Case studies on tropical benthic foraminifera assemblages in coastal marine environments under anthropogenic stress for the investigation of primary controlling factors in their diversity and distribution patterns

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

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Bonn, November 2022

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der

Rheinischen Friedrich-Wilhelms-Universität Bonn

Gutachter:	Prof. Dr. Martin R. Langer
Zweitgutachter:	Prof. Dr. Thomas Martin
Tag der Promotion:	16.12.2022
Erscheinigungsjahr:	2023

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Zusammenfassung

Benthische Foraminiferen kommen in allen Meeresbereichen vor, von brackigen Flussmündungen bis zu den Tiefseebecken. Sie sind unschätzbare Werkzeuge in der Paläoklimatologie, Paläozeanographie, Biostratigraphie und in der modernen ökologischen Forschung, wo sie zur Untersuchung der Funktionsweise von Ökosystemen und ihrer Wechselwirkung mit anthropogenen Eingriffen verwendet werden. Darüber hinaus kann die strukturelle und numerische Analyse benthischer Foraminiferen-Ansammlungen tiefere Einblicke in die Kombination von Umweltvariablen liefern, die ihre Zusammensetzung steuern, mit neuen Perspektiven zur Rekonstruktion vergangener Umgebungen. Unter den von benthischen Foraminiferen besetzten Gebieten sind tropische Ökosysteme äußerst produktiv, erhalten die Artenvielfalt, schützen die Küsten, binden Kohlenstoff und bieten der Küstenbevölkerung Lebensgrundlagen. Diese Doktorarbeit untersucht die Rolle benthischer Foraminiferen mit dem Ziel, besser zu verstehen, wie diese Ökosysteme funktionieren und sich unter menschlichem Einfluss verschlechtern. Dies wurde erreicht, indem das benthische Foraminiferen-Inventar der untersuchten Standorte durch sorgfältige taxonomische Analyse erstellt und die Struktur und numerischen Parameter von benthischen Foraminiferen-Ansammlungen in Bezug auf physikalische und chemische Daten wie Temperatur, pH-Wert, Tiefe, potenziell toxische Elemente (PTEs) und insgesamt gelöste Feststoffe (TDS) untersucht wurden. Diese Untersuchungen ergaben, dass i. stark salzhaltiges Ozeanwasser, das ständig tief in Küstengewässer (Flussmündungen oder Lagunen) eindringt, die Zusammensetzung verschiedener, artenreicher Habitate strukturiert, und die Salinität als wichtigste treibende Kraft für die Zusammensetzung benthischer Foraminiferenfaunen angesehen werden muss. Der pH und die im Waser gelösten Feststoffe (TDS) spielen hierbei eine untergeordnete Rolle; ii. die strömungs-, wellen- oder anthropogen induzierte Umverteilung benthischer Foraminiferentaxa ist begrenzt; benthische Foraminiferen-Ansammlungen können die ursprünglichen Gemeinschaftsstrukturen und ausreichende Umweltinformationen bewahren, die für Biomonitoring und paläoökologische Studien nützlich sein können, iii. Es wurde festgestellt, dass die von anthropogener Verschmutzung betroffenen Standorte durch spezifische benthische Foraminiferenfaunen und Taxa gekennzeichnet sind, was auf eine erhöhte Toleranz gegenüber mehreren Stressoren hinweist und ein Repertoire an Bioindikatoren bereitstellt, die bei zukünftigen Studien mit Schwerpunkt auf Umweltstörungen hilfreich sein können, iv. Die Verwendung von Gesamtkonzentrationen von PTEs allein als Werkzeuge für das Biomonitoring wird in Frage gestellt. Insbesondere bio- und nicht bioverfügbare Fraktionen von PTEs sollten durch Korrelation mit benthischen Foraminiferenparametern, einschließlich Abundanz, Artenreichtum und Diversitätswerten von Foraminiferen-Ansammlungen, abgeglichen und genutzt werden.

Summary

Benthic foraminifera are found in all types of marine environments, from brackish estuaries to the deep ocean basins. They are invaluable tools in paleoclimatology, paleoceanography, biostratigraphy, and in modern ecological research, where they are used for investigating ecosystem functioning and its interaction with anthropogenic interference. Moreover, structural and numerical analysis of benthic foraminiferal assemblages can provide deeper insights into what combination of environmental variables control their composition with new perspectives to reconstruct past environments. Among realms occupied by benthic foraminifera, tropical ecosystems are extremely productive ones, maintaining biodiversity, protecting coastline, sequestering carbon and providing livelihood to coastal populations. This doctoral thesis looks into the role of benthic foraminifera with the goal of better understanding how these ecosystems function and deteriorate under human impact. This has been accomplished through establishing the benthic foraminiferal inventory of the sites under investigation through careful taxonomical analysis, and studying the structure and numerical parameters of benthic foraminiferal assemblages in relation to physical and chemical data, such as temperature, pH, depth, total dissolved solids (TDS) and potentially toxic elements (PTEs). This investigation resulted in establishing that i. highly saline ocean waters consistently penetrating deep into coastal water bodies (estuaries or lagoons) promote compositionally diverse and species-rich biotas of benthic foraminifera, therefore salinity is the main driving force structuring and separating benthic foraminiferal assemblages, whereas pH and TDS are not observed to have a major controlling effect, ii. the current-, wave-, or anthropogenically induced redistribution of benthic foraminiferal taxa is limited; benthic foraminiferal assemblages may preserve the original community structures and sufficient environmental information that can be useful in biomonitoring and paleoecological studies, iii. the sites impacted by anthropogenic pollution are found to be characterized by specific benthic foraminiferal assemblages and taxa, indicative for enhanced tolerance levels to multiple stressors, providing a repertoire of bioindicators that can assist in future studies focusing on environmental perturbations, iv. using total concentrations of PTEs alone as tools for biomonitoring may not be useful, but especially bio- and non-bioavailable fractions of PTEs should be utilized through correlation with benthic foraminiferal parameters including abundance, species richness and diversity values of foraminiferal assemblages.

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Acknowledgements

I would like to express my deepest gratitude to my *Doktorvater* Prof. Martin R. Langer for all the scientific and life experience he has been sharing since my very first day in Bonn and also for his endless patience with me. How lucky I am to have been his student for the last four years and what an honor to be part of his scientific legacy for the rest of my professional career.

I am also grateful for having the pleasure of getting to know and working with Prof. Philippe Steemans. I very much appreciate his generosity in sharing knowledge and providing support.

I would like to thank my dear colleagues, Dr. Olugbenga T. Fajemila, Dr. Michael Martinez-Colón, Dr. Anna Weinmann, Ruta Stulpinaite, Gustavo Pereira, Iliya Bauchi Danladi and Christoph Steinhoff for their collaboration, support and company during my doctoral studies. My thanks also go to Georg Oleschinski for his helpfulness and patience while teaching me how to use an SEM, to Peter Göddertz for all his technical help, to Elke Haque for her help and hospitality, to Beate Mühlens-Scaramuzza and Dagmar Hambach for all their help and for creating the loveliest secretariat I have ever seen, to Olaf Dülfer for turning my meteoritewrecked belemnite into a beauty and to Luis Pauly for preparing a nice vitrine for it, and to all my young colleagues who attended the classes I either helped with or taught myself, also to the ones with whom I attended field trips to various corners of Germany, for inspiring me.

I cannot end this chapter without thanking my beloved family: my parents, my brother, my cat and last but definitely not least, my partner. Their unending support and love always managed to give me the motivation I needed during this unique and transforming journey.

Chapter 1

Introduction

Research done for this thesis focused on the use of benthic foraminifera assemblages as proxies for environmental assessment from two localities using different aspects, such as structure composition, diversity, relationship with geochemical parameters of the water to assess the ecological state of the coastal environments from Lagos Lagoon (Nigeria) and Mamanguape River (Northern Brazil), and resulted in the publication of three articles (Fajemila et al., 2020; Sariaslan and Langer, 2021; Fajemila et al., 2022).

In the first chapter, an introduction to the study localities is given, with a history of previous studies that took place at them. In the next part, a comprehensive survey of benthic foraminiferal proxies established so far is given. The chapters following the first one, the major findings of the studies making up this doctoral thesis are given, with a discussion of these findings in light of most recently published relevant studies. In addition to my doctoral research focusing on modern environments, I have also conducted paleoenvironmental research on miospores from Devonian successions of the Eifel Mountains as a side project and the last chapter of my doctorate, which is in review process and soon to be published.

1.1. Study Localities

1.1.1. Lagos Lagoon (Nigeria)

Lagos Lagoon (Fig. 1) is part of a continuous system of lagoons and creeks situated along the coast of Nigeria. It is located between longitude 6° 29' 58" N and 3° 28' 48" E. It has been reported to have a surface area more than 6,000 km², extending from the border of the Republic of Benin to the Niger-Delta (Badejo et al., 2014). It cuts across the southern part of the metropolis, linking the Atlantic Ocean (in the West and South) and Lekki lagoon (in the East). It is tidal and shallow with an average depth of 1.5 m except for the channels that are

continually dredged to accommodate intense and heavy water traffic (Adejare et al., 2011). It experiences restricted marine and mainly low salinity, brackish and freshwater conditions. Salinity varies substantially with the wet and dry seasons and is strongly impacted by the introduction of fresh water from rain, rivers and saline water from the ocean. The river input is so large during the raining season, that the lagoon is fresh to brackish throughout and salinities in the central lagoon area drop to 3‰, to below 1‰ in the eastern sector and to 0‰ at the mouth of the Ogun River (Coastal Profile of Nigeria, 1997). Minimal salinity values are recorded during the high rainfall months (July, August, September) and higher values are present during the dry season, accompanied with bottom water hypoxia events in the eastern lagoon sector (Alo et al., 2010). In general, the western sector of the lagoon experiences higher salinity because of its interactions with the Atlantic Ocean. The lagoon floor is covered by a wide variety of mixed deposits containing different proportions of coarse sand, fine sand, silty mud, and mud that can be organic-rich and abundant in mollusk shells (Hill, 1958).

Lagos Lagoon represents one of Africa's largest estuarine ecosystems, bordered by one of the fastest growing megacities in the world with more than 20 million inhabitants, and is the ultimate repository of contaminants carried in industrial, municipal and agricultural wastes. This resulted in the deterioration of water quality posing serious risks to human health. Moreover, lagoon ecosystems and livelihood of the coastal population have been adversely affected. As a result, Lagos Lagoon has become the most polluted African ecosystem (data from WHO and Africa UN Environment). The most densely populated areas including clusters of industry are spread along the lagoon's southwestern and western shorelines. The industrial complex around Lagos Lagoon includes textile, brewery, petrochemical factories, logging and metal industry, power plants, paper mills, and sawmills from which untreated effluents drain into the lagoon through creeks and underground canals. This has resulted in high concentrations of heavy metals (e.g., copper, zinc, manganese, lead, iron, nickel), i.e. potentially toxic

elements (PTE). Polycyclic aromatic hydrocarbons (e.g., naphthalene, phenanthrene, pyrene) have been found at considerable levels within the polluted western section of the lagoon (e.g. Ekett et al., 2018, Sogbanmu et al., 2016). Excessive sand mining and dredging activities contribute further to the disruption of the lagoon ecosystem. The lagoon is heavily exploited by fishing activities and aquaculture, leading to further environmental degradation and accompanying changes in water quality with biological consequences for biotas in the environment.



Figure 1. Simplified map of Lagos Lagoon showing its non-parallel alignment to the Gulf of Guinea coastline over 237 km.

1.1.2. Mamanguape River Estuary (Northeastern Brazil)

The Mamanguape River estuary is located on the north coast of the Paraíba State (Brazil) and extends for 25 km in east-west direction and for 5 km in the north-south direction. It is part of the Environmental Protection Area (Área de Proteção Ambiental - APA) of Barra de Mamanguape (Fig. 2). The regional climate is classified by Köppen (Clark and Pessanha, 2015)

as *As-type* (hot and humid). The rainy season begins in February and lasts until July, with maximum rainfalls occurring from April to June, whereas the dry season occurs in the spring and summer, with the lowest rainfalls occurring between October and December. The average rainfall recorded in the area is between 1,750 and 2,000 mm annually, and the average temperature is approximately 24-26°C. There is a well-preserved mangrove forest in the area, composed of *Avicennia germinans* (Linnaeus), *Avicennia schaueriana* (Stapf and Leechman), *Conocarpus erectus* (Linnaeus), *Laguncularia racemosa* (Gaertn) and *Rhizophora mangle* (Linnaeus), which grows around the main channel and tidal creek and extends to 600 ha, in addition to Atlantic Forest remnants (Rocha et al., 2007). Endangered species, such as the seahorse, *Hippocampus reidi* Ginsburg, and the West Indian manatee, *Trichechus manatus* (Linnaeus), are also found in this estuary (Mourão & Nordi, 2003).



Figure 2. Simplified map of the 40 km eastern portion of Mamanguape River joining the Atlantic Ocean.

Mangroves play an important role in protecting biodiversity, preserving shorelines, and regulating carbon cycling and Brazil is home to approximately 15 % of the world's total mangal

forest areas. Nearly 6700 km (90 %) of Brazil's 7400 km of coastline hosts mangrove forests, that often occur in estuaries. The Mamanguape River estuary is the second largest estuary in the northeastern state of Paraíba (Brazil) covering a mangrove swamp area of more than 57 km² (Bezerra et al., 2012). But the release of effluents and untreated wastewater pose threats to ecosystems and marine biota. Mangrove ecosystems are subject to a variety of disturbances that originate from different sources or is the result of a combination of those (e.g., geological, physical, chemical, and biological). Situated at the interface between land and sea at low latitudes, these ecosystems occupy a harsh environment and are subject to daily tidal, temperature, and salinity variations as well as varying degrees of anoxia. Mangrove forests and their inhabitants are therefore rather robust and highly tolerant to life in their saline environments within warm, subtropical, and tropical seascapes (Alongi, 2008). The wet season in the Mamanguape River Basin runs from March to August with June being the month of greatest contribution to the basin's water supply; in contrast, the dry season goes from September to February with October being the driest month of the period. Seasonal variations remain the same with projections of fall and increase in precipitation by 20%; however, with the 20% reduction, the water supply of the basin is extremely disadvantaged and with increase is widely favoured (Santos et al., 2015). In addition to natural fluctuations, anthropogenic activities also affect these ecosystems. The estuaries of the northeastern ecoregion are among the most affected by the human occupation processes in Brazil and need actions that guide an integrated management to maintain ecosystem sustainability. Cities close to the Mamanguape River estuary have a total of nearly 40,000 inhabitants (Instituto Brasileiro de Geografia e Estatistica, 2019).

1.2. Previous foraminifera studies in the study areas

Foraminifera are broadly used as bioindicators because they have short life cycles, which provide quick response to environmental changes, and are abundant, largely diversified, with a widespread distribution and specific ecological requirements (Murray 2006; Laut et al. 2016). The distribution of benthic foraminifera is controlled by a multitude of factors, such as temperature, salinity, dissolved oxygen, sediment grain size (Murray 1991), and changes in the quality and amount of nutrients (Murray 2006). Sediment characteristics strongly influence their distribution that are, however, also affected by pollution in the sediment (e.g. Alve and Olsgard 1999; Alves Martins et al. 2015; Bhalla and Nigam 1986; Frontalini and Coccioni 2008; Martins et al. 2013). Benthic foraminifera are widely used as proxies for coastal environmental monitoring across a wide variety of marginal environments, such as estuaries (e.g. Alve 1995; Bhattacharjee et al. 2013; Debenay et al. 2005), marshes (e.g. Gehrels and Newman 2004; Horton and Murray 2007), and lagoons (e.g. Samir 2000; Martins et al. 2013).

1.2.1. Foraminifera studies in Lagos Lagoon (Nigeria)

Lagos Lagoon waters and sediments are continuously monitored and have received detailed attention in terms of pollution research. Following aspects of Lagos Lagoon have been studied so far: sediment trace metals and total hydrocarbons (Ajao et al., 1991), phthalate esters (Adeogun et al., 2015), sediments, heavy metals, total organic matter, salinity, spatial distribution of the macro-infauna, diversity and faunal indices of major animal taxa (Ajao and Fagade, 1990; Ajao, 1996; Bawa-Allah et al., 2018), polycyclic aromatic hydrocarbons (Benson et al., 2014). The first assessment of anthropogenic stressors on benthic foraminiferal assemblages in Lagos Lagoon was done by Adebayo et al. (2012), where they studied foraminiferal assemblages from thirty-three surficial sediment samples in relation to parameters such as water depth, pH, temperature and salinity. In a recent study,

1.2.2. Foraminifera studies in mangrove estuaries of the Northern Brazil

There have been no foraminifera studies in the Mamanguape River Estuary, or in the estuaries of the Paraiba State for that matter, prior to this doctoral research study and southern Brazil has always been more popular for foraminiferal research compared to northern Brazil. Dias-Brito et al. (1988) generated, compared and discussed ecological models based on the distribution of recent ostracods and foraminifers from Sepetiba Bay, Rio de Janeiro. They divided the study area into three environmental domains and seven foraminiferal biofacies and discussed the relationship between biotic and abiotic components. Levy et al. (1995) analysed qualitatively and quantitatively the shallow water benthic foraminiferal fauna of the Fernando de Noronha archipelago, located offshore northeast to the state of Paraiba. They identified about 150 species, composed of 74 porcelaneous, 64 hyaline, and 10 agglutinated. Burone et al. (2011) obtained foraminiferal data from 66 samples of box cores on the southeastern Brazilian upper margin to evaluate the benthic foraminiferal fauna distribution and its relation to selected abiotic parameters, from areas with different primary production regimes. They also established a set of mathematical functions for analyzing the vertical foraminiferal distribution patterns, providing a quantitative tool that allowed to correlate the microfaunal density distributions with abiotic factors. Eichler et al. (2015) studied benthic foraminiferal assemblages in the marine area close to the Landulpho Alves Oil Refinery situated in Todosos Santos Bay (Bahia, Brazil). They assessed the environmental quality by statistical methods on foraminiferal assemblages, to establish which species were tolerant to low continental influence and to high organic matter. Laut et al. (2016), established the relationship between foraminifera and thecamoebian species and bacterial respiratory activities in the estuarine regions of Brazil. They showed that the bacterial biomass, as well as their respiratory activities, played an important role on the distribution, richness, and diversity of the foraminiferal and thecamoebian assemblages. Passos et al. (2017) established five different benthic foraminifera

groups in Itapanhaú River, Bertioga, and their relations with environmental parameters including salinity and sediment altitude. Eichler and Rodrigues (2018) discussed foraminiferal microhabitats in freshwater-rich zones over the continental shelf and interpreted these zones to be indicative of anthropogenic impact, since most of the pollutants and contaminants are dumped into water bodies that eventually reach and accumulate in the ocean. In a similar work, Eichler (2019) studied foraminiferal distribution patterns from two intertidal mangrove ecosystems located in the Bertioga Channel (São Paulo, Brazil) and their correlation with environmental gradients. Here, she associated the zones with mainly tidal influence and salinity differences. Raposo et al. (2018) separated the lagoon in three sectors based on benthic foraminifera distribution and concluded that this was primarily related to the biopolymeric concentration and to sediment grain size. These assemblages were found to be associated with conditions of i. low density with high impact/enriched in organic matter of low quality and ii. high richness to well oxygenated systems and the seawater exchange. Belart et al. (2019) studied the benthic foraminifera assemblages of the Saquarema Lagoon System (SLS) on the southeastern coast of Brazil in four lagoons, a tropical coastal lagoon in Rio de Janeiro State, to understand their seasonal variation during summer and winter. They analysed physicochemical parameters of the SLS and ecological indices of the foraminiferal assemblages in relation to those parameters.

