSAF values are used to classify an organism as: macro-concentrator (BSAF > 2), micro-concentrator (1 < BSAF < 2), and de-concentrator (BSAF < 1) (Dallinger, 1993). Traditionally the BSAF uses the bulk PTE concentration in the sediment (e.g., Kumari et al., 2018) but for purposes of this study the $F1_{Tess}$ and $F4_{Tess}$ sediment fractions are considered to be the most bioavailable to *U. rapax*. The bioconcentration factor (BCF) was calculated to determine the ratio of the PTE concentration in one non-sediment matrix (MLL) to another (*U. rapax*) and values > 1 are indicative of higher and active accumulation of the PTEs (e.g., Wang et al., 2019) into the fiddler crab.

The following equations were implemented to calculate:

$BSAF_{F1Tess} = PTE_{Ur}/PTE_{F1Tess}$(3)

$BSAF_{F4Tess} = PTE_{Ur}/PTE_{F4Tess}$(4)

$BSAF_{F1+F4} = PTE_{Ur}/(PTE_{F1Tess} + PTE_{F4Tess})$(5)

$BCF_{Ur\text{-}MLL} = [PTE_{Ur}]/[PTE_{MLL}]$(6)

The $[PTE_{Ur}]$ is the concentration of a given PTE in *U. rapax*, while $[PTE_{F1Tess}]$ and $[PTE_{F4Tess}]$ represent the PTE concentration in the exchangeable and organic-bound sediment fractions respectively. The $[PTE_{MLL}]$ represents the concentration of a given PTE in *A. germinans* mangrove leaf litter. The following PTEs were not considered for BSAF and BCF due to having two matrices below detection limits (BDL) in most stations which will render inconclusive results: Hg-Pb-Cr-Ni. Zinc was excluded only from BSAF.

A total of 40 variables were analyzed per sample. All PTEs were analyzed in each type of matrix ($PTE_{F1Tess/F4Tess/MLL/Ur}$). The PTE concentrations in each matrix, including TOC, was log-transformed (Log_{10} [1 + X]) (Parker and Arnold, 1999) and a One-way Analysis of Variance (ANOVA) was performed. The *F*-value (32.67) demonstrated that there are significant differences between the medians within a <0.001 confidence. A non-parametric Spearman analysis (alpha = 0.60, n = 9, CI = 95%) using PAST (Paleontological Statistics) was then performed to determine the strength of any associations between variables within each site. The following PTE were omitted from the Spearman analysis because of BDL in two of the matrices: Hg-Pb-Cr-Ni.

4. Results

4.1. Sediment samples

The TOC varied throughout the study sites (Table 2) with an almost three-fold increase ranging from 3.7% on the SE (Site 4) to 10.3% on the NW (Site 2) (Fig. 2). The pH values ranged from 4.3 to 7.2 with Site 7

Table 2
Percent total organic carbon (TOC), pH, and median grainsize. ND = no data. *= sediment "pellets".

Sample	Location (Lat/Long)	TOC	pH
1	17.9602, −66.2121	8.5/10.7*	6.1
2	17.9457, −66.2345	10.3	5.2
3	17.9585, −66.2186	5.7	4.3
4	17.9318, −66.1984	3.7	6.1
5	17.9197, −66.2532	8.9	ND
6	17.9510, −66.2506	9.6	6.4
7	17.9334, −66.2515	5.3	7.2
8	17.9358, −66.2048	3.7	6.8
9	17.9284, −66.2021	7.7	ND

being the only station with slightly alkaline soils. For PTE concentrations, not all metals of concern were found in each sample matrix and sample sites (Supplemental Material Table A.1). In the $F1_{Tess}$ sediment fraction (exchangeable), PTE concentrations varied as follows: Cd (0.002–0.10 ppb); Ba (1.36–35.16 ppb); Hg (BD); Pb (BD); V (1.22–19.87 ppb); Cr (BD); Co (0.09–0.32 ppb); Ni (BD); Cu (0.36–3.80 ppb); Zn (BD); As (0.67–2.64 ppb); and Se (0.91–2.42 ppb) (Fig. 3). Barium in Site 6 was found to have the highest concentration (Fig. 4). In the $F4_{Tess}$ sediment fraction (oxidizable) (Fig. 3), the following ranges were observed: Cd (0.20–3.72 ppb); Ba (57.56–1236.18 ppb); Hg (0.07–262.85 ppb); Pb (0.06–258.73 ppb); V (127.70–1180.34 ppb); Cr (32.47–94.46 ppb); Co (6.50–89.71 ppb); Ni (4.84–229.85 ppb); Cu (13.77–639.85 ppb); Zn (74.20–599.75 ppb); As (3.64–66.80 ppb); and Se (4.43–11.40 ppb). In this fraction Ba has the highest concentration in Site 3 (Fig. 5). In the MLL, the following ranges were observed (Fig. 3): Cd (0.36–3.97 ppb); Ba (28.76–246.89 ppb); Hg (0.60–53.49 ppb); Pb