Guanabara Bay (GB, Rio de Janeiro), due to its economic importance, has been studied fairly often compared to other localities in Brazil. It is a good representation of densely populated and economically active coastal regions in Brazil, which is comparable to the Mamanguape River Estuary. It similarly has a vivid ecosystem with extensive mangroves. Eichler et al. (2003) investigated benthic foraminiferal distribution patterns in Guanabara Bay in relation to temperature, salinity, dissolved oxygen and organic carbon content. They listed the principal environmental factors which appear to most strongly influence the habitat of different species. Donnici et al. (2012) studied 88 surficial sedimentary samples to characterize environmental variations through the use of benthic foraminifera. They also incorporated grain-size analysis and geochemical data into their interpretation. They established a list of stress-tolerant foraminiferal taxa that indicate pollutants from either natural or anthropogenic sources. Martins et al. (2016) analysed the influence of organic matter and enrichment of metals in relation to the structure of living benthic foraminiferal assemblages in the northeastern sector of GB. They showed that benthic foraminifera can live and reproduce in the NE region of Guanabara Bay, where the sediments have high total organic carbon content. However, their living assemblages extend across a small dimension and have low diversity and equitability, which suggests the presence of stressing environmental conditions in this region. Similarly, Delavy et al. (2016) also reported reduced diversity and density of benthic foraminifera in the impacted NE sector of GB. Examples to other benthic foraminiferal studies in Rio de Janeiro region include Vilela et al. (2011), who recognized low abundance values and the absence of foraminifera in five samples in the inner area (N/NE) in the Rodrigo de Freitas Lagoon (Rio de Janeiro) and interpreted it to possibly be caused by high levels of heavy metals and organic matter. Debenay et al. (2001) reported the benthic foraminiferal assemblages in the hypersaline lagoon Araruama (Rio de Janeiro) to be dominated by miliolid species. They concluded that anthropogenic stressors are not a reason for morphological abnormalities, and suggested that high salinity conditions and salinity changes are possibly responsible. However, higher proportions of Ammonia tepida in the more impacted northern part of the lagoon were interpreted to probably be due to human impact.

1.3. Previous studies employing benthic foraminifera for the assessment of ecosystems

Benthic foraminifera are widely used as tools to understand different aspects of ecosystems. This chapter is intended as a compilation and brief description of the different methods developed to this end.

1.3.1. Foraminiferal Index (FI)

Coral reefs are arguably the most biodiverse ecosystem on the Earth. They provide extensive recreational and commercial value to millions of people around the world; therefore, their deterioration poses a great threat to our planet. Unfortunately, since the 1970's, new diseases, bleaching in response to temperature stress, and physical and chemical impacts of hurricanes, ship groundings and pollution have caused coral reef communities to decline. It is of utmost importance to be able to assess water quality conditions around them, to see if foraminifera support reef development, even in the absence of healthy coral populations following mass mortality events. Even when corals would exchange their lost symbionts with more heat- or light-tolerant strains in the aftermath of a bleaching event, or one day scientists are able to genetically engineer heat- and disease-tolerant strains of corals and zooxanthellae (e.g. coral transplants), water quality of reefal environments must be maintained at a certain quality level to avoid the decline of mixotroph-based communities.

For assessing the water quality, larger benthic foraminifera were chosen as a good candidate, as they appear to be less vulnerable to coral-specific diseases and recover much more quickly from physical impacts than long-lived coral populations (Cockey et al., 1996), and also record the conditions in ecosystems they live in (Boesch and Rosenberg 1981; Messer et al. 1991). They are not dependent upon corals per se, but commonly associated with coral reefs where water quality is high. They are important in reef ecosystems as they are prolific carbonate producers, excellent indicators of water quality and environmental engineers with the potential

to stabilize future reef frameworks. Moreover, due to their enormous rate of reproduction they may be able to stabilize beaches impacted by rising sea level (e.g. Langer, 2007; Langer 2008; Langer et al., 2012).

Hallock et al. (2003) devised a "FORAM Index" (FI) as a straight-forward and cost-effective metric to assess whether water quality is suitable for mixotroph-based (i.e., algal-symbiotic-dominant) communities; either for recovery or for the continuation of reef growth, depending on the initial state of the reef (Table 1). For this, they first identify foraminifera specimens at least down to genus level and group them into three: i. symbiont-bearing larger benthic foraminifera, ii. opportunistic foraminifera, iii. Heterotrophic small foraminifera. Then they enter these values in a formula they developed to compute a FORAM Index for the environment and interpret it according to which interval it falls into among (0-2), (2-4), [3-5] and >4.

Table 1. Calculating the Foram Index (FI; Hallock, 2003)

Step 1. From each subsample examined, sort all foraminiferal specimens by genus, count, and record in a spreadsheet, with genera arranged by functional group. (See Table 1.)

c) $P_h = N_h/T$, where subscript "h" represents other small, heterotrophic foraminifers

Step 3. Weight proportions to calculate the FORAM Index (FI):

 $FI = (10 \text{ x } P_{s}) + (P_{o}) + (2 \text{ x } P_{h})$

Step 4. Interpretation:

FI > 4 indicates environment conducive to reef growth

FI varying between 3 and 5 indicates environmental change (Coefficient of Variation > 0.1)

2 < FI < 4 indicates environment marginal for reef growth and unsuitable for recovery

FI < 2 indicates stressed conditions unsuitable for reef growth

Hallock et al. (2006) stated that the key differences between bleaching in corals and *Amphistegina* are corals typically bleach by expelling their symbionts, while *Amphistegina* bleach when damaged symbionts are digested, and that mass coral bleaching requires high light

Step 2. Calculate the proportion (P) of specimens for each functional group by summing the specimens of each genus of that group (N) and dividing by the total number of specimens counted (T).

a) $P_s = N_s/T$, where subscript "s" represents symbiont-bearing foraminifers

b) $P_o = N_o/T$, where subscript "o" represents opportunistic foraminifers

but correlates most consistently with elevated temperatures, while bleaching in Amphistegina is induced by light. Amphisteginids are particularly sensitive to the shorter (300-490 nm) wavelengths of solar radiation, which increased in intensity relative to longer visible wavelengths (>490-700 nm) in clear reef waters over the past 30 years, as a consequence of stratospheric ozone depletion. FI was revisited by Prazeres et al. (2019) to outline its strengths and limitations, also to enhance its application across different geographical regions. They stress the importance of benthic foraminifera in enabling to distinguish between local (e.g. terrestrial runoff and nutrification) and global (e.g. ocean warming and acidification) stressors, so that ways to reduce local impacts to increase resilience to global stressors can be developed. As the FI was designed to be a relatively simple indicator of whether water quality supports calcifying organisms that host algal endosymbionts, there may be some species that do not behave exactly as predicted by a-priori assignment to a functional group. Regional taxonomic differences may further hamper the establishment of globally relevant metric thresholds for the FI. Nonetheless, the FI has demonstrated the validity and reliability of its basic premise in a number of different applications. Among these are assessing water quality on inshore coral reefs of the Great Barrier Reef in comparison with a composite index of 13 water quality variables (Fabricius et al., 2012), their use as bioindicators to evaluate nutrification-induced decline in the water quality of the northeastern Mediterranean coastal environments (Koukousioura et al., 2011), assessing changes in assemblage structure and composition in a 17-year data set to demonstrate the influence of climatic variability associated with the El Niño /La Niña - Southern Oscillation (Kelmo and Hallock, 2013). The need for additional studies to determine how relevant proposed FI thresholds are and where the FI should not be used, is also stressed by the authors.

Spezzaferri et al. (2018) aimed to apply the FI improved by Ramirez (2008; termed *Amphistegina* Bleaching Index, ABI) and to test whether the visual response to stressors of

Amphistegina populations - combined with ecological data and physical parameters - has the potential to serve as a low-cost risk-assessment tool for the Maldivian reefs in view of climate change or local anthropogenic impacts. They recognized a good example and showed how benthic foraminifera and corals differ in the way they bleach. For this they studied a 2015 El Niño event at the North Ari Atoll which triggered coral bleaching associated with anomalous temperatures that affected several coral taxa. However, the moderate photo-inhibitory stress, as shown by bleaching in *Amphistegina* in April–May 2015, occurred when solar irradiance and water transparency were highest, indicating elevated photo-oxidative stresses predating the extreme thermal El Niño pulse heralding a mass bleaching event. This study further demonstrated how benthic foraminifera respond to stressors in reef environments and what their potential can be to predict coral bleaching.

1.3.2. Foram Stress Index (FSI)

Dimiza et al. (2014) developed the Foram Stress Index (FSI), which in principle is based on the above-described FORAM Index (FI; Hallock et al., 2003) and the model of the foraminiferal response with distance from a point-source (Fig. 3; Alve, 1995).

Benthic foraminifera can occupy an extremely diverse range of niches (Murray, 2006). This spectrum has on one end taxa that can thrive in very clear, nutrient-poor waters (e.g. reefal waters; Langer and Hottinger 2000; Thissen and Langer, 2017; Förderer et al. 2018) as their algal symbionts can provide the energy they need (Prazeres and Renema, 2019; Narayan et al., 2022). Most of them live for months or years and produce relatively large shells (e.g., Hallock, 1985). On the opposite end there are smaller, faster-growing, stress-tolerant opportunistic taxa thriving in the presence of abundant nutrient supplies. These opportunistic taxa can tolerate environmental extremes including wide salinity fluctuations, low pH, hypoxia, and even concentrations of trace elements that are toxic to most eukaryotic organisms (e.g. Chatelet et

al., 2011; Coccioni, 2008; Coccioni et al., 2009; Duffield et al., 2015; Fajemila et al., 2020; Fajemila et al., 2022; Frontalini et al., 2009; Frontalini and Langer et al., 1990; Jorissen et al., 1992; Langer et al., 2022; Martins et al., 2015; Sariaslan and Langer, 2021).



Figure 3. Schematic diagram illustrating characteristic features of environmental modifications around point sources (modified from Alve, 1995).

FSI, analogous to the FI, is mathematically simple and based upon recognizing the spectrum described. However, different than FI, which is designed to employ taxa that host symbiotic algae and thus limited to certain depth and nutrient availabilities, FSI is aimed to be depth and food/nutrient-independent, therefore useful for ecological and environmental status assessments of soft-bottom benthic foraminiferal communities, including naturally stressed environments, such as lagoons or low salinity basins.

FSI utilizes heterotrophic foraminiferal taxa to characterize the gradient between extreme stress (abiotic) and unimpacted Mediterranean waters. Two benthic foraminifera groups are defined accordingly: one "sensitive" (Sen) and one "stress tolerant" (Str), then the index based upon their relative abundances is scaled: FSI = (10 * Sen) + (Str)

(Sen): Species that are sensitive to organic enrichment are present in unpolluted ecosystems, disappearing or occurring in low proportions under organic enrichment; mostly epifaunal and surface-dwelling (Langer 1988, 1993; Linke and Lutze, 1993; Murray, 2006).

(Str): Stress-tolerant taxa that may also be found under unaltered conditions. Because they are tolerant to environmental variability, they can act opportunistically and increase their relative abundance compared to the more sensitive taxa under organic enrichment; mostly infaunal (Châtelet et al., 2011; Jorissen et al., 1992; Jorissen, 1999; Jorissen et al., 1995, Murray, 2006). FSI varies from 1 to 10, 0 being only when the sediment is azoic (all groups zero). The boundaries enabling the distinction of the five Ecological Quality Status (ECoQ) classes according to the EU Water Framework Directive are shown in Table 2. Dimiza et al. (2019) confirmed the reliability of the FSI by correlating it with known FI's from other localities (e.g. Koukousioura et al., 2011).

Table 2. Classification scheme of soft bottom foraminiferal habitats based on FSI (Dimiza et al., 2014).

Pollution classification	FSI	Boundary EQR value	Ecological Quality Status (ECoQ)
Normal/pristine Slightly polluted, transitional	$\begin{array}{l} 9.0 \leq FSI \leq 10.0 \\ 5.5 \leq FSI \leq 9.0 \end{array}$	High/Good = 0.90 Good/Moderate = 0.55	High Good
Moderately polluted	$2.0 \le FSI \le 5.5$	Moderate/Poor = 0.20	Moderate
Heavily polluted	$1.0{\leq}FSI{<}2.0$	Poor/Bad = 0.10	Poor
Azoic	Azoic conditions	-	Bad

1.3.3. Studies of Hayward on pollution and shallow-water benthic foraminifera

Hayward et al. (1999) studied samples from brackish-water and shallow marine water environments around New Zealand. They identified specimens to species-level and statistically (cluster and correspondence analyses) analysed assemblages to define the factors most influential in the faunal distribution of benthic foraminifera (Table 3; factors listed in descending order of importance).

Table 3. Summary of the findings of Hayward et al. in brackish- and shallow-water marine environments of New Zealand (1999).

Type of	No. of species	No. of faunal	Factors determining
environment	identified/No. of	associations	faunal distribution
	samples	defined	
Brackish water	89/131	10	salinity, tidal exposure, and
			presence of intertidal
			vegetation
Shallow-marine	327/197	18	biogeography, bottom
(<100 m)			water oxygen
			concentrations and
			substrate type

Based on their total (dead plus live) assemblages, Hayward et al. (1999) showed that these are largely determined by a combination of salinity and tidal elevation. Their results verify what

was previously shown by Hayward (1993), and Hayward and Hollis (1994): salinity and secondly tidal exposure are the two environmental factors having the strongest influence on foraminiferal distribution within brackish settings. Moreover, Hayward (1993) reported that faunas display a marked zonation from freshwater to near-normal salinity in the rich foraminiferal faunas of a small estuary at Helena Bay, Northland. The general trends with increasing salinity are listed as decreasing abundance of agglutinated species, increasing abundance of porcelaneous species, calcareous perforate species, and planktic species, increasing diversity, and increasing mixing by post-mortem, out-of habitat current transport of tests. Four associations defined by Hayward from low to high salinity belonged to: i. extreme high tide, ii. tidal upper reaches of the estuary, iii. beneath the mangrove forest and in swamp, and iv. intertidal banks and sandflats within 100 m of the estuary mouth. The trends they identified in benthic foraminiferal assemblages from the more stressed, high tidal or low salinity habitats at the head of the harbour or estuary towards the more open sea at the mouth are: i. from dominantly agglutinated to dominantly calcareous (Rotaliina) faunas; ii. increasing diversity expressed by higher S, a, H values

The highest proportions of exotic tests (more abraded and robust) of normal salinity benthic foraminifera are mostly found in the entrance channels and just inside the sea mouths. These can be transported up into the estuary depending on factors as the strength of tidal currents, proximity of the setting to an exposed, stormy coastline and shape of the estuary or harbour mouth.

In shallow-water marine sample associations, the influencing factors include temperature (= latitude), wave and current energy, bottom water oxygen concentration, and other factors related to depth. Similar to the brackish faunas, post-mortem and out-of-habitat transport of tests into or out of the native environment is to be expected. Faunas in high energy environments are commonly observed to have a low abundance of small tests, indicating

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winnowing by strong currents. These faunas are commonly dominated by robust, abraded tests that have survived strong current transport processes.

In a follow-up study, Hayward et al. (2004) investigated the change in foraminiferal assemblages in four, late Holocene cores (two localities) since the arrival of humans (ca. 1300 AD), from the low tidal, estuarine fringes near New Zealand's largest city Auckland. This study has direct relevance and importance for this doctoral thesis as total assemblages were employed and shifts in the foraminiferal assemblages caused by anthropogenic impacts were investigated. The authors found that i. the foraminiferal faunas have switched from dominantly calcareous to dominantly agglutinated, ii. a two-step change is evident, with an intermediate mixed calcareous-agglutinated faunal zone (1950s–1970s), iii. Canonical correspondence analyses of the foraminiferal and environmental proxy data from the cores and the modern estuary transect indicate that decreasing salinity, and lowered pH are primarily responsible for faunal changes, iv. Increased nutrients as TOC, N and P to some extent have an effect on faunal changes, whereas sediment grain size and increasing heavy metal concentrations (Pb 40-100 ppm; Zn 130-250 ppm) have no considerable influence, v. Complete dissolution of calcareous foraminifera tests occur upstream where pH is lower than 7.5, vi. The decrease in salinity, indicated by faunal changes, is interpreted as a result of increased freshwater runoff with forest clearance in Polynesian (ca. 1300-1840) and early European arrival times (1840-1900). A prime example of nutrient-driven distribution is provided in a recent study on Jullienella foetida, the largest shallow-water foraminifera in modern oceans (Langer et al. 2022). J foetida has been found to be widespread along the western coast of Africa, including between Western Sahara to Ghana, Mauritania, Senegal, Gambia, French Guinea, Sierra Leone, Liberia, Ghana, and Côte d'Ivoire. The overall distribution coincides well with large parts of the Canary Current Upwelling System (CCUS), an area that extends from the Iberian Peninsula to Guinea, and constitutes one of the most productive coastal upwelling systems in the world. Not surprisingly,

the species has a biomass that is greater than that of other xenophyophore specimens. The relatively restricted distribution of this species off the north-west African coast is strongly related to the elevated, upwelling-related surface productivity along this margin, which provides enough nutrients and food to sustain this high biomass. In addition, this remarkable species plays a keystone role in the West African benthic ecosystems where it provides the only hard substrate on which other sessile organisms can settle.

1.3.4. FOraminiferal BIo-MOnitoring (FOBIMO)

The European Community Marine Strategy Framework Directive (MSFD) was adopted on June 17, 2008 to provide guidelines for monitoring the quality of marine ecosystems. However, a standardized methodology for benthic foraminifera surveys in assessing the status of marine environments (*bio-monitoring*) has not been developed so far. For that purpose, Schönfeld et al. (2012) presented a set of standard methods established in an expert workshop FOraminiferal BIo-MOnitoring (FOBIMO; June, 2011; Fribourg, Switzerland). They list a number of recommendations grouped as "mandatory" (to be followed if a study wants to qualify as sound and compatible according to norms) and "advisory" concerning the use of living (stained) benthic foraminiferal assemblages; these are given in Table 4. The application of their protocol by a large number of scientists was suggested by Schönfeld et al. (2012) to be the first step for a general acceptance of benthic foraminifera as a reliable tool in biomonitoring studies.

Table 4. Recommendations given by Schönfeld et al. (2012) concerning the use of living(stained) benthic foraminiferal assemblages.

Mandatory recommendations:	Advisory recommendations:
The interval from 0-1 cm below the	Sampling should take place in autumn.
sediments surface has to be sampled.	
For offshore surveys an interface or box	A sample size of 50 cm^2 or a tube of 8 cm
corer that keeps the sediment surface intact is	inner diameter should be used; ethanol
to be used .	(>70%) should be used as a preservative.
Grab samplers should not be deployed in soft	Rose Bengal at a concentration of 2 grams
sediments.	per litre for staining, and a staining time of at
	least 14 days required.
Three replicate samples are to be taken and	The split size should be defined by a target
analysed separately.	value of 300 specimens.
Samples are to be washed on a 63-µm screen,	Heavy liquid separation should be avoided.
and the living benthic foraminiferal fauna of	
the >125 μ m fraction is to be analysed.	
Splits are to be picked and counted entirely,	The 63–125 µm fraction of deeper sediment
and all counted foraminifera from at least one	levels may be considered in some
replicate per station have to be stored in	environments.
micropalaeontological slides.	
Census data, supplementary laboratory data	
and microslides have to be archived.	

1.4. Deep-sea benthic foraminifera as paleoceanographic proxies

Deep-sea benthic foraminifera are excellent paleoceanographic and paleoclimatic proxies, however the focus of this thesis is shallow benthic foraminifera. Their use and applications as proxy indicators is summarized below.

After their review of all paleoceanographical proxies based on deep-sea benthic foraminifera assemblage characteristics, Jorissen et al. (2007) classified *benthic foraminiferal assemblage data* into four different types: presence/absence data of various taxa, measures of faunal density, of biodiversity, or data on the morphology of dominant taxa. All of these have been utilized as *paleoceanographical proxies*. The reconstructed environmental parameters from these proxies can be grouped in two:

i. <u>Physico-chemical parameters</u>: temperature, salinity, carbonate saturation, hydrodynamics, or oxygen concentration of the bottom water. Such parameters may be expected to act as limiting factors, determining whether a foraminiferal species can live (actively, grow, calcify and reproduce) somewhere, and if all limiting factors are within the optimum range for a specific taxon, whether it will reach its maximum abundance (high for r-selected, low for K-selected taxa).

ii. <u>Resource parameters</u>: quality and quantity of the organic matter flux that directly influence the amount of food available to specific taxa. Resources are expected to act directly on the density of foraminiferal populations, but will also determine what species will dominate the fauna, because most taxa have maximal competitiveness within a specific flux range.

More specifically, the uses of benthic foraminifera as *paleoceanographic proxies* can be listed as follows:

1.4.1. Proxies of bottom water oxygenation and temperature

Bottom water oxygenation is a crucial aspect of marine ecosystems, not only for understanding the ecology but also as an important factor for the interdependence with ocean circulation, climate, and evolution of marine life.

The *Benthic foraminiferal oxygen index* (BFOI) of Kaiho (1994) is based on the hypothesis that in well-oxygenated bottom waters, dysoxic indicators live in poorly oxygenated deep infaunal microhabitats. In the case of hypoxic conditions at the sediment-water interface, less resistant taxa disappear and the "dysoxic indicators", formerly observed deep in the sediment, become dominant. The method used by Jannink et al. (2001) follows the same rationale reversed: oxygen penetration into the sediment increases with increasing bottom water oxygenation, leading to an increased volume of the niche potentially occupied by oxiphilic taxa. They use the cumulative percentage of oxiphylic taxa as a proxy for bottom water oxygenation. However, their approach omits the fact that the preference of a species to live close to the sediment-water interface is due to a combination of factors including bottom water oxygenation, as well as occurrence of high-quality food particles at the sediment-water interface.

Schmiedl et al. (2003) also proposed a formula to illustrate the change in bottom water oxygen content: (HO/(HO+LO)+Div)x0.5, where HO = relative abundance of high oxygen indicators (Miliolids-Articulina tubulosa + Cibicidoides pachydermus + Gyroidinoides orbicularis), LO = relative abundance of low oxygen indicators (*Fursenkoina* spp., *Chilostomella oolina*, *Globobulimina* spp.), and Div = normalized benthic foraminiferal diversity. Diversities are normalized relative to the maximum H(S) value found from the samples. The resultant is multiplied by 0.5 to distinguish between anoxic (minimum value = 0) and oxic (maximum value = 1) conditions. Through the addition of a factor independent of taxonomic composition,

they aimed to make the proxy more robust, and applicable in an array of areas with different faunal compositions.

The Ammonia-Elphidium Index (IAE) was suggested by Pregnolato et al. (2018) to assess the oxygenation level of coastal regions, as both genera are tolerant to and abundant in reducing environments, making this index useful to assess the impact caused by organic matter pollution. This index was originally developed by studies aiming to evaluate the current and past anoxia of the Long Island Sound and Chesapeake Bay coastal regions in the United States (Gupta et al., 1996; Gupta and Platon, 2006). In their study, Pregnolato et al. investigated the coastal area of Sergipe State (NE Brazil), that has been affected by petrochemical effluents released from the Petrobras Polo Atalaia Production complex. They evaluated the impact caused by these effluents on the density and diversity of living benthic foraminifera in the study area. Their results showed the Ammonia-dominated zones are rich in organic matter and organic compounds (hydrocarbons), but these zones are characterized by very low foraminiferal diversity. Because this trend is not consistent across habitats, Ammonia species were considered opportunistic where they tolerate adverse conditions in the presence of abundant food. The Ammonia- Elphidium index values tended to decrease progressively from the stations closest to the point source towards the further ones (Fig. 1), indicating increasing oxygen deficiency towards the point-source. They did not observe any species that are able to process petroleum residues as food source and it is likely that these compounds are highly toxic (Langer et al., 2016).

Benthic foraminiferal Mn/Ca ratio

Mn/Ca ratio in benthic foraminifera is a potential proxy for seawater oxygenation (e.g. Van Dijk et al., 2020). Manganese precipitates as a solid phase Mn-oxyhydroxide under oxygenated conditions, but at lower oxygen levels, Mn-oxyhydroxide is reduced and Mn²⁺ is released into

the surrounding seawater. Foraminifera incorporate dissolved Mn²⁺ (Reichart et al., 2003; Koho et al., 2015; Barras et al., 2018) and therefore the Mn/Ca of their shell is suggested to change with oxygenation conditions (Groeneveld and Filipsson, 2013; Koho et al., 2015; McKay et al., 2015; Ní Fhlaithearta et al., 2018; Petersen et al., 2019). To further develop this proxy, it is necessary to i) investigate potential other (environmental) parameters influencing Mn incorporation (Langer and Gehring, 1994) and ii) obtain species-specific calibrations by culture or field studies (Koho et al., 2015; Petersen et al., 2019), and ultimately iii) understand the incorporation pathways of Mn during biomineralization (Langer and Gehring, 1994).

Benthic foraminiferal Mg/Ca ratio

Benthic foraminiferal Mg/Ca ratio is a useful tool to estimate bottom water temperatures as shown from either core-top or cultured samples (BWT; e.g. Lear et al., 2002; Mawbey et al., 2020). The Mg/Ca–BWT relationship of three common *Cibicidoides* species can be described by an exponential equation: Mg/Ca = $0.867 \pm 0.049 \exp(0.109 \pm 0.007 \times BWT)$ (stated errors are 95% CI), which also allows comparing BWT estimations from recent and fossil foraminifera samples, even in cold bottom waters of the Arctic Ocean or in Antarctic margin settings, where data is scarce (Barrientos et al., 2018; Mawbey et al., 2020). Guo et al. (2018) used this ratio in combination with Mn/Ca and observed variations in both ratios when measured from different chambers within the same foraminiferal shell specimen. They interpreted these fluctuations as a likely response to changing ambient water dissolved oxygen and temperature during the specimen's growth; thus, they deemed combined use of foraminiferal Mn/Ca and Mg/Ca ratios significant for historical reconstruction of low oxygen conditions in seasonal hypoxia areas.
1.4.2. Proxies of paleoproductivity

Biological marine productivity depends on the availability of sunlight and required macronutrients (classically: carbon, nitrogen, phosphorus) and micronutrients (e.g. iron, manganese, zinc; Sarmiento and Gruber, 2006; Boyd et al., 2007). The intensity of sunlight useable by phytoplankton (marine algae, the dominant marine primary producers) varies depending on latitude, water depth, water clarity, and water column stability. Latitude controls the day length and the angle of the sun on the sea surface. As light intensity drops exponentially with depth and penetration is generally negligible below 50–100 m, any production over most of the ocean is dependent on populations of floating algae. This layer of floating algae remains close to the sunlit surface in deep waters, that are stable and resistant to vertical mixing. On the other hand, nearshore waters often have an unstable water column resulting from increased turbidity due to vertical mixing (Loubere, 2015). These phenomena cause the concentration of the essential nutrients at deeper parts of the oceans, so resupply for upper ocean plankton consumption depends on processes which bring deep water to the surface (Fig. 1; upwelling, deep vertical mixing; Sarmiento and Gruber, 2006).

Therefore, the main food source in deep sea benthic ecosystem is the downward flux of labile organic carbon (Gooday 1988, 1993 and references therein). The vertical flux of organic carbon to the sea floor is determined by the exported fraction of surface water primary productivity and losses due to degradation processes taking place in the water column (Suess 1980; Berger and Wefer 1990).

Ocean productivity is a vital aspect of marine ecosystems. It is equally important to study and understand ancient oceans, in the case of which it is termed *paleoproductivity*. Benthic foraminifera are an important tool for investigating paleoproductivity.

Hayward et al. (2002) investigated a suite of environmental factors and to what extent they influence the distribution patterns of benthic foraminferal tests (>63 μ m; between depths 90-

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4700 m) in a region in the east of New Zealand on the boundary between cold subantarctic water masses of the Southern Ocean and warm subtropical water masses of the South Pacific. They named two of the three most influential factors on the distribution of benthic foraminifera as sustainability of organic carbon flux rates and seasonality of food supply, both of which are directly related to marine productivity. Moreover, they observed that the composition of their abyssal associations (1200-4700 m) seems to be primarily controlled by the sustainability of food supply combined with bottom water type and associated ventilation and dissolution. Paleoproductivity proxies are developed based on the following principles:

Flux-dependence of individual species is realized by major faunal changes related to a specific organic flux level, such as shifts from faunas dominated by morphotypes considered typical of epifaunal lifestyles (trochospiral, milioline) to faunas dominated by morphotypes considered typical of infaunal microhabitats (rounded planispiral, flattened ovoid, tapered cylindrical, spherical, tapered flattened; Corliss and Chen, 1988). This approach is often adopted through the implementation of multi-variate analyses, however, its reliability is hampered by the small size of datasets in comparison to the number of species in the assemblage.

Benthic foraminiferal accumulation rate (BFAR)

Herguera and Berger (1991) proposed the BFAR (number of benthic foraminifera per unit of area per unit of time) as a paleoproductivity proxy. The underlying hypothesis of the method is that for every mg of organic carbon reaching the ocean floor, a fixed number of fossil foraminiferal tests is deposited. Their conditions are overly idealized, therefore the application of this proxy should be performed with caution. Their conditions are: i. a linear relation must exist between the organic matter flux and the number of fossilised foraminifera; ii. the flux of organic matter arriving at the ocean floor must in a linear way depend on surface water primary

production; iii. the sedimentation rate must be invariable or known in sufficient detail; and iv. there must be no significant carbonate dissolution.

Benthic foraminiferal distribution density

After analysing the vertical foraminiferal distribution patterns, Burone et al. (2011) proposed two functions that fit all the observed vertical distribution densities along the sediment layers to provide a quantitative tool that allows correlating the microfaunal density distributions with abiotic factors, such as primary productivity (PP), carbon flux, total organic carbon (C_{org}), d¹³C and grain size. They focused on areas with different primary production regimes on the southern Brazilian shelf and the upper slope, which is generally considered as an oligotrophic region.

These functions are as follows:

$$F(z) = k + Ae^{-z/\alpha} + Ce^{-(z-z_1/\beta)^2}$$
(1)

F(z) is the sum of a constant k, an exponential decay and a Gaussian function.

$$G(z) = (A_1 - A_2)/1 + e^{-(z - z_0)/\gamma} + A_2 \qquad (2)$$

In these equations, z represents the core depth and all the constants should be determined by fitting the function to the observed data. Although there is no theoretical model supporting the specific choice of the function defining F(z) and G(z), this is the first attempt for a mathematical description of the vertical foraminifera distribution.

In general, the cores that fit with pure exponential decaying functions were related to the oligotrophic conditions, prevalent on the Brazilian margin and to the flow of the Brazilian Current (BC). Different foraminiferal responses were identified in cores located in higher productivity zones, such as the northern and the southern region of the study area, where high percentages of infauna were encountered in these cores. The functions used to fit these profiles differ appreciably from a pure exponential function, as a response of the significant living fauna

in deeper layers of the sediment. One of the main factors supporting the different foraminiferal assemblage responses may be related to differences in primary productivity of the water column and, consequently, to the estimated carbon flux to the sea floor. Nevertheless, also bottom water velocities, substrate type and water depth need to be considered. The results obtained by Burone et al. (2011) are the first assessment to study the southeastern Brazilian margin using vertical benthic foraminiferal distribution patterns. Their results bring a stronger perspective on the potential for the utilization of benthic foraminiferal distribution to reconstruct the quantity, and also the quality of the organic input on the SW Atlantic continental shelf.

FORAM-AMBI

A benthic macroinvertebrate-based biotic sensitivity index commonly used in marine environmental monitoring (AMBI), applied to assess ecological quality status, was adapted for use on benthic foraminifera. As required by the AMBI formula, species were assigned to one of five ecological groups according to their sensitivity/tolerance to conditions along an increasing stress gradient (here increasing organic matter enrichment). They used 19 published data sets on fully marine NE Atlantic and Arctic fjord, continental shelf, and slope assemblages for which total organic carbon (TOC) data were available. They validated if the Foram-AMBI reflected changes in environmental disturbance along a gradient of increased organic carbon. The index was calculated for and tested on separate, independent data sets from the same geographic region, which clearly reflected an increasing organic carbon-induced stress gradient and showed promising results. Authors call for broader data sets in terms of higher numbers of benthic foraminifera assigned along wide organic carbon pressure gradients.

1.4.3. Proxies of bottom current velocity

Based on observations that a specific assemblages of benthic foraminifera occur in areas with elevated current velocities (Schönfeld, 1997, 2002a, 2002b), the idea of developing a proxy for bottom current velocity emerged. This proxy candidate also has the potential for paleocurrent studies to inform us about past variations of the deep-water circulation intensity.

Schönfeld (2002a) analyzed recent benthic foraminiferal assemblages in the Gulf of Cadiz, northeastern Atlantic, to study the impact of the saline and warm Mediterranean Outflow Water (MOW) undercurrent on the benthic environment. He found certain benthic foraminiferal species to seemingly prefer elevated microhabitats in the Gulf of Cadiz. Their abundance appeared to increase with the intensity of the ambient flow regime. The most likely explanation why certain epibenthic foraminifers settle at higher positions under stronger near-bottom currents is their individual capability to adapt their settling height to the level of maximum food supply (Linke and Lutze, 1993). He went on comparing the Gulf of Cadiz data with epibenthic foraminiferal abundances and current estimates from other regions to further constrain the boundary conditions of the elevated epibenthos versus current strength relation. The samples he used from the southern Portuguese and eastern Florida Margin were found to be largely comparable to distal settings in the Gulf of Cadiz. He also looked into samples from the English Channel and reported them to show more affinities to the proximal environments in the Gulf of Cadiz. To test the validity of this proxy candidate further, Schönfeld (2002a) also investigated the epibenthos percentages and estimated current velocities from the early and late Holocene. The gradual increase of current velocities with intermittent phases of weaker currents were found to be correlative with elevated epibenthos percentages and interpreted as a possible indication of shoaling and flow strength intensification in the upper MOW core layer. In a follow-up study, Schönfeld (2002b) investigated the impact of the MOW contour current, which flows northwards along the western Iberian Margin at 500 - 1500 m water depth, on the deep high-energy benthic environments in the Gulf of Cadiz (NE Atlantic). The four groups obtained from the living assemblages were found to trace the upper boundaries of MOW core layers and discern different biofacies under weak and strong near-bottom currents. Schönfeld attributed microhabitat preferences and faunal structure of benthic foraminifera under high and low current velocities to substrate stability. He further suggested that it may be a confining environmental variable for endobenthic and shallow epibenthic foraminifers. His observations also indicate that preferential settling height of epibenthic foraminifera is related to the highest lateral flux rates of food particles within reach from the sea floor.

With the aim to study the triggers and magnitudes of faunal response of benthic foraminiferal to environmental changes, Schönfeld and Mendes (2022) monitored benthic foraminiferal faunas annually at the Ria Formosa (Algarve, Portugal) coastal lagoon since 2013. They recorded distinct environmental changes in the tidal channel they observed, during the monitoring period, such as the relocation of a tidal inlet resulting in faster flushing, occurrence of higher tidal levels and stronger currents. As a result, the standing stock of the foraminiferal fauna declined in the next year due to food impoverishment. Thus, they showed that there is a much faster response from benthic foraminifera to environmental perturbations than in macro-organisms, which are often used for ecosystem status assessments in most European countries.

1.5. Aim and outline of this study

Benthic foraminifera, with their high reproduction rate and easy accessibility are well-known as excellent proxies and sensitive bioindicators of environmental disturbances. However, the ecosystems which are studied through benthic foraminifera are excessively complex and variable. Therefore, the need for comprehensive studies on the structure, distribution, diversity of benthic foraminifera increases every day as our tools become more and more advanced. The studies which constitute this doctoral thesis aimed to assess the impact of anthropogenic influence upon foraminiferal communities in the Lagos Lagoon and the Mamanguape River Estuary. For this, foraminifera were investigated on a lagoon-wide/estuary-long basis, whose results make up the main body of this thesis. It is aimed here to provide benthic foraminiferal data at an as-high-as-possible resolution and taxonomic precision. The ultimate aim of this thesis was to illustrate the entire faunas at species-level, identify potential bioindicators and sites of pollution, and highlight aspects to be considered in future in biomonitoring studies.

Chapter 2

Published in *PLOS One*

<u>Title</u>: Spatial distribution of benthic foraminifera in the Lagos Lagoon (Nigeria): Tracing the impact of environmental perturbations

Authors: Olugbenga T. Fajemila, Nisan Sariaslan, Martin R. Langer

Contributions: O.T.F. and M.R.L. designed the study. O.T.F., M.R.L. and N.S. wrote the manuscript.

Summary

Lagos Lagoon represents a unique environment for exploring how benthic foraminifera assemblages can be affected through pollution in a tropical coastal marine ecosystem. One of Africa's largest estuarine ecosystems, Lagos Lagoon is situated at the eastern side of one of the fastest growing megacities in the world (Lagos) and is the ultimate repository of contaminants carried in industrial, municipal and agricultural wastes. Lagos is the largest city in West Africa and the 21st largest city in the world. The mega city on the west coast of Africa is also one of the most densely populated cities on the planet, with an estimated 20 million people living on an area of less than 100 km², where the result is a suffocating mix of air pollution, single-use plastic pollution and solid waste. It is estimated that at least 30,000 people die every year in Lagos due to pollution (The World Bank, 2022). The high levels of pollutants have progressively deteriorated the water quality, adversely affected marine ecosystems, impacted the livelihood of the coastal population and pose serious risks to human health. To investigate the effects of these pollutants on Lagos lagoon ecosystem, benthic foraminifera assemblage structures, distribution and diversity parameters have been analyzed for the first time on a lagoon-wide basis to demonstrate their potential as proxies of environmental perturbations,

since they are already known as sensitive bioindicators of environmental disturbances. For this, sampled sediments were analyzed for a range of physicochemical properties via a multiparameter sensor probe-device including temperature, pH, depth and total dissolved solids (TDS) measurements. Quantitative analysis of 24 sediment samples yielded a total 3872 individuals of benthic foraminifera that belong to 42 species and 25 genera. They comprise 10 porcelaneous, 22 hyaline perforate and 10 agglutinated species. *Ammobaculites* aff. *A. exiguus*, *Ammotium salsum*, *Ammonia tepida*, *Ammonia parkinsoniana* and *Trochammina* sp. have been found to be the most abundant species. Heat maps were generated from abundance records for selected species to illustrate environmental preferences and relative resistance levels to individual forms of anthropogenic disturbance. The features recorded allow to delineate the spatial effects of hydrocarbon and heavy metal pollutants, urban sewages, and sand dredging activities. The data generated in this study can form the basis to assess the progressive deterioration of Lagos Lagoon environments from cores by using benthic foraminifera as bioindicators of environmental perturbation, as well as from recent samples by regular monitoring of the benthic foraminiferal communities in the future.

Chapter 3

Published in *Biogeosciences*

<u>Title</u>: Atypical, high-diversity assemblages of foraminifera in a mangrove estuary in northern Brazil

Authors: Nisan Sariaslan, Martin R. Langer

Contributions: N.S. and M.R.L. designed the study. N.S. performed the laboratory analyses, took the SEM pictures, prepared the graphics. N.S. and M.R.L. wrote the manuscript.