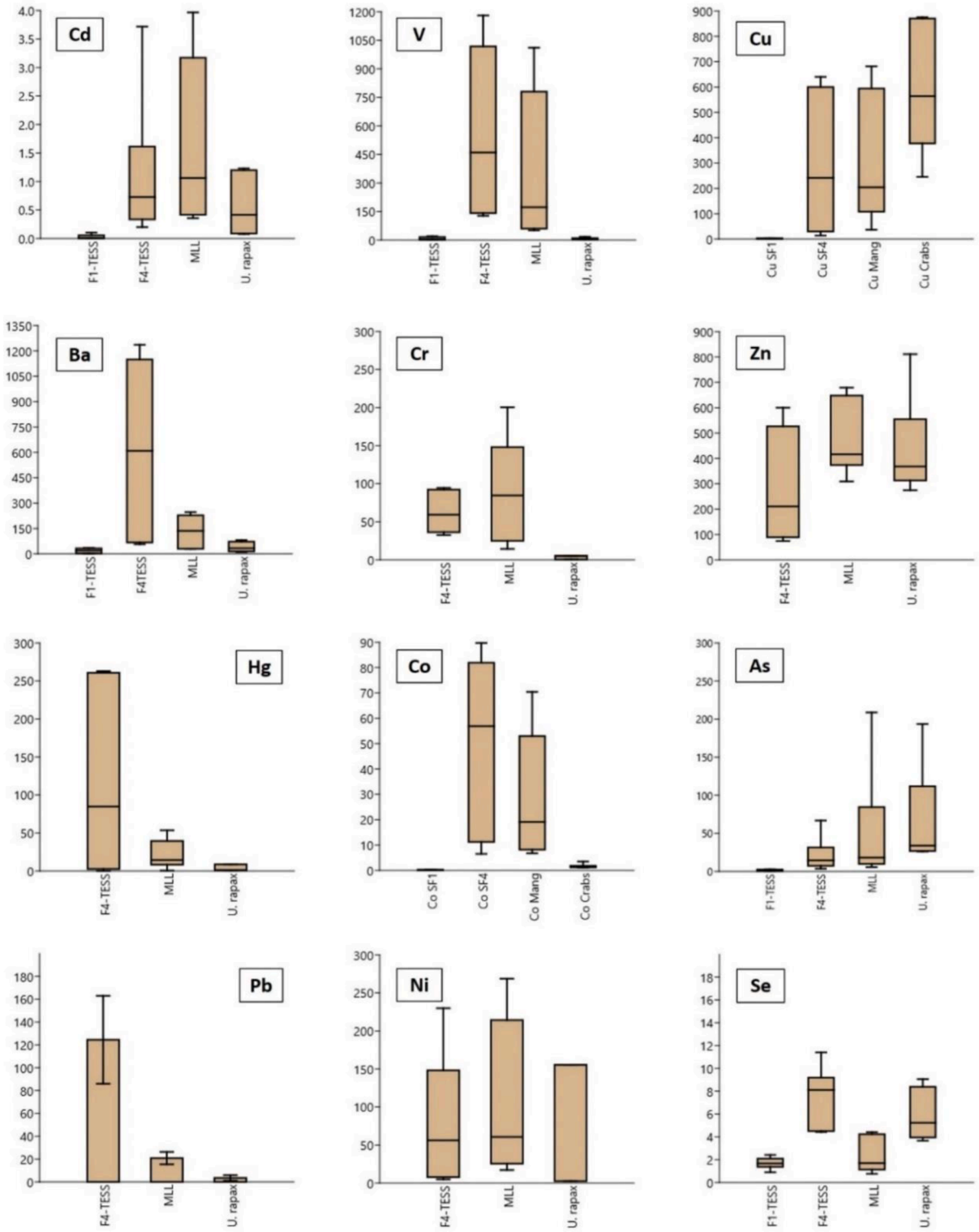

Fig. 3. Boxplots of each PTE per matrix type (in ppb).