Summary

Mangrove forests are biodiversity hotspots: they are hosts to fish nurseries between the meshed webs of roots. They are sources and sinks of organic carbon: they sequester carbon at a rate 2-4 times greater than mature tropical forests. They are extremely productive ecosystems: they provide livelihood sources to local communities from the marine life in and around the mangroves. They are shelters to people and animals (incl. corals): they are an invaluable protection from cyclones and tsunamis for those living on coasts, also refuge habitats for those seeking refuge from unfavorable environmental conditions (Stewart et al., 2021). However, mangroves suffer from human intervention, primarily due to conversion and land use change, being used as dump sites, resulting in prevention of natural regeneration of wetland vegetation and water pollution of varying intensities, as well as indirect effects of sediments and chemical runoff from catchments degraded by clearing of upland vegetation and associated agriculture. To investigate the interplay between anthropogenic effects, marine intrusion and the structure and diversity of benthic foraminifera assemblages in a mangrove swamps and mud flats along the

Mamanguape River in Paraíba/Northern Brazil. Brazil is home to approximately 15% of the world's total mangal forest areas but the release of effluents and untreated wastewater is threatening these important ecosystems along the coastline. Sampling points for foraminifera were selected to acquire information on the foraminiferal assemblages associated with different environmental conditions, on the mud banks and in the estuary. A total of ~ 100 species of benthic foraminifera were identified within the shallow mangrove habitats. The large number of identified mangrove taxa is the highest recorded so far for Brazilian mangrove habitats and rivals shallow-water assemblages recorded from nearby offshore and reef environments. Mangrove foraminifera require a number of physiological adaptations to overcome the problems of anoxia, high salinity and frequent tidal inundation. The high diversity recorded indicates that a particularly large number of species is capable to grow and flourish under conditions of multiple stressors. Numerical analysis of the faunal assemblages shows that specific taxa are abundant and indicative for specific habitats (inner mangroves-, channel-, brackish water environments). Tidal elevation is interpreted as an important factor in regulating species richness with a strong trend of decreasing diversity moving up the shore. Distribution, diversity and species-specific analysis will also provide guidance on the use of Brazilian mangrove foraminifera as indicators for sea-level reconstructions.

Chapter 4

Published in Water

<u>Title</u>: Contamination Levels of Potentially Toxic Elements and Foraminiferal Distribution Patterns in Lagos Lagoon: A Correlation Analysis

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Contributions: O.T.F., M.R.L. and M.M.-C designed the study including the methodology and performed the investigation. M.M.-C. and I.S.C. acquired the resources. O.T.F., M.M.-C., N.S., I.S.C. and T.O.K. performed the formal analysis. O.T.F., M.M.-C., N.S. and M.R.L. wrote the original manuscript; N.S. and M.R.L. completed the writing through review and editing. O.T.F., M.M.-C. and M.R.L. prepared the visuals. O.T.F., M.M.-C. and M.R.L. acquired the funding and supervised the study.

Summary

Sediments across Lagos Lagoon have been sampled and analyzed to investigate the extent and distribution of Potentially Toxic Elements (PTEs). For this, the bioavailable fraction of PTEs have been evaluated to explore the relationship between PTE concentration and the spatial distribution, composition, abundance, and species richness of benthic foraminifera biotas. The sediments have been found to show a wide range reflecting a diffuse contamination, where Contamination and Enrichment Factor suggest low to extremely polluted sediments. Our survey of the benthic foraminifera inhabiting Lagos Lagoon revealed diverse assemblages of benthic taxa, species-specific distribution patterns, gradients of species richness and abundance, and a disjunct distribution of agglutinated and hyaline-perforate/porcelaneous taxa along a pronounced salinity gradient. All PTE total concentrations have been shown to

positively correlate with mud and Total Organic Carbon (TOC) and two of the most abundant agglutinated taxa, *Ammotium salsum*, and *Trochammina sp. 1*, according to our correlation matrix analysis. Moreover, both species display significant positive correlations with Cr_{F4}-Co_{F2-F3-F4-total}-Cu_{F4-total}-Al_{F4-total}-Al_{F4-total}-Fe_{F3-F4-total}-Zn_{F3-F4-total}. On the other hand, both foraminifers correlate negatively with Pb_{F4}-Se_{F3}-Se_{total}. The overall significant positive correlation of these PTEs suggests that they behave as micronutrients when complexed with organic matter. No significant positive correlation with none of the PTEs in any fraction was found for neither species richness nor for the most abundant hyaline perforate species (*Ammonia aoteana*). Some PTE fractions were found to correlate either positively or negatively with individual foraminifera species, suggesting that they function as either micronutrients and/or stressors. The resulting Contamination Factor of the PTE total concentrations shows that only a few sample sites can be classified as "moderately" polluted for Cr, Zn, and Cu, and that all sampled sites are classified as "highly polluted" for Se. The highest concentrations for Cr, Cu, Ni, and Zn were found towards the industrialized western part, an area that is characterized by moderate to high diversity but low abundances of benthic foraminifera.

Chapter 5

Review in progress *Palynology*

<u>Title</u>: A complex Early Devonian palynoflora from the Waxweiler Lagerstätte (Klerf Formation, Rhenish Massif, western Germany): palaeobotanical implications DOI: <u>https://doi.org/10.1080/01916122.2022.2150904</u>

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Contributions: P.S., T.S., M.R.L. and N.S. designed the study including the methodology and performed the investigation. M.R.L., N.S. and T.S. acquired the resources. T.S. and N.S. performed the formal analysis. N.S., P.S., B.C.M. and M.R.L. wrote the original manuscript. N.S. and T.S. completed the writing through review and editing. N.S. prepared the visuals. P.S. and M.R.L. acquired the funding.

Summary

The Lower Devonian Klerf Formation is an exceptional Konservat-Lagerstätte, exposed at multiple sites in the Waxweiler region in the Eifel area, western Germany. It has been studied for its various fossils, mainly arthropods, fishes, plants, molluscs, brachiopods, and crinoids. At Waxweiler, the sediments are palaeoecologically interpreted as a prograding deltaic depositional system elongated from NW to SE in the Ardenno-Rhenish area. The Klerf Formation has, however, not been studied in full detail in terms of its microflora and microfauna. Our study of the sediments of the formation from two different quarries in the Waxweiler area yielded fairly diverse miospore assemblages dominated by abundant organic matter of varying degrees of coalification. The miospore assemblages are mainly composed of classical Lower Devonian taxa of the Old Red Continent (Laurussia). These belong, among

others, the Ambitisporites, Apiculiretusispora, Retusotriletes. to genera and Biostratigraphically more important species recovered include Acinosporites lindlarensis, Apiculiretusispora brandtii, Cymbosporites asymetricus, Diatomozonotriletes franklinii, Emphanisporites annulatus, Verruciretusispora dubia and Verrucosisporites polygonalis. In addition, Emphanisporites foveolatus, which is known only from a limited area in the Ardenno-Rhenish region, is also identified, indicating an earliest Pragian to middle Emsian age for the composite section. These assemblages are found to be accompanied by reworked phytoplankton to a much lesser extent. Our results reveal a much larger palaeobotanical diversity from the Rhineland than previously known, indicating a well-developed Psilophytontype vegetation with related plants. The results further suggest a likely presence of plants such as *Leclercqia* and *Pertica*.

Chapter 6

Discussion and Conclusions

The ecological studies on benthic foraminifera assemblages in the marginal marine environments of Lagos Lagoon (Nigeria) and in the Mamanguape River Estuary (Northeastern Brazil) yielded novel results that contribute to the understanding of the hierarchy of controlling factors in coastal tropical environments under anthropogenic influence. In this chapter, these will be discussed in comparison to previous studies.

In the first part of this research project, Fajemila et al. (2020), described the benthic foraminifera assemblages in Lagos Lagoon and their potential links to environmental perturbations. Our survey established that Lagos Lagoon is home to a much more diverse benthic foraminiferal fauna - 42 species belonging to 25 genera - than all other coastal environments studied so far in the Gulf of Guinea. Similar to other reports, low diversity, density, equitability and high dominance, indicative of high environmental stress, are observed in the most polluted parts of the Lagos Lagoon, along the highly populated western and southwestern coasts. Similar to our studies, Vilela et al. (2004) showed that their samples from Guanabara Bay (Rio de Janeiro, Brazil) revealed typical foraminiferal biotas found in many of the most polluted coastal environments from other parts of the world. The low values of abundance as well as the dominance of indicative stress-tolerant species, including but not limited to Ammonia tepida, reflects these polluted conditions. However, some other areas are characterized by comparatively higher species richness values. This may be due to pollution by organic matter rather than heavy metals, as found in the most confined zones of Ria de Aveiro, where pollution by organic matter is found to lead to a growth in foraminifera density, when biopolymer concentrations of high-quality increase, whereas the rise of available heavy metal concentrations caused a decline in foraminifera density and diversity (Alves Martins et

al., 2015a). This is mainly caused by the occurrence of marine taxa carried landwards to these parts through Atlantic waters. Even though Ammonia tepida was not identified in our taxonomic analysis, several Ammonia species, such as Ammonia aoteana and Ammonia convexa, occur in high percentages. Ammonia species are long known to characterize pollution in coastal environments, however in our study these species seem to follow the salinity contour lines rather than polluted sites in the lagoon. Similar marine-influenced patterns were observed in living assemblages of the Bertioga channel, Brazil (Rodrigues et al., 2020). Therefore, these two species present a significant potential to be considered as bioindicators in polluted coastal environments. However, this does not eliminate the problem of differentiating between different sources of pollution through these bioindicator species. As in our case and in most other cases, these biotic indicators were mostly identified in areas with diffuse organic matter pollution, where it is very difficult to obtain objective measures of anthropogenic stress. Nevertheless, such measures are needed to calibrate the faunal indicators/indices, and to identify boundaries between the various environmental quality classes (Muxika et al., 2005). As complicated as it can be to define ecological reference conditions, which are needed to assess environmental quality (Alve et al., 2009, Borja et al., 2012, Dolven et al., 2013, Martínez-Crego et al., 2010), it is also very difficult to differentiate between anthropogenic impact and natural background conditions and attribute any indicator species to a certain ecological group (Parent et al., 2021).

On an attempt to determine which faunal parameters could be relevant to adequately describe the ecosystem health, on a sample set collected from 31 stations for the study of benthic foraminiferal faunas along the erratically polluted French Mediterranean coast (>1000 km), Barras et al. (2013) looked at different faunal parameters, namely diversity indices, wall structure proportion, and indicative species groups. They investigated the possible relationship between these parameters and pollution by organic matter. They concluded that the use of indicator species, such as stress-tolerant or sensitive, is more relevant than the use of diversity indices for the evaluation of ecosystem quality (at least in rather oligotrophic areas such as the Mediterranean Sea). Moreover, they once again showed (previously by e.g. Schönfeld et al., 2012), the analysis of the uppermost layer (0–1 cm) of sediment is sufficient to obtain relevant information needed for biomonitoring purposes, as also adopted in our studies. We have not applied standardization to our stress-tolerant benthic foraminifera percentage, because as opposed to Barras et al. (2013), a strong sediment-size-controlled distribution of stress-tolerant taxa has not been observed in Lagos Lagoon. They stated, faunas living on clayed substrates are more adapted to naturally enriched conditions (eutrophisation), often characterised by increased organic matter concentrations and sometimes seasonal low oxygen concentrations. For this reason, their stations with muddy substrates tend to show an elevated proportion of stress-tolerant species, even if the concerned ecosystem is not subject to anthropogenic impact. Instead, distribution of our assemblages appears to be governed by the combined effects of salinity and pollution.

In Lagos Lagoon, stress-tolerant species *Ammonia aoteana* dominates the hyaline-perforate group sample material, by constituting 14.1% of the total population of benthic foraminifera recovered and percent abundances at individual sites ranging from 0-90%. Its occurrence is striking at the entrance and in the polluted western part of the lagoon, where water is mostly shallow (3-7 m). Second most important stress-tolerant species *Ammonia convexa* generally does not exceed 10% in the lagoon. These species are important for future biomonitoring studies in polluted coastal waters, as shown by Barras et al. (2013), percentage of indicator species, stress-tolerant in our case, is more discriminative, thus essential for the development of a biotic index of ecological quality status. Moreover, our hyaline-perforate taxa occur dominantly around Lagos Harbor and the Commodore channel where marine waters mix with the lagoon water, and at the same time where highest values of organic enrichment are expected

due to multiple sources of pollution. Similar to findings of Barras et al. (2013), where the percentage of hyaline-perforate foraminifera plots in the same area as organic matter content, which is associated with pollution, our hyaline-perforate assemblages reflect the superimposed effect of marine intrusion and the distribution patterns resulting from the pollution. Despite this combined effect, it is noteworthy to report yet more stress-tolerant species in Lagos Lagoon, which can be further investigated in biomonitoring activities in polluted coastal environments. In addition to *Ammonia aoteana* and *A. convexa*, these are species of *Elphidium*, *Nonion*, *Trochammina*, *Ammobaculites* and *Bolivina*. Nevertheless, salinity control brought by the intruding marine waters appears to be the dominant factor in the distribution of our assemblages in Lagos Lagoon, because in equivalent lagoon settings lacking marine intrusion, but having a mix of stressors such as PTE's and organic enrichment, benthic foraminiferal faunas exhibit low abundances and diversities associated with environmental stress.

In the follow-up of this study, benthic foraminiferal patterns governed by a selection of potentially toxic elements (PTEs), have been investigated in Lagos Lagoon (Fajemila et al., 2021). As revealed previously (Fajemila et al., 2020), foraminiferal assemblages collected across the highly polluted Lagos Lagoon exhibit a distinct separation of agglutinated and hyaline-perforate/porcelaneous taxa, largely oriented along the salinity contour lines. In this study, the anthropogenic influences, whose traces are superimposed on the salinity-driven distributional differences, have been looked into and an in-depth cross-correlation has been performed on the spatial distribution foraminifera and a selection of Potentially Toxic Elements (PTEs) in the bioavailable fraction. This revealed: i. the only negative correlation to be between benthic foraminiferal species and Pb_{F4} and Se_{F4} , ii. A negative correlation between the spatial distribution of foraminiferal species richness and diversity and Co_{F4} -Ni_{F4}-Fe_{F4}, again a feature that tracks the salinity gradients, iii. a positive correlation between Co_{F4} -Cu_{F4}-Ni_{F4}-Al_{F4}-Fe_{F4}-Zn_{F4} and the foraminiferal number distribution, suggesting that these PTEs behave as

micronutrients, iv. new information on the bioavailability of PTEs, especially of Se in Lagos Lagoon, which reaches the highest concentration within the central portion of the lagoon and correlates with the Ecological Risk Index and the Enrichment Factor, indicating moderate to severely polluted environmental conditions in this part. Shannon diversity values H(S) show significant negative correlations with various bioavailable PTEs in the F4 fraction (Co-Ni-Fe). It is uncertain to what extent Se affects the foraminiferal assemblages, since the abundance of Ammotim salsum and Trochammina sp. 1, and the FN are the only parameters showing significant negative correlations with the non-bioavailable fractions of Se. Comparing our results with those of Alves Martins et al. (2020), who studied the statistical relationship between benthic foraminifera and PTE's in the NE region of the Guanabara Bay (SE Brazil), there are major discrepancies observed. These are likely due to i. the aforementioned combined effect of marine intrusion and pollution, where marine intrusion seems to predominate, unlike it is in the Sepetiba Bay, which exhibits signs of severe pollution, ii. that Alves Martins et al. (2020) did not study the bioavailable fraction of the heavy metals they investigated, but instead the total concentrations, iii. as discussed by Martínez-Colón et al. (2009), salinity gradients will affect clay flocculation in estuaries which will allow PTEs to be more readily adsorbed or desorbed to/from organic matter at higher salinities, like in Lagos Lagoon. Thus, the dynamics and velocity of these processes that allow the temporary retention and/or release of heavy metals can also influence the degree of toxicity of PTEs and their impact on biota, which may have caused completely different relationships between potentially bioindicator species and PTE's in Lagos Lagoon and in Sepetiba Bay. Our study serves as a baseline for future studies investigating further these complex interactions among different variables in coastal environments affected by pollution through benthic foraminifera, faunal indices, and PTE's. In our large-scale survey on the benthic foraminifera community in mangrove environments of the Mamanguape River estuary (NE Brazil), a highly diverse assemblage of benthic

foraminifera including 33 porcelaneous, 53 hyaline-perforate, and 7 agglutinated species have been identified. This unprecedented result in a coastal environment in terms of species richness, composition of wall-structural types, presence of abundant hyaline-perforate and porcelaneous miliolid taxa, and in particularly low abundances of agglutinated species, shows great deviations from what is typically known to be a mangrove benthic foraminiferal assemblage (e.g. Barbosa et al., 2005; Eichler, 2019). The high diversity measures obtained from Mamanguape resembles those of coastal nearshore environments or back-reef settings where foraminiferal diversity is much higher than in mangroves (e.g. Fajemila et al., 2015; Minhat et al., 2020). Pararotalia cananeiaensis is also considered an indicator of saline water intrusion into paralic systems (e.g. Duleba et al., 2018; Rodrigues et al., 2020, Debenay, 2001). As opposed to classical and more recent mangrove studies, which claim mangrove foraminiferal assemblages to be of low-diversity and agglutinated-dominated (e.g. Abd Malek et al., 2020; Saha and Saha, 2021), the high-diversity benthic foraminiferal assemblages in Mamanguape were established to be autochthonous. This feature of our assemblages indicates much longer residence times for marine waters in the mangrove estuary than ever thought for these environments - so long that reaching even further upstream from where the sampling sites are found. Therefore, our results once again highlight the importance of salinity, which has long been recognized as a major driving force governing the species richness, composition of wall structural types, and diversity of foraminiferal assemblages (Debenay, 1990; Murray, 1991; Hayward and Hollis, 1994; Fajemila et al., 2020).

As clearly shown by the findings of Fajemila et al. (2020), where a distinct separation between agglutinated and calcareous taxa along a pronounced salinity gradient was reported, our overall results point to salinity, as the most prominent control factor, promoting heterogenous and diverse assemblages rich in calcareous benthic foraminiferal taxa. Moreover, Fajemila et al. (2022) hypothesized that the terrestrial organic matter, sourced by the mangroves, is not

optimal to support living benthic foraminifera (deemed as degraded organic matter - low food quality of Delavy et al., 2016), while marine organic matter may be a preferred source of food. Alves Martins et al. (2015b) also showed atypical benthic foraminiferal assemblages in Bizerte Lagoon, with taxa that are not commonly recorded in contaminated transitional environments. Their assemblages included several species common in continental shelf environments, in areas where the flux of organic matter is high. As our study area is considered to be under high marine influence, the organic matter potentially sourced by the ocean, together with high tidal mixing providing ventilation for benthic foraminiferal communities (unlike the case in Alves Martins et al., 2020), could have supported our diverse assemblages.

As shown by Richirt et al. (2020) in brackish waters of New Zealand (including mangroves), sites subject to seasonal anoxia with different durations are characterised by the presence of free sulphide (H₂S) in the uppermost part of the sediment, and their foraminiferal communities are impacted by the presence of H₂S in their habitat, with a stronger response in the case of longer exposure times. H₂S is one of the main components of pyritization processes in anoxic marine environments (Thiel et al., 2019). Many benthic foraminifera of the Mamanguape River Estuary were found to contain framboidal pyrite in their tests, revealing long exposure times (35 days) and suggesting that some of them are not negatively influenced by anoxic conditions. This further supports our observation that salinity outweighs other environmental controls in our assemblages and can be established as the main driving force governing the structure of our benthic foraminiferal assemblages. However, other controlling factors may become prominent in equivalent settings, just like when tidal current activity was considered to have a higher influence on foraminiferal biotope distribution through controlling substrate stability and patterns of erosion and accretion than the variations in temperature or salinity in Ria de Aveiro and Lagoon of Aveiro in Portugal (Alves Martins et al., 2014). These complex relationships should be further researched in a controlled manner with predefined ecological

reference conditions. The final indication of our results is that these assemblages may not be interpreted as mangrove assemblages in the fossil record as their composition, structure and diversity resemble fully marine biotas. Here the uniqueness for each environment comes into play and needs to be considered to infer detailed conclusions. One important aspect that affects the interaction between these controlling factors is the exposure time of the sediment to air along intertidal transects, which seems to exert a primary influence on the distribution of foraminiferal associations along intertidal transects (Semensatto Jr. et al., 2009).