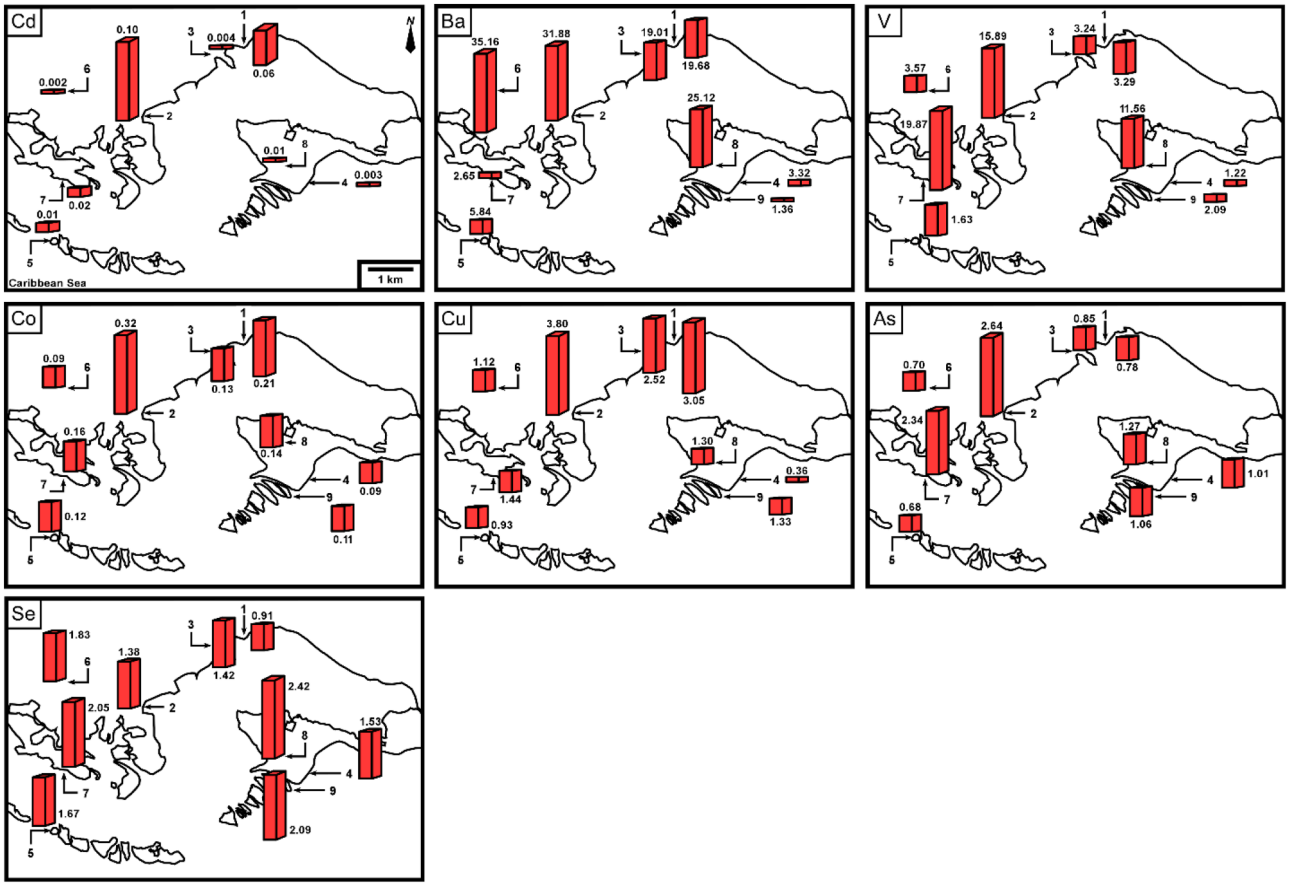

Fig. 4. Distribution maps of PTEs in the $F1_{TESS}$ exchangeable fraction. All concentrations are in parts per billion.

(0.27–47.96 ppb); V (51.08–1010.59 ppb); Cr (14.26–200.17 ppb); Co (6.74–70.40 ppb); Ni (17.22–268.78 ppb); Cu (36.52–681.77 ppb); Zn (309.36–679.06 ppb); As (5.64–208.82 ppb); and Se (0.77–4.42 ppb) (Fig. 3). The highest concentration in this matrix was seen for V and Cu in Site 5 (Fig. 6). In the *U. rapax* tissue fraction (Fig. 3), the following PTE ranges were observed: Cd (0.08–1.23 ppb); Ba (10.18–81.24 ppb); Hg (0.81–8.71 ppb); Pb (0.80–8.26 ppb); V (3.50–16.77 ppb); Cr (0.39–5.11 ppb); Co (1.11–3.49 ppb); Ni (2.58–155.40 ppb); Cu (245.50–875.22 ppb); Zn (274.64–811.08 ppb); As (26.18–193.51 ppb); and Se (3.65–9.05 ppb).

Copper and Zn had the highest concentration in Site 1 and 2 respectively (Fig. 7).

Overall a SE to NW concentration increase can be inferred for the PTEs in all the matrices except for As which is decreasing. The I_{geo} results determined for the combined $F1_{Tess}$ and $F4_{Tess}$ sediment fractions does not exceed a value of 0, deeming the sediments collected in JB as uncontaminated by PTEs. It is important to clarify that this result might be an "artifact" of only using $F1_{Tess} + F4_{Tess}$ fractions instead of total PTE concentration from bulk sediments.

A Spearman rho was calculated for all the PTEs (Supplemental Material Table A.2) of interest. Only As and Co-Se from the $F1_{Tess}$ sedimentary fraction had positive and negative correlation respectively with *U. rapax* tissue while Ba and Se from the same sediment fraction are correlated positively and negatively with MLL and $F4_{Tess}$ fraction respectively. Only three PTEs Cd-Ba-Cu in the $F4_{Tess}$ sediment fraction had a significant positive correlation with *U. rapax* while Cd-V from the same sediment fraction had a positive correlation with MLL. Arsenic in MLL is the only PTE to be positively correlated with *U. rapax*. The TOC had several positive correlations with PTEs in the $F4_{Tess}$ (Cd-V- Co-Cu-Se) and with Ba-Cu in the MLL matrix while Cu-Se in *U. rapax* tissue correlated positively with TOC. Arsenic in MLL and U. rapax tissue is the only contaminant to have a significant negative correlation with TOC.

4.2. Biota-sediment accumulation factor and bioconcentration factor

The BSAF for the PTEs considered in this study are summarized in Supplemental Material (Table A.3). We wanted to assess the contribution of both PTE fractions independently and combined to categorize at what level is *U. rapax* a bioconcentrator. In several of the sampled stations, *U. rapax* is a macro-concentrator for Cd-Ba-V-Co-Cu-As-Se ($F1_{Tess}$ fraction) while in three stations it is considered a de-concentrator in relation to Ba-V. For the $F4_{Tess}$ fraction the fiddler crab is a macro-concentrator (Cu-As), de-concentrator (Cd-Ba-V-Co-Se) in almost all the stations except for Cu in only two of the stations. The fiddler crab behaves then as a micro-concentrator of Cd-Cu-As-Se in only four stations. As suspected, *U. rapax* is categorized as a de-concentrator with respect to most PTEs in all the stations when combining the effects of the $F1_{Tess}$ and $F4_{Tess}$ fractions except for Cu-As which responds as a macro-concentrator in six stations and as a micro-concentrator in three stations.

The BCF values are shown in Supplemental Material (Table A.3). Only Cu (0.89–13.20)-As (0.37–10.49)-Se (0.95–5.56) have values > 1 in almost all the sampled stations. Copper with a 15-fold increase is the most bioconcentrated PTE in Site 4 followed by As in Site 7 with a 28-fold increase and Se at Site 2 with a 6-fold increase (Fig. 8).

5. Discussion

5.1. PTE transport and bioavailability

Following the nomenclature of Tessier et al. (1979), PTEs could be found in five different fractions based on the degree of operational bioavailability (see Martínez-Colón et al., 2009, 2018 for further

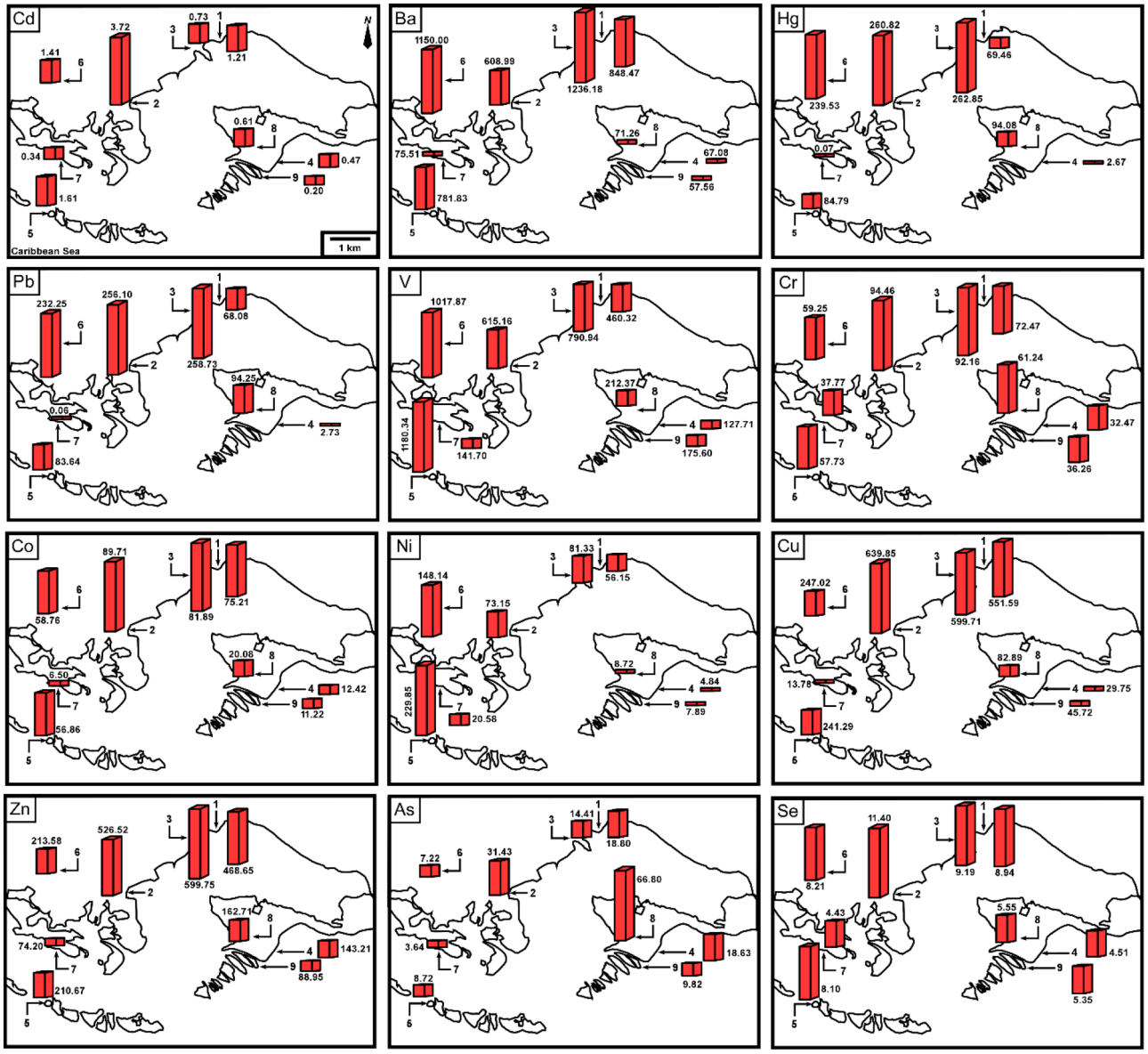

Fig. 5. Distribution maps of PTEs in the $F4_{Tess}$ organic-bound fraction. All concentrations are in parts per billion.

explanations). The choice of the $F1_{Tess}$ exchangeable and $F4_{Tess}$ oxidizable (organic-bound) fractions in this study was based on the relationship between sediment, *A. germinans*, and *U. rapax*. Mangrove zonation is integral in the distribution of sediments (e.g., Furukawa et al., 1997) and other physiochemical parameters (e.g., sediment pH, porewater salinity) that subsequently play an important role in the biogeochemistry of pollutants (Alegría et al., 2016; Marchand et al., 2016). In JB the sediments associated with *A. germinans* are organic sandy-silty muds. This sediment type coupled with organic matter content is crucial in the fate/transport of PTEs but most importantly on their bioavailability. Sediments serve as "sinks" of PTEs based on their surface area and chelating/cation exchange capacities (e.g., Martínez-Colón et al., 2009; Pinheiro et al., 2012). Depending on the chemical profile of the environment (e.g., pH, salinity, redox), PTEs could be immobilized into different chemical fractions (e.g., iron sulfides) which prevents absorption by flora/fauna (Lacerda et al., 1993; Pinheiro et al., 2012) or be more readily available if found in other fractions like adsorbed to clay-size sediments ($F1_{Tess}$) and organic matter ($F4_{Tess}$) (e.g., Bacon and Davidson, 2008; Zimmerman and Weindorf, 2010) or in solution (e.g., Lacerda et al., 1993; Martínez-Colón et al., 2009). Although sediments are not the only avenue in which PTEs enter food chains, vegetation plays a key role. For example, the mangrove's rhizosphere serves as a depuration mechanism of extracting PTEs, and other pollutants, from water and sediments that eventually are translocated from the root towards leaves and seeds (Lacerda et al., 1993; Lacerda, 1997; Ramos e Silva et al., 2006; Pinheiro et al., 2012; Alegría et al., 2016; Marchand et al., 2016). Vegetation translocation processes allow for PTEs bioconcentration in leaves from which subsequently they are relocated back to the soils as litter (e.g., MacFarlane et al., 2007) increasing their bioavailability for detritus feeders like *U. rapax*. For example, under anoxic conditions sulfate-reducing bacteria promotes the methylation of Hg making mangrove trees sources of methyl-mercury (Bayen, 2012). The PTEs found in the $F1_{Tess}$ fraction are readily adsorbed/desorbed (e.g., remobilized) due to pH and salinity changes (e.g., Martínez-Colón et al., 2009; Bayen, 2012) induced by tidal changes and as porewater floods the *U. rapax* burrows, the sediment–water interface is constantly migrating. The fact that fiddler crabs irrigate the sediments due to excessive bioturbation (Nielsen et al., 2003) also adds to the remobilization of PTEs due to redox changes coupled with sediment texture modification (Botto and Iribarne, 2000) among others although