Applications and the use of benthic foraminifera as tracers of environmental pertubations strongly depend on rigorous taxonomic species-level identifications, to identify the source, magnitude and nature of pollutants. Salinity has been identified as a major agent driving the composition, species-richness, diversity and distribution of shallow-water benthic foraminifera. The analysis of benthic foraminiferal indicators and patterns together with Potentially Toxic Elements (PTE's) is a means to identify potential sources, but benthic foraminifera react on species level to sources of pollution and the identification of the pollutants to assess the degree and magnitude of the pollutant for environmental assessment, still relies on high resolution taxonomy and expertise. Hohenegger *et al.* (2021) clearly showed that culturing in laboratory cannot substitute natural conditions in growth and pollution studies and the response of foraminifera was found to be not uniform and varied among species (Fujita *et al.* 2011; McIntyre-Wressnig *et al.* 2013; Hikami *et al.* 2011; Prazeres *et al.* 2015; Hohenegger *et al.* 2021). To document the response under natural - not laboratory - conditions, and to provide reliable assessments, past records from fossil core material are essential to distinguish between natural and anthropogenic forms of impact.

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APPENDIX

Chapter 2: Publication



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Citation: Fajemila OT, Sariaslan N, Langer MR (2020) Spatial distribution of benthic foraminifera in the Lagos Lagoon (Nigeria): Tracing the impact of environmental perturbations. PLoS ONE 15(12): e0243481. https://doi.org/10.1371/journal. pone.0243481

Editor: Marcos Rubal García, CIIMAR Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, PORTUGAL

Received: August 16, 2020

Accepted: November 21, 2020

Published: December 7, 2020

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Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: This work was supported by grants from Tertiary Education Tax Fund Nigeria (www.tetfund. gov.ng) to OTF (TETFund/16b/0014), and the German Science Foundation (DFG; www.dfg.de) to ML (LA 884/10-1, LA 884/5-1). The funders had no role in study design, data collection and analysis, **RESEARCH ARTICLE**

Spatial distribution of benthic foraminifera in the Lagos Lagoon (Nigeria): Tracing the impact of environmental perturbations

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Abstract

Lagos Lagoon is among Africa's largest estuarine ecosystems, bordered by one of the fastest growing megacities in the world and the ultimate repository of contaminants carried in industrial, municipal and agricultural wastes. The high levels of pollutants have progressively deteriorated the water quality, adversely affected lagoon ecosystems, impacted the livelihood of the coastal population and pose serious risks to human health. Benthic foraminifera are excellent proxies and sensitive bioindicators of environmental disturbances but comprehensive studies on the structure, distribution, diversity and impact of pollution upon foraminiferal communities have not yet been conducted in the Lagos Lagoon. To demonstrate the potential of foraminifera as proxies of environmental perturbations, benthic foraminifera were investigated on a lagoon-wide basis. Lagos Lagoon comprises areas that range from low levels of direct impact to those of severely affected by various forms of anthropogenic disturbance. The goals of this study are to analyze patterns of distribution and species richness, to document foraminiferal community structures, and to identify taxa that track documented records of pollution in Lagos Lagoon sediments. Heat maps were generated from abundance records for selected species to illustrate environmental preferences and relative resistance levels to individual forms of anthropogenic disturbance. Sediments were analyzed for a range of physicochemical properties, via a multi-parameter sensor probedevice, including temperature, pH, depth and total dissolved solids (TDS). Quantitative analysis of 24 sediment samples yielded a total 3872 individuals of benthic foraminifera that belong to 42 species and 25 genera. They comprise 10 porcellaneous, 22 hyaline perforate and 10 agglutinated species. Ammobaculites exiguus, Ammotium salsum, Ammonia aoteana, Ammonia convexa and Trochammina sp. 1 have been found to be the most abundant species. For the first time, the complete present-day foraminifera fauna is illustrated here via scanning electron microscopy. The features recorded allow to assess the spatial effects of pollution upon foraminiferal assemblages on a lagoon-wide basis. The data generated may ultimately form the basis to assess the progressive deterioration of Lagos Lagoon ecosystems from cores by using benthic foraminifera as bioindicators of environmental perturbation.

decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Lagos Lagoon (Nigeria) is the largest lagoon system in the Gulf of Guinea with more than 6,000 km² of surface area. The lagoon is situated between the Atlantic Ocean and Lagos, one of the fastest growing megacities in the world (Fig 1). The rapid population growth and industrial development, has made Lagos the economic hub and financial focal point of Nigeria with one of the largest seaports along the African coastline. Surrounded by a population of more than 20 million people, the lagoon has become the ultimate sink for the disposal of industrial, agricultural and domestic wastewaters. Lagos Lagoon ranks first among the most polluted African ecosystems (data from WHO and Africa UN Environment) and is primarily impacted by effluents from the oil and textile industry and urban sewage carried by the Ogun and Osun rivers. These led to high concentrations of heavy metals (e.g., copper, zinc, manganese, lead, iron, nickel) in the lagoon environment. Polycyclic aromatic hydrocarbons (e.g., naphthalene, phenanthrene, pyrene) have been found at considerable levels within the polluted western section of the lagoon (e.g., [1-4]). Excessive sand mining and dredging activities contribute more to the disruption of the ecosystem in the lagoon. Moreover, the lagoon is heavily exploited by fishing activities and aquaculture, leading to further environmental degradation and accompanying changes in water quality with biological consequences for biotas in the environment. With the expansion of Lagos City, large-scale destruction, deforestation and pollution of the mangroves forests have severely modified ecosystems along the western lagoon shores and resulted in the domination of tidal swamps by floating water-hyacinth (Eichhornia crassipes), saltgrasses and weeds [5]. These observations constitute the motivation for this study, which aims to investigate the structure, distribution and composition of foraminiferal assemblages in order to better understand the degree of environmental perturbation and to identify potential taxa as tracers of pollution. Previous studies on modern foraminifera from the Lagos Lagoon are limited, restricted to a few selected sites and mostly focused on the western part [6-8]. In a recent study, Philipps et al. [9] identified 20 species of benthic foraminifera from the western part of Lagos Lagoon and considered pollution as a driving force for harbor samples that are barren of foraminifera. Here we provide the first lagoon-wide analysis of present-day benthic foraminifera, illustrate the entire fauna, identify potential bioindicators and sites of pollution and highlight aspects to be considered in future in biomonitoring studies.

Environmental setting

Lagos Lagoon is separated from the Atlantic Ocean by a long sandspit and drains its water via the comparatively narrow Commodore Channel into the Atlantic. The most densely populated areas including clusters of industry are spread along the lagoon's southwestern and western shorelines. Due to the limited exchange with marine waters, the Lagos Lagoon system experiences restricted marine and mainly low salinity, brackish and freshwater conditions [8, 10, 11]. Currents in Lagos Lagoon are strongly constrained by the tidal regime and freshwater discharge from Ogun River [10]. At high tide, incoming waters flow from the Atlantic Ocean into the harbor and the lagoon through the Commodore Channel and the Five Cowries Creek and are mainly directed towards the east. At low tide, the direction of the currents is reversed. Moreover, salinity varies substantially with the wet and dry seasons and is strongly impacted by the introduction of fresh water from rain, rivers and saline water from the ocean (Fig 1). Rivers Ogun and Osun empty into the lagoon through the northern and eastern corridors, reducing the salinity at these sectors tremendously and create fan-deltas. Minimal salinity values are recorded during the high rainfall months (July, August, September) and higher values are present during the dry season. In general, the western sector of the lagoon experiences higher salinity because of its interactions with the Atlantic Ocean. During the dry season, the



Fig 1. Location of the sample sites and generalized salinity contours in Lagos Lagoon, Gulf of Guinea (Nigeria). Salinity in Lagos Lagoon varies by season and location, with the freshest water being in the northern and the eastern portion of the lagoon. The salinity contours provided cover the range of seasonal variation [8, 10, 11]. Salt water entering the lagoon via the main Atlantic entrance channel and the Five Cowries Creek mingles with lagoon water and creates a brackish water environment with decreasing values towards the distal ends of the lagoon. The Osun River discharges its waters into Lekki lagoon, a large expanse of shallow freshwater situated to the east of Lagos Lagoon, and connected to it via a narrow corridor.

influx of river water is low and salinities rise to about 30‰ around the entrance channel, to \sim 16‰ in the southwestern area, and to 8–10% in the central part. Towards the east and near the mouth of the Ogun River, salinities decrease further. The river input, however, is so large during the raining season, that the lagoon is fresh to brackish throughout and salinities in the central lagoon area drop to 3‰, to below 1‰ in the eastern sector and to 0‰ at the mouth of

the Ogun River [11]. During the dry season, bottom water hypoxia events were recorded in the eastern sector of Lagos Lagoon [12].

The lagoon is known to have a wide range of sediments from mangrove swamp to muddy and sandy foreshores with either slight or pronounced wave action according to the degree of exposure, depth and the extent of the open water [9, 10]. Thus, a wide variety of mixed deposits containing different proportions of coarse sand, fine sand, silty mud, and mud cover the lagoon floor. Sediment samples were found to consist of dark grey, organic-rich muddy sand and fine- to coarse-grained sand containing various abundances of mollusk shells.

Material and methods

Sampling was carried out between May 24–26, 2019, which corresponds to the beginning of the rainy season in Nigeria. The sampling was conducted in collaboration with the Nigerian Institute of Oceanography and Marine Research (NIOMR), as necessitated by the overall extent of the lagoon and the laborious logistics required (no specific permits were required for the described field studies). A total of twenty-four sites were sampled by boat with a Van Veen grab sampler and sediment was scraped off from the top 2 cm. The material was stored in plastic containers, transported to the laboratory, washed over 63 µm sieves and dried at room temperature [13]. The locations of individual sampling sites were precisely georeferenced via GPS and site-specific information is provided in Table 1 and Fig 1.

Foraminifera were then picked but not every sample yielded the standard amount of 300 specimens, as the abundance of benthic foraminifera varied from sample to sample. Benthic

Table 1. Sample site information including *in situ* measurements of depth, pH, Total Dissolved Solids (TDS) and sea surface temperature recordings and sediment type (CS = Coarse Sand; FS = Fine sand; SM = Silty Mud; M = Mud).

STATION	LATITUDE	LONGITUDE	DEPTH (m)	pН	TDS (ppm [mg/l]	Temp (^O C)	Sediment Type
1	6°23'59.73"	3°23'58.79"	20	6.9	8.4	29.8	CS
2	6°26'13.37"	3°23'58.59"	15	6.8	7.67	29.2	FS
3	6°27'32.27"	3°22'31.60"	20	6.5	7.83	29.1	FS
4	6°29'14.99"	3°24'12.62"	10	6.4	8.06	29.0	SM
5	6°31'3.77"	3°24'36.41"	5	6.9	1.31	29.2	М
6	6°32'43.78"	3°24'54.41"	7.5	6.6	1.22	29.2	М
7	6°34'32.38"	3°26'2.30"	3	6.6	0.96	27.5	М
8	6°35'24.84"	3°28'27.54"	4	6.5	0.31	27.3	FS
9	6°33'3.53"	3°27'44.75"	6	6.5	0.99	26.2	FS
10	6°30'56.07"	3°27'3.26"	7	6.6	1.01	27.8	М
11	6°28'27.27"	3°26'19.18"	7	6.4	0.98	27.7	М
14	6°28'6.16"	3°28'9.68"	12	6.5	1.76	27.7	М
15	6°27'42.21"	3°30'6.88"	12	5.8	1.68	26.3	SM
16	6°31'39.16"	3°33'8.84"	5	6.8	7.53	25.9	SM
17	6°29'44.65"	3°33'3.96"	4	6.7	8.21	25.1	SM
18	6°28'27.02"	3°33'14.44"	3	6.8	7.72	24.5	SM
19	6°30'40.31"	3°34'35.70"	4	6.7	8.72	24.9	SM
20	6°32'43.70"	3°34'34.85"	3	6.8	8.69	24.1	SM
21	6°32'5.01"	3°36'42.58"	3	6.6	7.65	24.2	SM
22	6°33'0.55"	3°38'25.59"	3	6.8	7.40	25.1	М
23	6°32'19.31"	3°41'49.52"	4	6.6	7.40	24.3	М
24	6°35'46.04"	3°41'44.34"	2	6.5	7.35	24.3	М
25	6°36'28.48"	3°45'37.70"	3	6.8	7.62	24.5	М
26	6°36'21.51"	3°49'37.21"	5	6.7	7.81	24.2	М

https://doi.org/10.1371/journal.pone.0243481.t001

foraminifera were then identified to species level and individual taxa were documented by the Scanning Electron Microscopy (SEM), identified and assembled into a catalogue of taxa. For our analysis, living foraminifera were grouped with dead tests because our aim was to provide a general environmental and lagoon-wide data set useful in paleoecology. Our samples are thus time-averaged, and as such provide an effective means to compare changes recorded in the fossil record [14].

Individual species were counted and percent abundances were calculated for each taxon and for wall structural types (Table 2, Fig 2). Heat maps, showing the abundance of taxa, were then generated for selected species to document and analyze their distribution. The composition and structure of benthic foraminiferal assemblages were then analyzed from individual sites and further examined for diversity indices namely Fisher α , Shannon H (log base 10), Dominance D and total species richness.

To determine the structure in our foraminiferal data set, statistical analyses (Cluster, Principal Component [PCA] and Detrended Correspondence Analysis [DCA]) were carried out using the PAST3 software [15]. These techniques group samples with similar faunal assemblages and reveal a typology of environmental signatures embedded in foraminiferal assemblages. PCA and DCA are helpful in a multivariate analysis to structure and visualize larger data sets by reducing a large number of variables to a few linear combinations (principal components). In addition, the foraminiferal number (FN) per gram of treated sediment sample was counted for all the sites [16–18].

Sample Stations	Taxa_S	Individuals	Dominance_D	Shannon_H	Fisher_a	% Hyaline	% Agglutinated	FN g ⁻¹
ST1	11	27	0.2016	1.937	6.92	48.1	40.7	7.7
ST2	23	68	0.1025	2.67	12.23	73.5	7.4	13.3
ST3	3	3	0.3333	1.099	0	100	0	0.6
ST4	8	189	0.5629	0.9499	1.694	89.4	10.1	61
ST5	12	237	0.4461	1.234	2.668	10.1	88.6	184.1
ST6	3	15	0.4489	0.9276	1.128	40	60	19.5
ST7	7	263	0.7499	0.6026	1.321	3.8	96.2	821.9
ST8	6	50	0.2928	1.485	1.78	12	88	33.4
ST9	7	68	0.6181	0.8894	1.957	13.2	86.8	34
ST10	7	258	0.5348	0.9502	1.327	5.4	94.6	345.8
ST11	6	302	0.6124	0.7636	1.061	1	99	523.4
ST14	2	2	0.5	0.6931	0	0	100	3.4
ST15	21	65	0.1569	2.352	10.76	69.2	24.6	16.7
ST16	23	213	0.3244	1.838	6.548	24.9	67	51.9
ST17	2	20	0.505	0.6881	0.5532	45	55	5.7
ST18	2	68	0.8153	0.3315	0.3864	100	0	44
ST19	8	84	0.3611	1.4	2.174	63.1	35.7	23.5
ST20	10	129	0.4766	1.232	2.531	19.4	76.7	43.9
ST21	13	196	0.4092	1.289	3.13	75	23	74.1
ST22	8	328	0.767	0.5517	1.48	2.1	97.9	1,012.3
ST23	9	335	0.5471	1.044	1.702	12.2	87.8	327.5
ST24	6	349	0.5596	0.9157	1.029	4.6	95.4	502.2
ST25	7	262	0.5438	0.9732	1.322	7.3	92.7	333.3
ST26	6	341	0.5776	0.8993	1.034	7.9	92.1	703.1

Table 2. Quantitative faunal analyses of foraminiferal assemblages from the Lagos Lagoon, Southwest Nigeria, Gulf of Guinea.

Numerical data include diversity and dominance indices of individual samples, percent abundances of hyaline and agglutinated foraminifera specimens, and foraminiferal number (FN) per gram sediment.

https://doi.org/10.1371/journal.pone.0243481.t002



Fig 2. Percent abundances of agglutinated, hyaline-perforate and miliolid foraminifera across the Lagos Lagoon. Circle diagrams represent 100 percent of the total assemblage at the individual sites (for details see Table 2).

A Hanna HI 9813-6N multi-parameter sensor probe-device was used to record the physico-chemical and environmental data including depth, temperature, pH and total dissolved solids (TDS = combined content of all inorganic and organic substances). Environmental data recordings were conducted at the water surface and are provided in Table 1). For species identifications we have applied the concepts of the nearest complete faunal studies from Mikhalevich [19, 20], Debenay and Basov [21], Debenay and Redois [22], Langer et al. [13, 23] and Fajemila and Langer [24, 25], Thissen and Langer [26], Langer et al. [27], Hayward et al. in press [28].

Results

Physico-chemical measurements

Surface water temperature measurements revealed a range between 29.8 and 24.1 °C across the lagoon. Temperatures were found to be generally high (>29 °C) around the entrance channel and along the southwestern shore (ST1-6). Towards the northwestern sector, near the Ogun River, and in the central parts of the lagoon, temperatures drop to ~26 °C and become successively lower towards the easternmost sector (>24 °C).

Ph values were found to be largely homogenous across the lagoon and range between 5.8 and 6.9 (see also [29]). The lowest values were recorded in the northwestern sector near the Ogun River mouth (<6.7). Highest values were recorded in the entrance channel and around Lagos harbor (>6.8, ST1, ST2), where marine waters enter the lagoon system. Medium and high values were recorded at a few selected sites in the central part of the lagoon (Table 2).

Total dissolved solids were found to range between 0.31 to 8.72 mg/l. Highest values (>7.35) were recorded around the entrance channel and along the southwestern shore (ST1-4)

and in the murky waters of the eastern sector (ST16-ST26). Medium values were recorded in the western lagoon, and lowest values were found in the northwestern area (ST7-9) near the mouth of the Ogun River.

The physicochemical measurements, however, only provide a snapshot of environmental conditions at the time of collection and do not account for seasonal variations related to the wet and dry season and runoff from the Ogun River. As such they are of limited value for the analysis of total assemblages.

Composition of foraminiferal assemblages

A total of 3872 benthic foraminifera specimens were picked and identified to species level whenever possible. This resulted in the identification of 42 species belonging to 25 genera. The foraminiferal assemblage comprises 10 porcelaneous, 22 hyaline perforate and 10 agglutinated taxa. Agglutinated foraminifera are the dominant group with an abundance of 77.9%, while the hyaline-perforate species make up 21.1% of the total assemblages. The remaining 1% belongs to the porcelaneous taxa (Table 2).

Agglutinated foraminifera dominate the foraminiferal assemblages over large parts of Lagos Lagoon (Fig 2, Table 2) and constitute ~90% of the total assemblage at many sites in front of the Ogun River (ST5, ST7, ST8, ST9, ST10, ST11), make up more than 67% along the northern shore (ST16, ST20), and comprise mostly more than 90% of the fauna in the easternmost sector of the lagoon (ST22-ST26).

Foraminifera with a hyaline perforate test are dominant around Lagos Harbor and the Commodore Channel (ST1- ST4), where the lagoon empties its waters into the Atlantic, and marine waters mix with brackish lagoonal waters. High abundances of perforate foraminifera where also found along the lagoonal shore off Lekki.

Miliolid foraminifera are generally rare within the entire lagoon and are mostly represented by species of the genus *Quinqueloculina*. The abundance of porcelaneous species corresponds to only 1% of the total population of benthic foraminifera of the Lagos Lagoon. Highest occurrences of miliolids were recorded near the entrance channel (<19%), where open ocean and brackish waters mix and salinity values are commonly higher than in the lagoon. Miliolids are absent in the easternmost parts of the lagoon and near the mouth of the Ogun River, where low salinity and freshwater conditions are predominant for most of the year. The distribution of miliolids, hyaline-perforate, and agglutinated foraminifera neither covaries with pH nor with lagoon surface water temperature recordings (see Table 1).