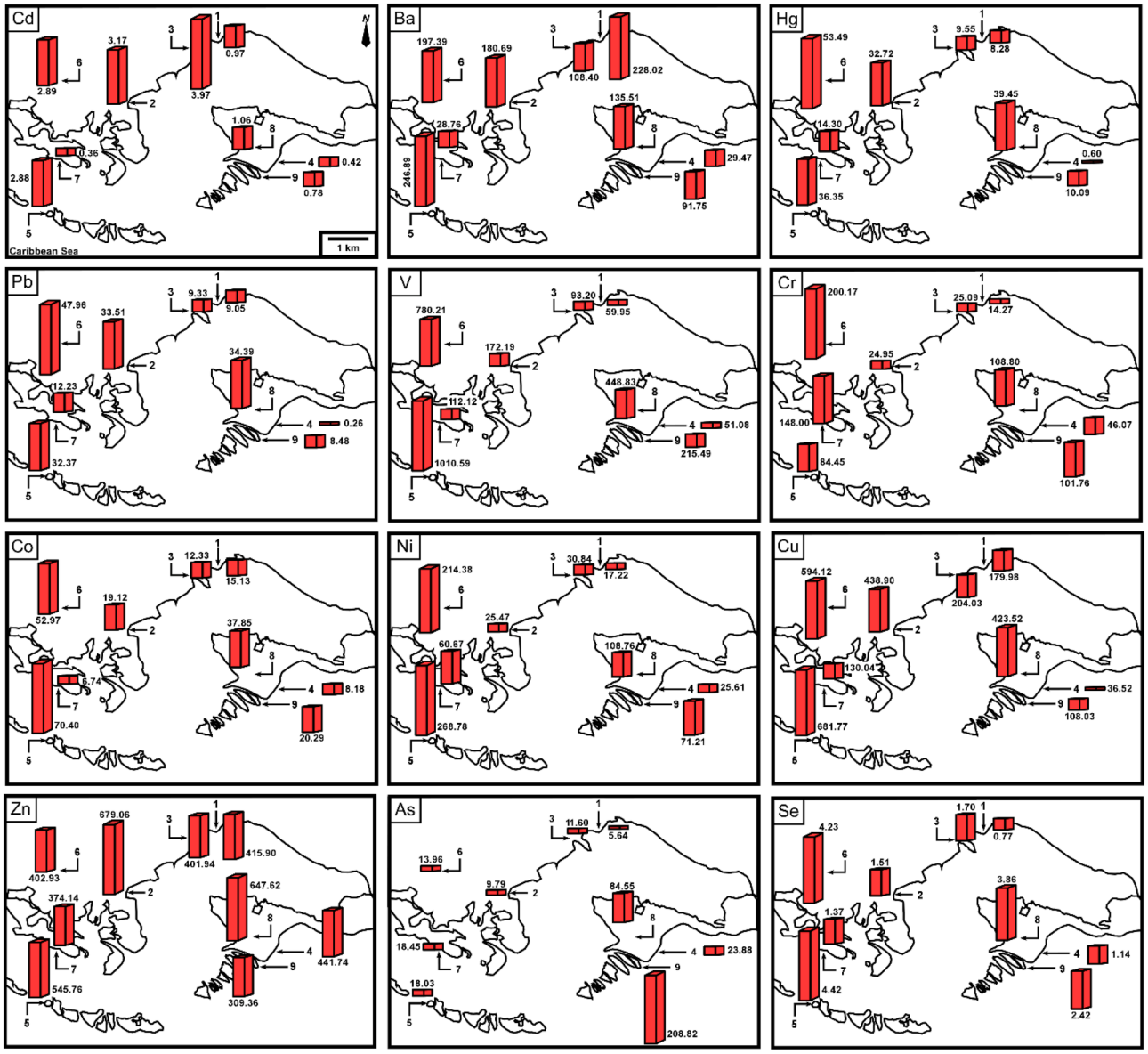

Fig. 6. Distribution maps of PTEs in MLL (mangrove leaf litter). All concentrations are in parts per billion.

Michaels and Zieman (2013) concluded that burrows do not have a noticeable effect on sediment aeriation. Fiddler crabs are known deposit feeders foraging on different food sources, but discrepancies exist on the type of food supply. It has been reported that leaf litter serve as a food source (e.g., Kristensen, 2008) for fiddler crabs and it has been reported that 20% of the *Uca Pugnax*'s diet is vascular plants (Ringold, 1979). For example, in JB it was found that sediments associated with red mangroves (*Rhizophora mangle*) consist mostly of C3 vascular plants (Demopoulos et al., 2007). It is logical to assume in JB that the MLL derived from *A. germinans* dominated mangrove sediments serve as an autochthonous food source especially since these have relatively high amounts of organic matter (Table 2). However, isotopic work in JB and in Joyuda Bay (Puerto Rico west coast) demonstrated that the food used by fiddler crabs consists partly of leaf litter (equivalent to MLL), particulate organic matter (equivalent to $F4_{Tess}$), and benthic microalgae (France, 1998; Demopoulos et al., 2007). The production of MLL is expected to reduce the dissolve oxygen of the surface sediments and within burrows which plays an important role in the fate and transport of PTEs (Ramos e Silva et al., 2006) especially when burrows are made in thick accumulations of decomposed leaf litter (Supplemental Material Fig. A.1). Although it has been reported that burrows promote sediment oxidation (e.g., Bertness, 1985), Michaels and Zieman (2013) concluded that salt marshes burrowed by *Uca* have a minimal effect on aeration especially when burrows are waterlogged (very limited oxygen diffusion) and if not, they retain pore- as well as hygroscopic water due to capillary forcing. Examining the PTE concentrations in the organic-bound $F4_{Tess}$ as well with $F1_{Tess}$ is a promising approach since each provides a different perspective on bioaccumulation and potential biomagnification under the assumption that the two sediment fractions are most bioavailable to *U. rapax* rather than relying on bulk or total PTE concentrations.

The amount of TOC is relatively high between the sampled sites. Most of the PTEs (except Ba-Zn-As) are found to be complexed with organic matter in the $F4_{Tess}$ fraction as supported by the positive correlation with TOC. In particular, the Sites 1–2, 5, 6, and 9 were expected to have the highest PTE concentration due to their elevated TOC values (8.5–10.3%) (Supplemental Material Fig. A.1). However, this interpretation was only observed in Sites 1–2 and 6. The most likely scenario is that these three sites have a greater influence of terrestrial organic matter in addition to their proximity to point- and non-point sources of

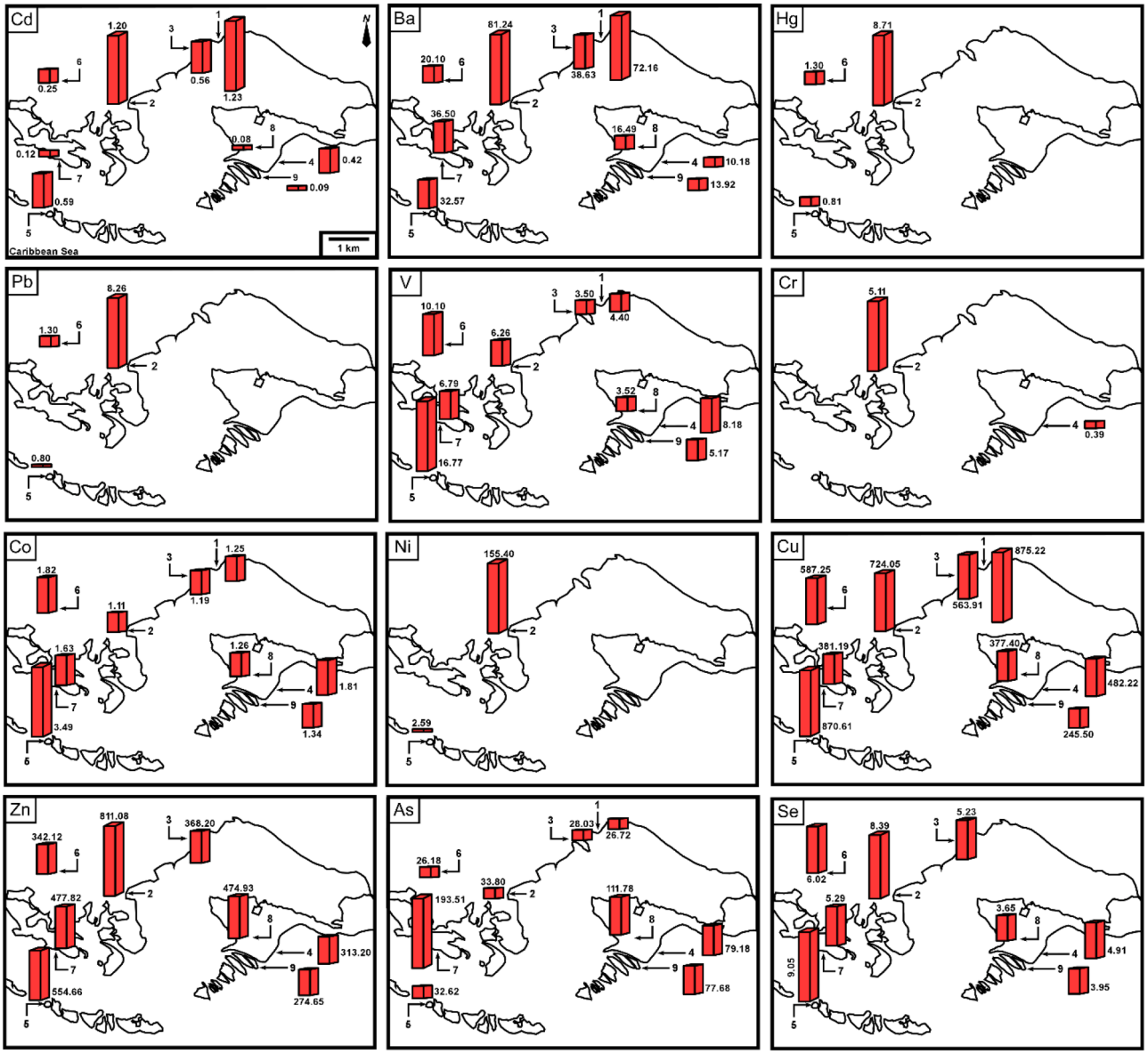

Fig. 7. Distribution maps of PTEs in *U. rapax* whole body soft tissue. All concentrations are in parts per billion.

pollution (e.g., agriculture) in JB (Fig. 2). If considering the amount of organic matter, sediment pH (mostly acidic), and the nature of redox conditions in mangrove forest soils, it is possible that these conditions limited PTE adsorption towards mud-size sediments and preferentially allowed the sequestration and/or co-precipitation of PTEs with Fe-Mn-Al oxyhydroxide minerals (e.g., goethite) or Fe-sulfides (e.g., pyrite) rendering the pollutants with a much lower bioavailability (e.g., Martínez-Colón et al., 2009).