Agglutinated species of the genus *Ammotium* dominate the assemblages within the lagoon at many sample sites (Table 2 and Fig 3A) and account for about 62.8% of the total population of benthic foraminifera. *Ammotium salsum* alone frequently reaches abundance levels of more than 50% in some samples (ST5, ST7, ST10, ST11, ST16, ST20, ST22, ST23, ST24, ST25, ST26). It is particularly abundant in shallow waters (< 7m) in the northwestern part of the lagoon near the mouth of the Ogun River (> 65%) and in the eastern areas of Lagos lagoon. Both areas are characterized by low salinity but salinity varies with the season (0–10‰). Along the densely populated western shore, around the harbor and other industrial structures, and in southern shallow waters off Lekki, abundance values drop substantially and commonly range below 25%. Within the deeper and highly saline Commodore entrance channel, percent abundance values of *Ammotium salsum* display lowest values and range below 5%.

The agglutinated taxon *Trochammina* sp. 1 is also fairly abundant (Fig 3B) and frequently constitutes more than 10% to the population at many sample sites (ST5, ST6, ST10, ST11, ST24, ST25). The species occurs throughout the Lagos Lagoon, its abundance maxima, however, are antagonistic to those of most other agglutinated taxa and differ significantly from



Fig 3. Heat maps showing color-coded percent abundances with interpolated distribution contours for A.) Ammotium salsum, B.) Trochammina sp. 1. and C.) Ammonia aoteana, within the Lagos Lagoon, Nigeria.

https://doi.org/10.1371/journal.pone.0243481.g003

those of *Ammotium salsum*. The species reaches its highest total abundance values at site ST6 and ST14 (60% and 50%). Site ST6 is located south of dense mangrove forests at the mouth of Ogudu Creek, where sewage, industrial wastewater and heavy metal concentrations accumulate. *Trochammina* sp. 1 is abundant where most other agglutinated taxa (*Ammotium salsum*, *Miliammina fusca*, *Ammobaculites exiguus*, and *Textularia* sp.) have minimal or rare occurrences.

Ammonia aoteana dominates the group of hyaline-perforate foraminifera in the Lagos Lagoon sample material. It constitutes 14.1% of the total population of benthic foraminifera recovered and percent abundances at individual sites range between 0 and 90%. It is prominent at some sites along the densely populated western part of the lagoon (<20%, ST4, ST6), in the entrance of the Commodore Channel (ST1 and ST2), and strikingly abundant in the shallow central parts SE of Lekki (ST15, ST17, ST18, ST19, ST21; Fig 3C). Abundance peaks of *Ammonia aoteana* were mostly recorded in shallow waters (3-7m) with highest value of 90% at sample station ST18 (3m). The distribution of the second species of *Ammonia, Ammonia convexa*, matches the distribution of *Ammonia aoteana*, but percent abundance values of *A. convexa* are generally low and rarely exceed 10%. *Ammonia convexa* was absent at site ST3, ST7, ST11, ST14 and ST17. In total, the species accounted for about 4% of the population of all benthic foraminifera recovered.

Miliammina fusca, a miliolid foraminifer with an agglutinated test, was recorded in low numbers at a few sites within the lagoon. Its presence, however, is to be restricted to the north-westernmost area near the Ogun River mouth (ST7-ST9) and to two sites in the low salinity areas in the eastern sector of Lagos Lagoon (ST22-ST23).

Miliolid foraminifera are generally rare in the lagoon and contribute ~1% to the total assemblage. Highest abundances (~19%) were recorded in close proximity to the Atlantic entrance (ST1, ST2) and moderate values were recorded at site ST15, ST16 and ST19 (<8%). At all other sites, miliolids are extremely rare and occur only sporadically (Fig 2 and Table 2 and S1 Appendix).

Only 4 individuals of larger symbiont-bearing foraminifera were recovered from the entire lagoon material. They belong to *Pararotalia* and *Amphistegina*, and constitute only ~ 0.1% of the total assemblage. The few individuals recovered were recorded at sample stations ST10, ST15, ST16, three sites that receive marine water via the main entrance channel and the Five Cowries Creek.

Foraminiferal numbers

The number of foraminifera per gram sediment (FN) varies substantially among individual samples and ranges from 0.6 to more 1000 g⁻¹ (Table 2). Highest values (>300) were recorded in the eastern sector (ST22-ST26), at the northwesternmost end near the mouth of the Ogun River (ST7), and in the central and southern part of the lagoon (ST10, ST11). Low and medium values (>20–200 g⁻¹) were found at two sites on the western lagoon shores (ST4, ST5), in the northwestern area (ST8, ST9), and at the transition from the central lagoon to the eastern sector (ST 16, ST 18-ST21). Lowest values (<20 g⁻¹) were found at the mouth and in the entrance channel of the lagoon (ST1-ST3), at the mouth of the Ogudu Creek (ST6), and at two sites along the southern shore of Lagos Lagoon (ST14, ST15). The FN covaries neither with pH nor with surface water temperature recordings (see Table 2, Fig 4).



Fig 4. Number of foraminifera per unit gram of sediment (FN) in the Lagos Lagoon. Note consistently high values in the eastern sector medium to high values along the continuation of the Ogun River outflow.

https://doi.org/10.1371/journal.pone.0243481.g004

Diversity

The number of benthic species of foraminifera present in Lagos Lagoon was counted at all sites and the results are illustrated in Fig 5. Species richness ranges between 2 and 23 across Lagos Lagoon. Highest values were recorded near Lagos Harbor (ST2, 23 species), were lagoonal waters mix with the adjacent Atlantic Ocean, on the southern shore near Lekki (ST15, 21 species), and at site ST16 (23 species) near Baiyeku. Species richness in the eastern sector of the lagoon (ST22-ST26) was considerably lower and ranges from 6–9. In the northwestern sector, near the mouth of the Ogun River (ST7-ST10), the number of taxa was also recorded to be low and ranges from 6–7. Particularly low species richness values were noted along the western shore near the outlet of Ogudu Creek and in Lagos Harbor (ST6, ST3; 3 species each), and at sites ST14, ST17 and ST18 (2 species), which possibly result from either intense pollution or dredging activities taking place in these localities.

Fisher α diversity values range between 0 and 12.23 in the Lagos samples. The highest Fisher α values were recorded ST1, ST2, ST15, and ST16. The lowest Fisher α index values were noted at ST3, ST14, ST17 and ST18. The high and low Fisher α recordings are congruent with maximum and minimum species richness values recorded at these sites. Shannon (H) values vary between 0.33 and 2.67; the highest values were observed at ST1 (1.937), ST2 (2.67), ST 15 (2.352), and ST 16 (1.838). The lowest Shannon (H) values were recorded at ST7, ST14, ST17, ST18, and ST22. Dominance values range between 0.1 and 0.81. Shannon (H) and Dominance index values are in general accordance with species richness values. Neither species richness nor Fisher α index values covary with pH or surface water temperature recordings (see Table 1).



Cluster analysis

Cluster analysis (R- and Q- mode), analyzing the similarity, composition and abundance of foraminifera at individual sample stations were performed on the 13 most abundant benthic foraminifera recorded throughout the lagoon (Table 3). The 13 species selected represent 98.3% of the total population of all foraminifera counted. Samples ST3 and ST14 were excluded from the Q-mode cluster analysis, because they did contain sufficiently high numbers of specimens (<4).

Cluster analysis (Q-mode), comparing the composition and abundance of foraminifera assemblages from all sample sites, revealed the presence of two units (cluster A-B, Fig 6) and an outlier at ST6. The dendrogram shows that individual clusters occupy different sectors of Lagos Lagoon that are characterized by specific environmental conditions. Cluster A comprises the sample sites that are located near the entrance channel (ST1-ST4) and all sample stations that are located on the southern lagoon shores in the central part of the lagoon (ST15-ST21). Cluster B covers the northwesternmost sites where freshwater from the Ogun River drains fan-like into the lagoon (ST5, ST6-ST11), sites along the northern lagoon shores (ST16, ST20) and all sites located in the eastern lagoon sector (ST22-ST26). The outlier site ST6 is situated at the mouth of the Ogudu Creek, where saltgrass meadows and mangrove trees flourish along the lagoon coastline.

The R-mode analysis resulted in a dendrogram that also revealed two major clusters (Clusters R1 and R2, Fig 7). Cluster R1 comprises a total of 8 species and contains exclusively taxa with hyaline-perforate or porcelaneous wall structure types. Species characterizing cluster R1 have their highest abundances along the industrial areas in southwestern lagoon area, in the Commodore entrance channel and at sample stations that are located on the southern lagoon shores and in the central parts of the lagoon. The distribution recordings of species contained in cluster R1 show that these taxa favor marine and avoid low salinity, brackish or freshwater conditions.

SST	Α	В	С	D	E	F	G	Н	I	J	K	L	М
ST1	0	1	9	1	0	1	0	0	1	1	0	7	0
ST2	0	6	16	1	0	6	6	0	2	8	2	3	0
ST3	0	0	0	0	0	1	0	0	0	1	0	0	0
ST4	0	24	139	12	0	1	4	0	0	0	0	0	7
ST5	14	4	13	151	2	0	4	0	2	0	1	0	43
ST6	0	2	4	0	0	0	0	0	0	0	0	0	9
ST7	14	1	9	227	6	0	0	5	0	0	0	0	1
ST8	6	4	2	24	8	0	0	6	0	0	0	0	0
ST9	3	3	5	53	2	0	0	1	1	0	0	0	0
ST10	13	8	5	182	2	0	0	0	0	0	0	0	47
ST11	19	1	2	231	3	0	0	0	0	0	0	0	46
ST14	0	0	0	1	0	0	0	0	0	0	0	0	1
ST15	0	9	21	9	0	1	4	0	1	0	1	5	0
ST16	17	7	21	117	3	6	7	0	3	3	3	10	4
ST17	0	0	9	11	0	0	0	0	0	0	0	0	0
ST18	0	7	61	0	0	0	0	0	0	0	0	0	0
ST19	8	4	47	15	5	2	0	0	0	0	0	0	2
ST20	9	3	15	87	0	4	0	0	2	1	4	3	0
ST21	2	21	117	39	2	1	0	0	0	7	1	2	0
ST22	26	4	3	286	3	0	0	3	0	0	0	0	1
ST23	15	5	29	244	6	0	7	2	0	0	0	0	26
ST24	28	8	8	255	3	0	0	0	0	0	0	0	47
ST25	19	14	5	189	2	0	0	1	0	0	0	0	32
ST26	31	20	7	255	1	0	0	0	0	0	0	0	27

Table 3.	Thirteen (13) m	ost frequent and	l abundant	benthic foram	ninifera from	the lagoon.
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They correspond to 98% of the entire population of the foraminifera counted: A- Ammobaculites exiguus; B- Ammonia convexa; C- Ammonia aoteana; D- Ammotium salsum; E- Ammotium sp.1; F- Hanzawaia cf. H. nipponica; G- Cribroelphidium mirum; H- Miliammina fusca; I- Neoeponides sp. 1; J- Nonion fabum; K- Quinqueloculina seminulum; L- Textularia sp. 1; M- Trochammina sp. 1; ST-Sample Station.

https://doi.org/10.1371/journal.pone.0243481.t003

Cluster R2 comprises a total of 5 species and is dominated by taxa with an agglutinated wall structure (*Ammotium*, *Ammobaculites*, *Trochammina*). Cluster R2 is associated with low-salinity, brackish water and freshwater conditions. Among the species of cluster R2, we also find *Miliammina fusca*, *a* species that is often the last identifiable species to survive under marginal marine and low salinity conditions.

Principal component analysis

Principal Component Analysis (PCA) is a dimension-reduction tool that can be used to minimize a large set of variables, which helps to describe and classify our extensive foraminiferal occurrence dataset. Like the cluster analysis, the principal component analysis was conducted with the 13 most abundant benthic taxa. The species are shown as vectors and their lengths represent the importance of individual species as calculated by their eigenvalues. It revealed two major groups, that are largely separated by structure (Fig 8). The agglutinated group, which is prevalent in the eastern sector of the lagoon, occupies the B ellipsoid. The *Ammotium salsum* vector is strongly related to those sample stations and includes all sample sites from ST22 to ST26. The second vector includes the sites that are dominated by specimens of the hyaline-perforate genus *Ammonia* (ST4, ST21, ST18, ST19, ST15, and ST2). The sites occupy the densely populated western part and the central southern shores of the lagoon.



Detrended correspondence analysis (DCA)

The detrended correspondence analysis revealed three groups (A-C, Fig 9) that are mainly separated by the wall structural types and sample sites. Group A is dominated by *Ammonia aoteana* and *Ammonia convexa*, two species that have abundance maxima in the southwestern areas of Lagos Lagoon (see also Fig 3C). Group B is dominated by *Ammotium salsum* and other species with agglutinated tests (*Trochammina* sp. 1, *Ammotium* sp. 1, *Ammobaculites exguus*). *Miliaminna fusca* is also associated to group B. Group C contains a mixture of heterogenous and mostly rare species with different types of wall structures from different genera (e.g., *Hanzawaia, Textularia, Nonion, Elphidium, Quinqueloculina*, Fig 9). Members of group C are typical marine taxa, and their distribution in the lagoon is mainly restricted to the area that connects the Atlantic Ocean with Lagos Lagoon (ST1-ST3) and the southwestern and central area, that receives marine waters via the Five Cowrie Creek (ST15, ST16, ST19-ST21).

Discussion

This study provides the first quantitative, species-level and lagoon-wide survey of modern benthic foraminifera present in Lagos Lagoon. A total of 42 species and 25 genera of benthic foraminifera were documented (Figs 10-13). The species recorded include 10 agglutinated, 10 porcelaneous, and 22 hyaline perforate taxa.

Spatial distribution: Salinity

Our data revealed a distinct separation of assemblages dominated by agglutinated and hyalineperforate/porcelaneous taxa across the lagoon (Fig 2). Assemblages with predominantly high percent abundances of agglutinated taxa prevail along the northern lagoon shores, in freshwater diluted waters along the fan-like wedge created by the outflow of the Ogun River and in the



Fig 7. Species dendrogram produced by the R-mode cluster analysis using the correlation coefficient matrix. Note that R-mode clusters reflect test wall types (agglutinated versus hyaline-perforate/porcelaneous).

eastern sector of Lagos Lagoon. Throughout the year, these areas show the lowest salinity values within Lagos Lagoon ranging from freshwater at the mouth of the Ogun River to low saline brackish water conditions (~10‰). Agglutinated foraminifera were recorded to constitute



Fig 8. PCA of the foraminiferal fauna of the Lagos Lagoon showing principal components 1 and 2 (A, B and R1, R2 refer to the assemblages defined in Figs 6 and 7).

~90% of the assemblages at all sites in the eastern sector and in front of the Ogun River mouth and more than 67% along the central northern lagoon shores. Assemblages dominated by hyaline-perforate taxa prevail around the harbor and in Commodore entrance channel, in shallow waters of the densely populated southwestern lagoon area, and along the central southern shores of Lagos Lagoon. The latter areas also contain minor fractions of porcelaneous taxa. All sites that are dominated by hyaline-perforate taxa are marked by higher salinity values (16– 30‰) and characterize the areas that are influenced by Atlantic waters entering the lagoon through the Commodore Channel, the Five Cowries Creek including portions of the central lagoon area.

The transition from characteristic agglutinated assemblages to hyaline-perforate/porcelaneous biotas appears to occur relatively abruptly, even though transitional zones are observable. Culver [30] and Langer and Lipps [31, 32] reported that such changes may occur within a few meters and that zonations are controlled by salinity [33, 34].



Fig 9. Detrended correspondence analysis of the benthic foraminiferal species recovered from the Lagos Lagoon (A, B and R1, R2 refer to the assemblages defined in Figs 6 and 7).

In general, marginal marine environments were previously shown to be dominated by agglutinated taxa [see <u>34</u> for review] and only a few calcareous taxa (e.g., ammoniids, elphidiids), survive under permanently low salinity conditions (e.g., [<u>35</u>, <u>36</u>]). Typical end-members along decreasing salinity gradients are a few agglutinated foraminifera, including *Jadammina*, *Miliammina*, *Ammotium*). Habura et al. [<u>37</u>] argue that the combination of reduced salinity and low pH conditions disfavor calcification in foraminiferans, as they are dependent on the local carbonate concentration [<u>38</u>], which generally decreases in low salinity environments (see also [<u>39</u>]).

Foraminiferal wall structure types were found to have a marked salinity-dependent distribution throughout the lagoon. This phenomenon is observed in total assemblages (Fig 2) and also feature prominently in the antagonistic distribution patterns of the agglutinated species *Ammotium salsum* and the hyaline-perforate *Ammonia aoteana* (Fig 3A and 3C). While *Ammotium salsum* was found to be the dominant constituent in the low salinity northern and eastern sectors, *Ammonia aoteana* prevails along marine influenced southwestern and south-central lagoon sites. The results of antagonistic distribution patterns recorded in total assemblages are in accordance with observations on living foraminifera in the respective areas [7, 8].

Ammotium salsum is known for its tolerance to salinity fluctuations, and a typical representative in marginal marine environments (e.g., [13, 24, 30, 34, 40-42]). Similar to our findings, Debenay [40] reported the species to be the dominant component (>90%) within the



Fig 10. Scanning electron micrographs of benthic foraminifera from the Lagos Lagoon: 1–7. *Ammotium salsum* (Cushman and Brönnimann, 1948); scale bar for Fig 10.2 is 200 μm; **8**, **9**. *Ammotium* sp. 1; **10–12**. *Ammobaculites exiguus* Cushman and Brönnimann, 1948; **13**. *Textularia candeiana* d'Orbigny, 1839; **14–17**. *Textularia* sp. 1; **18–20**. *Textularia* sp. 2; **21**, **22**. *Siphotextularia* sp. 1; **23–26**. *Caronia exilis* (Cushman and Brönnimann, 1948); scale bar for Fig 10.26 is 50 μm; **27–30**. *Milliamina fusca* (Brady, 1870); **30–36**. *Trochammina* sp. 1. Scale bar is 100 μm for all magnifications.

hyposaline (5–10‰) innermost parts of Ebrie Lagoon (Ivory Coast, W-Africa). Within the low saline Camaronera Lagoon (Gulf of Mexico), a lagoon that has not direct link to the ocean and is connected to the Gulf only via a small channel over the Alvarado Lagoon, Phleger and Lankford [43] documented that *Ammotium salsum* also constitutes ~90% of the total assemblages. In the hyposaline Ologe Lagoon (Nigeria) Fajemila and Langer [24] reported *Ammotium salsum* to represent up to 85% of the total assemblage.

Among the calcareous taxa recovered are two species of *Ammonia (Ammonia aoteana, Ammonia convexa)*. Species of *Ammonia* are well known for their tolerance to salinity fluctuations [27, 28, 44]. They are present in almost every shallow-water marginal marine and tidal



Fig 11. Scanning electron micrographs of benthic foraminifera from the Lagos Lagoon: 1, 2. *Trilocularena patensis* Closs, 1963; **3–5**. *Quinqueloculina debenayi* Langer, 1992; **6–9**. *Quinqueloculina cf. Q. cuvieriana* d'Orbigny, 1839; **10**, **11**. *Quinqueloculina seminulum* Linné, 1758; **12–14**. *Quinqueloculina cf. Q. seminulum* Linné, 1758; **15**, **16**. *Quinqueloculina cf. Q. vandiemeniensis* Loeblich and Tappan, 1994; **17**, **18**. *Quinqueloculina sp.* 1; **19**. *Quinqueloculina* sp. 2; **20**. *Quinqueloculina* sp. 3; **21**, **22**. *Quinqueloculina* sp. 4; **23–27**. *Triloculina cf. T. verspertilo* Zheng, 1988; **28**. *Pseudotriloculina* sp. 1; **29**, **30**. *Edentostomina* sp. 1; **31**, **32**. *Miliolinella* sp. 1; **33–35**. *Neoeponides* sp. 1. Scale bar is 100 µm for all magnifications, and 50 µm for Figs 11.2, 11.11, 11.15, 11.16, 11.24, 11.31, and 11.32.

influenced estuarine environments, under normal and brackish water conditions and in places where significant loads of freshwater river discharge mix with marine waters [28, 31, 34, 45]. Along the transect studied in the Ebrie Lagoon (Ivory Coast), Debenay [40] reported *Ammonia* to be present to salinity levels of ~10‰, but lacking at values below this threshold. Similarly, Fajemila and Langer [24] did not find *Ammonia* in the hyposaline Ologe Lagoon west of Lagos, but widely present along Atlantic coastline sites in the Gulf of Guinea [13, 23, 25].