An unexpected finding was made at Site 1. Two sizes of sediment "pellets" were observed in proximity to *U. rapax* burrows. The "pellets" with the largest size were collected for TOC and PTE analysis ($F1_{Tess}$ and $F4_{Tess}$). In the literature these "pellets" have been described as "feeding balls", "pseudo-feces" or excavated "mud balls" with the latter being the largest in size (e.g., Ringold, 1979; Weis and Weis, 2004). These "pellets" contained up to 4x the concentration of certain PTEs when compared to the values in the $F1_{Tess}$ fraction: Cd (2.5x), Co (2.4x), and Se (3.6x) (Supplemental Material Table A.1). The other PTEs had slightly higher concentrations in the same fraction except for Zn which had an insignificant lower concentration (−1.04x). In the $F4_{Tess}$ fraction, PTEs had insignificantly higher (<1.2x) or lower (<−1.3x) concentrations.

This is puzzling as an explanation could be related to how the fiddler crab engineer's its burrow which varies in depth between 10 and 40 cm (Kristensen, 2008). It is uncertain from what depth these "pellets" were excavated from, but it is hypothesized that could have come from depths >4 cm (deeper than normal sampling depth) below the redox boundary. Although the average high tide is 12 cm (Zitello et al., 2008), Site 1 is not flooded due to being on an irregular topographic high (17 cm above sea level). However, it is located adjacent to a tidal channel, so seawater does migrate into the *A. germinans* zonation via groundwater movement thus potentially creating a sediment redox stratification that fluctuates with groundwater tidal intrusion. Gueiros et al. (2003) and Banerjee et al. (2012) described the vertical migration of the redox boundary in mangrove forests from Brazil and India respectively as an effect on the fate and transport of PTEs resulting in co-migration of these contaminants between oxidized and anoxic sediment layers. Assuming that a similar situation is happening at Site 1, PTEs could be constantly remobilized between sediment chemical fractions (e.g., clay-oxyhydroxide adsorption/desorption; carbonate-bound PTE dissolution) and be re-sequestered and complexed by organic matter within the burrows. Although TOC values in the "pellets" (10.7%) were higher than

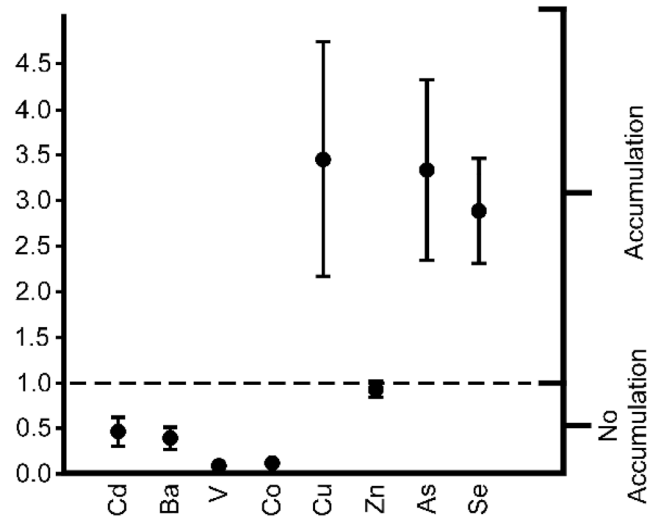

Fig. 8. Box plot of PTE mean values.

in the sediment (8.1%), the $F4_{Tess}$ PTEs in these "pellets" have insignificant higher concentrations suggesting anoxic conditions triggering co-precipitation with sulfide minerals (e.g., pyrite) which lowers their bioavailability within the burrow. In addition, this suggests that those "pellets" where, at the time collection, recently excavated so not enough time had passed for the sulfide minerals to be oxidized and the PTEs to be leached out and remobilized. In the case of $F1_{Tess}$, numerous authors have described how mud-size sediments and clay mineralogy have a strong affinity with PTEs (e.g., Martínez-Colón et al., 2009; Bayen, 2012) and how these help in organic matter flocculation which also increases adsorption (e.g., Lacerda et al., 1993; Marchand et al., 2006). Lacerda et al (1993) reported higher PTEs (up to 10x) in the exchangeable fraction ($F1_{Tess}$ equivalent) from the rhizosphere of *Avicennia* when compared to *Rhizophora* mangroves in Brazil. It has also been described that *Avicennia* can oxidize the sediments within its rhizosphere thus "aerating" (oxygen diffusion) them which limits PTE-bound sulfide precipitation (Marchand et al., 2006) and enhances sulfide dissolution and subsequent PTE adsorption onto mud-size exchangeable sediments (Lacerda et al., 1993). In the case of Site 1, it is speculated then that the fiddler crabs while excavating, are translocating organic-rich mud-size sediments from an undetermined depth from which there is a strong variability in oxidation and reduction processes that preferentially allows a higher adsorption of Cd-Co-Se to the $F1_{Tess}$ fraction.

5.2. PTE bioaccumulation and biomagnification

The fate and transport of pollutants from the environment towards an organism is controlled by several abiotic (e.g., redox) processes that determines their bioavailability. This is also enhanced by biological processes such as active (e.g., food chain) and passive (e.g., diffusion) incorporation of pollutants (e.g., Martínez-Colón et al., 2009 and references therein) which are key factors on how an organism becomes inadvertently a bioconcentrator within its environment. Based on the I_{geo} values, the studied sites are considered not polluted with respect to the bioavailable PTEs found in the $F1_{Tess} + F4_{Tess}$ fractions. Nonetheless, *A. germinans* and *U. rapax* have bioaccumulated PTEs (Fig. 3). In assessing the potential level of accumulation, the average BSAF values are not uniform between matrices as expected. Supplemental Material (Table A.4) show contrasting results as seen that *U. rapax* behaves like a macro- and de-concentrator for the same PTE when considering the $F1_{Tess}$ and $F4_{Tess}$ fractions. Given that PTEs in these two fractions are considered more bioavailable, the BSAF calculations combining both fractions produced realistic results. In this new light, *U. rapax* is behaving as a de-concentrator for most PTEs except for As-Cu which is behaving as a macro-concentrator. Arsenic is the only PTE in the $F1_{Tess}$ fraction to be positively correlated with *U. rapax* suggesting that the main source of this pollutant is coming from this fraction as is being retained more in their soft tissue (retention > depuration) (As concentration in *U. rapax* is 50x greater than in $F1_{Tess}$). Arsenic has been reported in marine organisms in its organic form- arsenobetaine which is non-toxic (e.g., Cullen and Reimer, 1989) and comprises 75–100% of the total As found in crustaceans (Popowich et al., 2016). In the case of Co, it is explicit that it is aggressively bioaccumulated but there is no evidence to suggest biomagnification. It is the only pollutant with a negative correlation which suggests a greater depuration process (e.g., molting) when compared to the other PTEs. Like As, Cd-Ba-Cu in $F4_{Tess}$ are positively correlated with *U. rapax* suggesting that organic matter is one source especially for Cu (Supplemental Material Table A.2) which has also a positive correlation with TOC. However, Cd-Ba are readily deconcentrated (retention < depuration) while Cu is being bioaccumulated (retention > depuration). It is important to highlight that Cu is an essential nutrient as it is found in the hemocyanin molecule that facilitates oxygen transport in the bloodstream of decapods (e.g., Mangum, 1993). It is very probable that the observed Cu correlation between sediment fractions, TOC, and *U. rapax* and its behavior as a macro-concentrator is due to the natural background concentrations found in hemocyanin. However, Capparelli et al. (2016) found PTEs bioaccumulated in the gills and hepatopancreas of *U. rapax* with Cu having the highest concentrations attributed to sediment pollution. Capparelli et al. (2017) reported that *U.* (*Minuca*) *rapax* is capable of regulating Cu but concluded that bioaccumulated (gills, hepatopancreas, and hemolymph) waterborne Cu (>150 ppb) is an osmoregulatory toxicant. Given the high bioaccumulated amounts of Cu (245–875 ppb) found in the whole soft body tissue and not knowing the background concentration in hemocyanin, it is difficult to assess if Cu is biomagnified even though *U. rapax* has 2x the average concentration of that found in the $F4_{Tess}$ organic fraction.

Our data supports that *A. germinans* (MLL detritus) is bioaccumulating PTEs such as Cd-Ba-V as demonstrated by their positive correlation with $F1_{Tess}$, $F4_{Tess}$, or TOC (Fig. 3, Supplemental Material Table A.2). It has been reported that *Avicennia* accumulates higher amounts of PTEs in its leaves when compared to *Rhizophora* (Lacerda et al., 1986, 1993) but it is ~one-two orders of magnitude lower than surrounding sediments (MacFarlane et al., 2007). Given the strong affinity of these PTEs towards Cl-ion ligands, the formation of metal-chloride complexes could be an accidental depuration mechanism in *A. germinans* (salt excretion) which has been described in *Avicennia* marina (MacFarlane et al., 2007). This could explain why Cd-Ba-V are not bioconcentrated in *U. rapax* (Supplemental Material Table A.4). It is only Cd-As-Se to be found bioconcentrated in *U. rapax*. However, PTE biomagnification (Fig. 8) is inferred solely for As given that it is the only bioconcentrated pollutant to be correlated positively with MLL and *U. rapax*.

6. Conclusion

Our results indicate that the sediments from the sampled sites in JB are not polluted with respect to Cd-Ba-V-Co-Cu-Zn-As-Se when considering I_{geo} values which is supported by low PTE concentrations (ppb) found between the fiddler crab *U. rapax* and the matrices $F1_{Tess}$-$F4_{Tess}$-MLL. In addition, the physiochemical characteristics of the sediments (e.g., redox, salinity) further limited the bioavailability of the PTEs. This study showed that the black mangrove *A. germinans* has indeed bioaccumulated PTEs found in the surrounding sediments which has been translocated to its leaves (MLL) thus subsequently rendering these pollutants more bioavailable so they could be bioaccumulated by *U. rapax* aside from the other different matrices. However, treating the bioavailable $F1_{Tess}$ and $F4_{Tess}$ fractions separately just to assess biota-sediment transfer of PTEs was limiting in scope and contradicting

since for example *U. rapax* cannot de-concentrate the same pollutant from different matrices. Combining both matrices determined that *U. rapax* is indeed a macro-concentrator of just Cu-As and coupled with Se they are found to be bioconcentrated in the soft tissues of the fiddler crabs. Arsenic is the only PTE that seems to be biomagnified via trophic transfer mechanism when *U. rapax* relies on *A. germinans* detritus (MLL) as part of their diet.

CRediT authorship contribution statement

Michael Martínez-Colón: Conceptualization, Methodology, Investigation, Formal analysis. **Henry Alegría:** Conceptualization, Investigation, Writing - review & editing. **Ashley Huber:** Writing - original draft, Investigation, Formal analysis. **Hatice Kubra-Gul:** Formal analysis. **Perihan Kurt-Karakus:** Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.107038.

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