Our finding of largely disjunct distribution patterns of agglutinated and hyaline-perforate/ miliolid foraminifera are strongly supported by independent lines of evidence. This includes Q- and R- mode cluster, DCA, an, PCA analyses, showing distinct separations along the lines



Fig 12. Scanning electron micrographs of benthic foraminifera from the Lagos Lagoon: 1–5. *Rosalina* cf. *R. orientalis* (Cushman, 1925); 6–9. *Hanzawaia* cf. *H. nipponica* Asano, 1944; 10–13. *Planulina* sp. 1; 14–16. *Nonion fabum* (Fichtel and Moll, 1798); 17–19. *Pararotalia sarmientoi* (Redmond, 1953); 20–24. *Elphidium* sp. 1; 25–30. *Cribroelphidium mirum* Langer and Schmidt-Sinns, 2006; 31, 32. *Porosononion* sp. 1. Scale bar is 100 µm for all magnifications and 50 µm for Figs 12.10–12.13, 12.31 and 12.32.

of different wall structures. This suggests, that the general distribution pattern and division of agglutinated and hyaline-perforate/porcelaneous foraminiferal biotas, are mainly driven by the salinity gradient.

Abundance

The number of foraminifera per gram sediment (FN) was found to vary substantially among individual samples. The general pattern recorded revealed highest numbers of specimens $(>300 \text{ g}^{-1})$ in the eastern sector and in proximity to the fan-like outflow of the Ogun River, where gravitational settling promotes the deposition of fine-grained, organically enriched sediments and where seasonal bottom water hypoxia are known to occur (ST22-ST26; [12, 23]).



Fig 13. Scanning electron micrographs of benthic foraminifera from the Lagos Lagoon: 1, 2. *Rotorbis*? sp. 1; **3**, 4. *Globocassidulina*? sp. 1; **5**, **6**. *Bolivina* cf. *B. persiensis* Lutze, 1974; **7–9**. *Bolivina striatula* Cushman, 1922; **10**, **11**. *Bolivina* sp. 1; **12**, 13. *Bolivina* sp. 2; **14–16**. *Rectuvigerina phlegeri* Le Calvez, 1959; **17–22**. *Ammonia convexa* Collins, 1978; **23–27**. *Ammonia aoteana* (Finlay, 1940); **28**, **29**. *Cibicides pseudolobatulus* Perelis and Reiss, 1975; **30**. *Amphistegina* sp. 1. Scale bar is 100 µm for all magnifications and 50 µm for Figs 13.1, 13.2, 13.9 and 13.11.

FN numbers in these areas were found to be distinctly higher than in all other habitats and may reach up to 1000 individuals per gram sediment. The Lagos Lagoon areas that revealed the lowest FN values ($<20 \text{ g}^{-1}$), cover the industrial areas around the Commodore entrance channel, the Lagos Harbor and three sites along the southern shores near Lekki. A low FN number (19.5 g⁻¹) also characterizes site ST6, a locality that is in the immediate vicinity of dense mangrove forest. Sample site ST6 was also found to stand out as an outlier in the Q-mode analyses, based on its distinct faunal composition. The site is situated at the mouth of the Ogudu Creek, where untreated municipal and industrial effluents from densely populated areas are discharged into the lagoon system. Its neighboring site ST7, in turn, a locality that is not under the impact of the Ogudu Creek, shows an FN value of 821 g⁻¹. The FN numbers

reported by Phillips et al. [8] were found to range between 0.6–45.8 g⁻¹, agree with our findings from the polluted western lagoon sites, but are considerably lower than the high numbers reported here from less-polluted sites. Whether the striking FN differences of high and low FN are indeed a consequence of pollution, or a result of higher survivability or preservation rates, requires further study.

Diversity

Species richness and Fisher α diversity index values recorded across the Lagos Lagoon were found to largely correlate with the disjunct distribution pattern of agglutinated and hyalineperforate/porcelaneous taxa, with highest values around the Lagos Harbor and decreasing values towards the distal eastern sector and the northwestern area in front the Ogun River. Foraminiferal diversity thus traces the general Lagos Lagoon salinity pattern, with high diversity in areas that are under the influence of marine waters and low diversity in the low salinity areas (see also [8, 46]). Indicator taxa for the latter include Ammotium salsum, Ammotium sp. 1, Ammobaculites exiguus, and Miliammina fusca (see also [23]). Species indicative for areas characterized by higher salinity (>16‰), include members of Quinqueloculina, Miliolinella, Elphidium, Bolivina, Rectuvigerina, Triloculina, Textularia, Porosononion, Nonion, Neoeponides and others (see [8, 9, 46]). Almost all of them have a calcareous test and are typical constituents of the shallow water marine fauna in the Gulf of Guinea [13, 25]. The high diversity recorded around the heavily polluted harbor area, appears to be contradictory at first glance, as impacted sites commonly display a decrease in the number of species, reduced abundances and a selection towards pollution-resistant taxa [47-50]. Due to the proximity to the Atlantic Ocean, the harbor is subjected to diurnal tidal fluctuations and is regularly flushed by marine waters entering through the main channel. The high diversity recordings around the harbor area is mainly driven by the presence of typical marine taxa (see also [9]), and promoted by favorable salinity and pH conditions. The extent to which seawater enters the lagoon depends on the season and brackish water conditions may exist up to >30 km into the lagoon and creeks. Within the lagoon, waters are rapidly diluted and foraminifera species richness is substantially reduced, ultimately resulting in low-diverse agglutinated assemblages (Fig 5; see also [6]). Low-diverse agglutinated assemblages, similar to the biotas recorded near the Ogun River, were recently recorded as far as 40km inland [24]. In 2012, Phillips et al. [8] recorded only 9 species of benthic foraminifera across the lagoon, with a 95% dominance of Ammonia in the total assemblages. In a more recent study, the number has increased to 20 benthic taxa [9]. Benthic foraminiferal species richness recorded here was found to exceed 40 and thus more than doubles previous species counts.

Anthropogenic Influences Superimposed on the salinity-driven distributional differences between agglutinated and hyaline-perforate/porcelaneous assemblages are a multitude of stressors related to increasing anthropogenic influences. The City of Lagos has experienced tremendous growth over the past 50 years, reaching ~20 million from just 1.4 million in 1970. The exponential population growth goes hand in hand with the environmental degradation of Lagos Lagoon, where present-day water pollution levels often exceed compliance levels of regulatory health standards (e.g., [3, 51–56]). Pollution levels of the lagoon were reported to be greatest in the Lagos Harbor area, along the densely populated western coast, and decreases towards the northern and the eastern sector [57]. The Lagos Lagoon ecosystem therefore includes areas that cover the full range from strongly impacted by human activities to those having low levels of direct impact [58]. The environmental impact includes pollution from untreated wastewater discharge, hydrocarbon pollutants and petroleum exploitation wastes, chemical contaminants, widespread and unregulated practice of coastal solid waste dumping,

uncontrolled chemicals used by local fisherman, ineffective sewerage systems, industrial discharges, heavy metal pollution, wood residue leachates, and sand dredging activities [1, 56, 59– 62] and pose a serious threat to biodiversity and the aquatic ecosystem.

Influence of pollution

Studies on the concentration of heavy metal sediment contamination (Cd, Co, Cr, Cu, Ni, Pb, Zn), indicated i.) highest values in close proximity to anthropogenic activities and near point sources from industrial effluents and domestic sewage, ii.) spatial variation, and iii.) concentrations via bioaccumulation that exceed toxicity levels with considerable risks to aquatic systems and biotas [11, 55, 59, 63, 64].

The spatial distribution of individual species recorded along the polluted western coast of Lagos Lagoon revealed species abundance patterns that highlight selected taxa as potential bioindicators of stressed environments (Fig 3). *Ammotium salsum*, a typical representative of brackish water lagoons, was found to dominate the northwestern and eastern sector of the lagoon, where relatively lower degrees of pollution and more pristine waters are found. The species is significantly less abundant along the polluted western coast, and largely absent from the Lagos Harbor, the Commodore Channel and in shallow waters north of Lekki.

Among the sample stations situated along the polluted western coastline is site ST6, a site that is located in front of the Ogudu Creek, south of the northern mangrove area. The creek discharges municipal and industrial effluents, wastewater from the cottage industry and is used a dumpsite for solid wastes [3, 51, 65]. Q-mode cluster analysis has identified site ST6 as a distinct outlier and contains only *Trochammina* sp. 1 and two species of *Ammonia*. Besides having extremely low diversity, the site is characterized by particularly low foraminiferal abundances (FN). Studies by Ejimadu et al. [66] revealed very high levels of suspended solids (4170 ppm) that possibly result from upstream artisanal sand mining activities. Moreover, the levels of sulphate, phosphate and the concentrations of heavy metals (Fe, Cu, Pb and Zn) were found to exceed the standards of the National Environmental Standards and Regulations Enforcement Agency (NESREA). ST6 is strikingly different from its neighboring site ST7, a locality that is also near the same mangrove area but not under the impact of the Ogudu Creek. Site ST7 shows a 40-fold higher FN value and contains 8 species.

Ammonia aotena was found to be the dominant taxon along the highly polluted area north of Lagos Harbor (ST4), a finding that is concordance with the presence of living individuals from this area [7, 8]. The area is impacted by highest concentrations of both carcinogenic polycyclic aromatic hydrocarbons (PAH), heavy metal pollutants, trichloroethylene and characterized by higher sediment oxygen demand (SOD) and total organic matter (TOM) levels in proximity to the heavily industrialized port area near Apapa [4, 58, 64, 67]. Studies on the microbial assemblages indicated that the bacterial communities around this area differ substantially from non-polluted sites in the lagoon [58] and revealed lower diversity and evenness in the microbial communities. *Ammonia aoteana* and *A. convexa* often co-occur together along the industrialized western (sampling sites 1, 2, 3 and 6) and southern coast (sampling sites 15, 18 and 21) of the lagoon. Even when they occur separately, these species are most prominent in the western and southern parts of the lagoon (Fig 2 - A. aoteana at sampling sites 17 and 19; *A. convexa* at sampling site 8).

Species of *Ammonia* were widely reported to be among the most pollution-tolerant benthic taxa [47, 48, 50, 68]. The resilience includes tolerance against industrial and municipal sewage outlets, chemical and thermal effluents, fertilizer byproducts, oil discharges, mining effluents, land reclamation activities, and pollution site outfalls discharging heavy metals. Depending on the type and degree of pollution, the stress-related response of *Ammonia* was shown to vary

from site to site, and often involves either an increase or decrease in abundance and various forms of test deformation [47, 48, 50]. The resilience of some species of *Ammonia* is indicative for higher rates of survivability under conditions of environmental perturbation, and the taxa were regarded as endmembers under extreme conditions and as potential bioindicators of pollution [47].

Other known bioindicators of pollution [47] were also recorded in Lagos Lagoon (Table 3; [7–9]). This includes species of *Elphidium*, *Nonion*, *Trochammina*, *Ammobaculites* and a few bolivinid taxa. Most of them were found to be rare but their occurrence is largely restricted to the polluted western portion of the Lagoon (Fig 2) and most of them are absent from the less-polluted eastern sector and along the northwestern outflow of the Ogun River. Species of these genera were found to tolerate discharges from both industrial and domestic effluents, including drainage of heavy metals, toxic oil-based components, sewage, and agricultural and fertilizer pollutants (reviewed in [47] and [50]). Increased abundances of pollution-associated foraminifera around impacted sites were attributed to enhanced tolerance levels, where species profit from the reduced competitive ability over more sensitive taxa, through reduced competition or predatory pressure [47, 69, 70]. Recordings of the above-mentioned genera from the innermost harbor areas (not studied here but see [9]) deserve particular attention in future biomonitoring studies.

A general pattern that emerged from this study was that the polluted western coast sites (ST1-ST4, ST6) displayed low and very low FN numbers that range from 0.6 to 61 g⁻¹. The finding is in accordance with a recent study, that revealed FN numbers ranging from 0.6 to 45.8 g⁻¹ in the most polluted area harbor areas [9]. Low foraminiferal abundances were also recorded from other sites impacted by oil discharges (PAH's) and heavy metals [68, 71–74] and commonly goes hand in hand with a decrease in species richness [48, 50]. In severely polluted but regularly flushed harbor areas, where rates of seawater exchange are high and water residence time remains short, high diversity assemblages, composed of both marine, brackish and marginal marine biotas, continue to be promoted [75]. The presence of marine taxa and comparatively high species richness recorded around the Lagos Harbor area (Table 2, Fig 5), are in agreement with this finding and attest that threshold values of pollution are limited to the extent, that allow a selected number of species to thrive under the impact of antropogenically induced perturbations. Reports of harbor samples barren of foraminifera [9], indicate, however, that source-point specific biomonitoring deserves particular attention.

Redistribution of species

The present-day Lagos Lagoon is a highly dynamic system, where sediments are injected from fluctuating river flows, disperse and settle along prevailing current regimes, are remobilized, redeposited, and accumulate on the lagoon floor or mix with incoming marine sediments from the Atlantic around the Commodore entrance channel or through the Five Cowries Creek. In general terms, the distribution of lagoon sediments follows the energy systems, with the coarsest sediments near Ogun River mouth and around the Atlantic entrance channel and the finest sediments in the innermost reaches and eastern sector, where current velocities are low and approach zero. The dissemination and redistribution of taxa is therefore of concern for biomonitoring studies, especially when working with total assemblages. The following findings, however, support the notion that collected foraminiferal biotas represent mostly autochthonous assemblages and that large scale-redistribution effects can be excluded.

1. The transition from characteristic agglutinated assemblages to hyaline-perforate/porcelaneous biotas was found to be consistent and comparatively abrupt.

- 2. Numerically abundant species with restricted distribution ranges (e.g., *Miliammina fusca*, *Hanzawaia* cf. *H. nipponica*, *Nonion fabum*, *Quinqueloculina* species) do not show random or scattered occurrences, but remain limited to confined environments.
- 3. Visual inspection of the foraminiferal material revealed that test preservation within the lagoon generally ranged between well-preserved and excellent. This also includes taxa with particularly fragile tests (e.g., *Miliammina fusca*) Near the Atlantic entrance and in the Commodore entrance channel, test preservation was moderate to low, indicative reworking and horizontal transport. Previous studies have shown that the degree of test preservation in foraminfera can be used as an approximation for transport rates, where well preserved tests are indicative for the living or *in situ* fauna, whereas poorly preserved tests show allochthonous origins or reworking [76, 77].
- 4. Hyaline-perforate and porcelaneous taxa are largely restricted to environments that are under the influence of marine waters with salinities above ~16‰.
- 5. Larger symbiont bearing foraminifera were found to be extremely rare (only 4 specimens), thus excluding large-scale transport of fully marine species into the lagoon habitat through the entrance channel.
- 6. High abundances of agglutinated foraminifera were recorded in shallow waters off Banana Island, a man-made island developed by land reclamation involving dredging activities from around the northwestern Ikorudu area. Typical foraminiferal indicator taxa present around the northwestern city of Ikorudu (ST8), were also found at site ST11 off Banana Island (e.g., *Ammotium salsum, Ammobaculites exiguus*). Whether sand dredging is indeed the source for the high abundance of agglutinated assemblages off Banana Island, or if the assemblage at ST11 is autochthonous, requires further study. Other than this, we do not have evidence that sand dredging has caused large-scale distortions in the general distribution pattern of foraminiferal assemblages.

In summary, this implies that the current-, wave-, or anthropogenically induced redistribution of taxa is limited and that the benthic assemblages may preserve the original community structures and sufficient environmental information to be useful in biomonitoring studies.

Conclusions

Lagos Lagoon is the ultimate sink for its metropolitan residential and industrial discharges and a significant repository of pollutants. Our study on the spatial distribution, species richness, structural composition and abundance of individual taxa of benthic foraminifera, leads to the following major conclusions:

- 1. Lagos Lagoon houses a total of 42 species of benthic foraminifera including 10 porcelaneous, 22 hyaline perforate and 10 agglutinated species. Our research constitutes the most comprehensive study on benthic foraminifera with species records that more than double previous species counts.
- 2. Foraminiferal assemblages recorded across the lagoon display a two-part pattern that is separated along the lines of wall structural types. Agglutinated foraminifera strongly dominate in the low saline eastern and northwestern portion the lagoon and foraminifera with a hyaline-perforate or porcelaneous test are mainly present in the marine influenced areas. The spatial separation of lagoonal biotas into two domains is supported by independent lines of evidence, including cluster, PCA and DCA analysis, and features prominently in the antagonistic distribution patterns of the two most abundant taxa (*Ammotium salsum*, *Ammonia*

aoteana) and in FN recordings. The spatial separation is largely oriented along salinity contour lines, does not co-vary with pH and TDS, and appears to be largely driven by salinity.

- 3. Areas with high pollution along the highly populated western and southwestern coasts were found to be characterized by low FNs but comparatively higher species richness values. High diversity recordings around the polluted harbor area is mainly driven by the presence of marine taxa, indicative for the influence of Atlantic waters entering the harbor area through the main entrance channel.
- 4. Analysis of total assemblages shows the foraminiferal biotas to be largely autochtonous, and thus preserve the original community structures and sufficient environmental information to be useful in paleoecology.
- 5. The sites impacted by pollution were found to be characterized by specific assemblages and taxa, indicative for enhanced tolerance levels to multiple stressors, and provide a repertoire of bioindicators to assist in future studies on environmental perturbations.

Supporting information

S1 Appendix. Benthic foraminifera count from the sediments of the Lagos Lagoon sample stations.

(DOCX)

Acknowledgments

Sampling was conducted in cooperation with the Nigerian Institute of Oceanography and Marine Research (NIOMR). The authors gratefully acknowledge support from Dr. SIna Adegbie for field assistance. We are also grateful to the referees and the associate editor for their constructive comments and suggestions.

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Chapter 2: Supplement
Supplementary Table S1

Species	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST11	ST14	ST15	ST16	ST17	ST18	ST19	ST20	ST21	ST22	ST23	ST24	ST25	ST26
Ammobaculites																								
exiguus	0	0	0	0	14	0	14	6	3	13	19	0	0	17	0	0	8	9	2	26	15	28	19	31
Ammonia																								
convexa	1	6	0	24	4	2	1	4	3	8	1	0	9	7	0	7	4	3	21	4	5	8	14	20
Ammonia aoteana	9	16	0	139	13	4	9	2	5	5	2	0	21	21	9	61	47	15	117	3	29	8	5	7
Ammontium																								
salsum	1	1	0	12	151	0	227	24	53	182	231	1	9	117	11	0	15	87	39	286	244	255	189	255
Ammotium sp.1	0	0	0	0	2	0	6	8	2	2	3	0	0	3	0	0	5	0	2	3	6	3	2	1
Amphistegina sp.																								
1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Bolivina cf. B.																								
persiensis	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Bolivina striatula	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bolivina sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Bolivina sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Caronia exilis	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0
Cibicides																								
pseudolobatulus	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Cribroelphidium																								
mirum	0	6	0	4	4	0	0	0	0	0	0	0	4	7	0	0	0	0	0	0	7	0	0	0
Edentosomina sp.																								
1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elphidium sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Globocassidulina?																								
sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Hanzawaia cf. H.																								
nipponica	1	6	1	1	0	0	0	0	0	0	0	0	1	6	0	0	2	4	1	0	0	0	0	0
Miliammina fusca	0	0	0	0	0	0	5	6	1	0	0	0	0	0	0	0	0	0	0	3	2	0	1	0
Miliolinella sp.1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Neoeponides sp.	1	2	0	0	2	0	0	0	1	0	0	0	1	3	0	0	0	2	0	0	0	0	0	0
Nonion fabum	1	8	1	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1	7	0	0	0	0	0
Pararotalia																								
sarmientoi	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Planulina sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Porosononion sp.																								
1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Pseudotriloculina																								
sp. 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quinqueloculina																								
cf. Q. cuvieriana	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Benthic Foraminifera count data obtained from Lagos Lagoon sediment samples

Quinqueloculina																								
cf. Q. seminulum	1	3	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0
Quinqueloculina																								
debenayi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Quinqueloculina																								
seminulum	0	2	0	0	1	0	0	0	0	0	0	0	1	3	0	0	0	4	1	0	0	0	0	0
Quinqueloculina																								
sp. 1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Quinqueloculina																								
sp. 2	0	2	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Quinqueloculina																								
sp. 3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quinqueloculina																								
sp. 4	1	2	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Rectuvigerina																								
phlegeri	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Rosalina cf R.																								
orientalis	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Rotorbis? sp. 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Siphotextularia																								
sp. 1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Textularia																								
candeiana	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0
Textularia sp. 1	6	2	0	0	0	0	0	0	0	0	0	0	5	10	0	0	0	3	2	0	0	0	0	0
Textularia sp. 2	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Trilocularena																								
patensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Triloculina sp. 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trochammina sp.																								
1	0	0	0	7	43	9	1	0	0	47	46	1	0	4	0	0	2	0	0	1	26	47	32	27

Chapter 3: Publication



Atypical, high-diversity assemblages of foraminifera in a mangrove estuary in northern Brazil

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Received: 1 March 2021 – Discussion started: 29 March 2021 Revised: 31 May 2021 – Accepted: 1 June 2021 – Published: 7 July 2021

Abstract. Mangrove forests are extremely productive ecosystems, are sources and sinks of organic carbon, and provide essential services both to the marine environment and people. We have studied the composition and species richness of modern benthic foraminiferal assemblages from mangrove swamps along the Mamanguape River in Paraíba, northern Brazil. Sampling points for foraminifera were selected to acquire information on the composition of foraminiferal assemblages from dense mangrove stands collected along a river transect. Almost 100 species of benthic foraminifera were identified within the shallow mangrove habitats. The large number of identified mangrove taxa is the highest recorded so far for true mangrove habitats. The high species richness rivals shallow-water assemblages recorded from nearby offshore and reef environments and indicates that a particularly large number of species is capable of growing and flourishing under multiple stressor conditions. Numerical analysis of the faunal assemblages shows that specific taxa, which were previously known to be uncommon in mangrove environments, are abundant in the Mamanguape River estuary. The atypical foraminiferal fauna found in the Mamanguape River estuary resembles shallowwater offshore assemblages, is characterized by high percent abundances of perforate and miliolid taxa, and contains only very few of the otherwise typical and numerically abundant agglutinated mangrove taxa. The unusual structure of the assemblages recorded provides insight into what combination of environmental variables controls their composition and novel perspectives to reconstruct past mangrove environments. Distribution, diversity, and species-specific analysis will provide guidance on the use of Brazilian mangrove foraminifera as indicators for the strength of tidal activity, pollution, and anoxia in coastal waters and sea-level reconstructions.

1 Introduction

Mangrove ecosystems around the world play an important role in protecting biodiversity, preserving shorelines, and regulating carbon cycling. They respond actively to coastal processes and sediment input and are considered one of the best geological indicators for the detection of modifications in coastal zone dynamics (Cunha-Lignon et al., 2009). Brazil is home to approximately 15% of the world's total mangal forest areas but the release of effluents and untreated wastewater pose threats to ecosystems and marine biota. Nearly 6700 km (90%) of Brazil's 7400 km of coastline hosts mangrove forests. The Mamanguape River estuary is the second largest estuary in the northeastern state of Paraíba (Brazil) covering a mangrove swamp area of more than 57 km² (Bezerra et al., 2012).

Mangrove ecosystems are subject to a suite of disturbances that vary in their intrinsic nature (e.g., geological, physical, chemical, and biological) in time and space. Inhabiting the interface between land and sea at low latitudes, these ecosystems occupy a harsh environment and are subject to daily tidal, temperature, and salinity variations as well as varying degrees of anoxia. Mangrove forests and their inhabitants are therefore rather robust and highly tolerant to life in their saline environments within warm, subtropical, and tropical seascapes (Alongi, 2008). In addition to the natural fluctuations, anthropogenic activities also affect these ecosystems. The estuaries of the northeastern ecoregion are among the most affected by the human occupation processes in Brazil and need actions that guide an integrated management to maintain ecosystem sustainability. Cities close to the Mamanguape River estuary have a total of nearly 40 000 inhabitants (Instituto Brasileiro de Geografia e Estatistica, 2019) and extensive sugarcane and shrimp aquaculture fields place

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environmental pressure on the mangrove ecosystem. The synergistic effects of multiple stressors may cause broadscale changes in estuarine and coastal ecosystems impacting the abundance, species richness, and distribution of functionally important taxa. The interactions of multiple stressors are likely to increase as climate change and anthropogenic pressures will alter the delivery of freshwater and associated nutrients and pollutants to estuarine and coastal ecosystems (Scavia et al., 2002; Paerl et al., 2006; Gillanders et al., 2011; Schiedek et al., 2008). Understanding the response of benthic communities to key stressors is vital for managing mangrove environments and the first step towards setting ecologically relevant limits.

Foraminifera in tropical mangrove environments of South America and the islands nearby have been studied since the late 1940s in Trinidad (Cushman and Brönnimann, 1948a, b; Wilson et al., 2008), the Gulf of Paria (Todd and Brönnimann, 1957), Bahia (Zaninetti et al., 1979; Hiltermann et al., 1981; Eichler et al., 2015; Laut et al., 2016), Rio de Janeiro (Brönnimann et al., 1981; Debenay et al., 2001; Barbosa et al., 2005; Laut et al., 2016; Martins et al., 2016; Gasparini and Vilela, 2017; Belart et al., 2019), Colombia (Boltovskoy and Hincapié de Martínez, 1983), Puerto Rico (Culver, 1990), French Guiana (Debenay et al., 2002, 2004), Santa Catarina (Laut et al., 2016), Sao Paulo (Eichler et al., 2007; Passos et al., 2017; Eichler et al., 2019), and Rio Grande do Sul (Laut et al., 2016; Damasio et al., 2020; Semensatto et al., 2009). Most foraminifera assemblages studied from true mangrove settings were previously reported to be dominated by agglutinated species in a low-diversity ensemble (e.g., Boltovskoy, 1984; Culver, 1990; Debenay, 1990; Murray, 1991; Brönnimann et al., 1992 and references therein). However, we were persuaded to perform an in-depth investigation of our samples upon observing highly diverse and calcareous-dominated assemblages in our preliminary results. The objectives of the present study were (i) to provide detailed documentation on the structure and species richness of foraminiferal assemblages in mangroves of the Mamanguape River estuary of northern Brazil, (ii) to understand the driving forces contributing to the unusual composition and high diversity of these assemblages, and (iii) to discuss implications for interpreting the fossil record of foraminiferal mangrove assemblages.

2 Regional setting

The Mamanguape River estuary system is located on the coast of Paraíba State in northeastern Brazil and bound by latitudes $6^{\circ}43'02''$ S to $6^{\circ}51'54''$ S and longitudes $35^{\circ}07'46''$ W to $34^{\circ}54'04''$ W (Fig. 1). It is situated in an incised river valley, formed along a graben structure and oriented perpendicular to the coastline (Bezerra et al., 2001). The Mamanguape River estuary belongs to the Northeastern Marine Ecoregion (NEME; Spalding et al., 2007), covers an area of $\sim 658 \text{ km}^2$, and is characterized by different rainfall regimes with varying rates of precipitation and duration during the wet and dry periods. As a result, a pronounced environmental variability among NEME estuaries is observed, where the highest reported pH (9.5) was recorded in the Mamanguape River (see Table 3.1 in Rafaela et al., 2018).

Radiocarbon dates collected from core sample material provide evidence that the inundation of the estuary occurred over the last 6000 years and more abruptly within the last 1000 years (Alvez, 2015). The dissolved oxygen levels in the estuary are controlled by a balance between the deoxygenation caused by the intense vegetation and tidal cycles and the oxygenation brought by bioturbating organisms. It is likely that hypoxia is a common condition of intertidal Mamanguape mangrove environments due to the mineralization of a large amount of organic matter produced by mangrove trees, which is responsible for a high consumption of oxygen by bacteria (Alongi et al., 2004) but also due to the exchange of porewater between sediments and the water column, known as "tidal pumping" (Li et al., 2009; Gleeson et al., 2013; Call et al., 2015; Leopold et al., 2017). According to Nordi et al. (2009), the tidal cycle is semi-diurnal on the northeastern coast of Brazil, producing two floods and two ebbs per day with a tidal range of approximately 2.8 m (Paludo and Klonowski, 1999). The estuary is heavily bioturbated by macrofaunal organisms such as oligochaetes, polychaetes, gastropods, bivalves, and shipworms. In addition, crabs and fish are diverse and abundant and are part of the vibrant ecosystem found in the estuary (Leonel et al., 2002; Nascimento et al., 2016; Van der Linden et al., 2017). Thus, sediments of the Mamanguape River estuary are heterogenous in terms of their oxygen content; they rapidly become anoxic below the sediment surface, but bioturbation results in localized oxygenation of sediments around macrofaunal burrows (see also Langer et al., 1990).

3 Material and methods

Sediment samples were collected from the top 2 cm in September 2006 along the Mamanguape River estuary (Fig. 1). This time of the year falls within the dry season (August to November; Debenay et al., 2004) and the year 2006 is considered a "very dry" year and the driest between 2002 and 2006 (Santos et al., 2015). The sampling sites selected are located along the main and tributary mangrove channels and are scattered along a 2 km traverse upstream (Fig. 2). Samples were taken during a post-conference field trip at FORAMS 2006, the largest international symposium on foraminifera promoting the exchange of foraminiferal material under permission of the national science community. Sampling was conducted within dense mangrove stands and with distance from the channels (> 10 m) to avoid potentially allochthonous species transported by currents along the channel. Sampling points are located directly around the



Figure 1. Map of the Mamanguape River estuary with sample site locations (modified from Dolbeth et al., 2016).



Figure 2. Fisher α diversity index graph for foraminiferal samples from the Mamanguape River estuary in comparison to total assemblages reported in previous mangrove studies (data from Brönnimann et al., 1981 (Guaratiba, Brazil); Rouvillois, 1982 (Casamance, Senegal); Lipps and Langer, 1999 (Jellyfish Lake, Palau); Debenay et al., 2002, 2004 (Kaw River, French Guiana); Langer and Lipps, 2003 (Madang, Papua New Guinea); Langer and Lipps, 2006 (Moorea, French Polynesia); Wilson et al., 2008 (Trinidad); Fajemila et al., 2015 (Moorea, French Polynesia); Langer et al., 2016 (Akanda, Gabon); Fajemila and Langer, 2017 (Sao Tome and Príncipe); Eichler, 2018 (Sao Paulo, Brazil); and Semensatto et al., 2009 (Trapandé Bay, Brazil)).

roots within the mangrove trees and the sediments collected are composed of organic-rich mud and silt.

The samples were washed over $63 \,\mu\text{m}$ sieves and dried at room temperature; a total of ~1352 foraminifera specimens (death assemblage) were picked from these samples (Table 1). To standardize the number of individuals recorded in individual samples and to avoid potential pitfalls (Patterson and Fishbein, 1989; Murray, 2006), picking was limited to a weight of 0.05 g of washed and dried sediment. All specimens were identified to species level, illustrated by scanning electron microscopy (SEM), and arranged into plates using Adobe Illustrator CS5 (Figs. 3–9). The total number of individuals was then counted for each species (Table 1) and their abundance and distribution patterns were analyzed.

As a measure of diversity, species richness was determined for each sample and is illustrated by the Fisher α diversity index (Fig. 2, Table 2; Fisher et al., 1943; Murray, 1973). To this end, the total number of individuals has been plotted against the total number of species via the PAST software (Hammer et al., 2001) to compare the foraminiferal assemblage diversity and species richness with respect to the sampled locations. In addition, the Shannon diversity index (*H*) was calculated to characterize species diversity in foraminiferal communities. The Shannon diversity index accounts for both abundance and evenness of the species present. The proportion of species *i* relative to the total number of species (p_i) is calculated and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species and multiplied by -1:

$$H = -\sum_{J=1}^{s} p_{J} \ln(p_{J}).$$
(1)

Species richness and Fisher α diversity values were then compared to other mangrove studies from around the world to place our study in perspective. Lastly, the foraminiferal fauna was documented on plates (Figs. 3–9). All specimens are deposited in the micropaleontological collection at the Institute of Geosciences, University of Bonn (Germany).

Table 1	 Alphabetical list, 	abundance of a	all species	of foraminifer	a identified,	and total	abundance	of specimens,	genera,	and specie	s in the
samples	s from the Mamang	guape River esti	uary.								

Species	Sample 1	Sample 2	Sample 3	Sample 4
Abditodentrix rhomboidalis	2	2	2	4
Adelosina milletti	3	4	2	3
Ammonia advena	2	3	3	1
Ammonia veneta	23	11	11	20
Amphistegina lessonii	1	1		
Amphistegina radiata	1			
Angulogerina cf. A. occidentalis	2	7	5	7
Anomalinulla glabrata	1	2		
Anomalinulla sp. 1	25	17	18	17
Arenoparella mexicana				1
Articulina alticostata				1
Astrononion gallowayi	2	1		
Bigenerina sp. 1	1	2	2	1
Bolivina brevior	2		1	
Bolivina densipunctata	4	3		
Bolivina ordinaria	9	9	2	2
Bolivina striatula	4	5	1	7
Bolivina sp. 1		2		
Bolivina variabilis	1	1		7
Bolivina cf. B. variabilis	6	6	13	10
Buliminella elegantissima	1		1	
Cassidelina sp. 1	2	2	1	2
Cornuspira involvens	5	5	3	1
Cornuspira planorbis			1	1
Cribroelphidium mirum	2	6	12	5
Cushmanina bricei			1	2
Discorbis williamsoni	1			1
Discorbitina pustulata		1	1	2
Edentostomina sp. 1	2	3	2	3
Elphidium sagrum		2	3	3
Elongobula parallela	4	10	9	7
Eoponidella pulchella	4	1		
Fischerina sp. 1	1			
Fissurina sp. 2	2	1	2	1
Fissurina bispinata			1	
Fissurina colomboensis	1	2	2	3
Fissurina semimarginata		2	1	2
Fissurina ? sp. 1	1			
Glabratella carinata	2	4	2	3
Glabratella mirabilis	2	1	1	2
Globocassidulina crassa	2	2	5	4
Globocassidulina rossensis	2	4	3	
Hauerina atlantica				1
Inaequalina sp. 1				1
Laevipeneroplis bradvi				1
Lagena tenuis				1
Loxostomina costulata	5	1	1	
Miliolinella webbiana	-	1	2	3
Miliolinella sp. 1	1	-	1	1
Mychostomina revertens	1		1	1
Neoconorbina radiatogranulata		1	1	2
Neoconorbina sp. 1	3	3	4	4
Neoconorbina terauemi	c.	5	1	1
Nonionoides grateloupii	8	5	2	4
0 <i>r</i>	5	č	-	•

Table 1. Continued.

Species	Sample 1	Sample 2	Sample 3	Sample 4
Orbitina carinata	12	25	30	22
Pararotalia cananeiaensis	81	76	75	71
Procerolagena oceanica				1
Pseudolachlanella eburnea	1	2	2	3
Pseudolachlanella bermudezi	9	6	6	12
Pseudotriloculina sp. 1	4	2	3	2
Pseudotriloculina sp. 2	2	2	1	2
Pseudotriloculina sp. 3	2	1	2	3
Quinqueloculina cf. Q. bosciana	5	5	2	3
Quinqueloculina cf. Q. carinatastriata	3	3	3	3
Quinqueloculina cuvierina	10	8	16	15
Quinqueloculina moynensis	19	22	15	17
Quinqueloculina poeyana	1			
Quinqueloculina cf. Q. rebeccae	1	1		
Quinqueloculina samoaensis	3	5	4	4
Quinqueloculina tantabiddyensis	1	1		
Quinqueloculina sp. 1	2		1	2
Quinqueloculina quinquecarinata	1		1	1
Quinqueloculina sp. 2			3	2
Quinqueloculina sp.3	2	2	3	3
Quinqueloculina sp. 4		1		
Quinqueloculina cf. Q. compta		1	1	2
Quinqueloculina cf. Q. zhengi	1			
Rosalina sp. 1		2	2	1
Rosalina sp. 2	15	14	8	11
Rosalina bradyi		7		1
Rotaliammina trumbulli	2		1	
Rotorbis auberii	4	5		3
Sagrina pulchella		1	3	3
Sigmoilinita costata	2		2	1
Sigmavirgulina tortuosa	1	3	6	2
Svratkina acuta	2	1	1	2
Siphonina reticulata	4	3	4	5
Spirillina grosseperforata			1	1
Textularia sp. 1		1	2	1
Textularia sp. 2		2	1	1
Textularia cf. T. semialata		1		
Trochammina inflata	1		1	
Wiesnerella auriculata	4	5	5	
Total number of specimens	335	346	328	343
Number of genera	40	38	38	44
Number of species	65	66	66	70

4 Results

4.1 Composition of foraminiferal assemblages

A total of 1352 benthic foraminifera specimens belonging to 93 species and 53 genera, including hyaline-perforate, porcelaneous, and agglutinated taxa, were recovered from the samples collected in the Mamanguape estuary (Fig. 1 and Table 1). The foraminiferal tests obtained were found to be particularly well preserved, with even the most fragile forms in good condition, lacking signs of dissolution, abrasion, or breakage. Many specimens were found to contain pyrite framboids inside their tests, a feature considered to be indicative of anoxic conditions in the surrounding sediment (Fig. 9, figs. 22–23). Hyaline-perforate foraminifera comprise 34 genera (64.2% of the total assemblage) and 53 species (57% of the total assemblage), porcelaneous types account for 14 genera (26.4%) and 33 species (35%), and agglutinated taxa are represented by 5 genera (9.4%) and 7 species (8%). Percent abundances of wall structural types (agglu-



Figure 3. (1–2) Trochammina inflata (Montagu); (3–4) Rotaliammina trumbulli Seiglie; (5–6) Arenoparrella mexicana (Kornfeld); (7) Bigenerina sp. 1; (8–9) Textularia sp. 1; (10) Textularia cf. T. semialata Cushman; (11) Textularia sp. 2; (12) Mychostomina revertens (Rhumbler); (13–14) Spirillina grosseperforata Zheng; (15–16) Cornuspira involvens Reuss; (17) Cornuspira planorbis Schultze; (18–19) Fischerina sp. 1; (20–21) Wiesnerella auriculata (Egger); (22–23) Edentostomina sp. 1; (24) Adelosina milletti (Wiesner); (25) Articulina alticostata Cushman; (26–27) Pseudotriloculina sp. 1; (28–29) Pseudotriloculina sp. 2; (30–33) Pseudotriloculina sp. 3. The scale bar is 100 and 50 µm for figs. (8)–(14), (17)–(19), (21)–(24), and (26)–(29).

tinated, hyaline-perforate, and porcelaneous) do not show any significant variation among the sampling sites. Hyalineperforate foraminifera range between 72 % and 75 %, porcelaneous foraminifera constitute 23 % to 27 %, and agglutinated species contribute only 1 % or 2 % to the total assemblage at each site (Table 2). The total number of benthic foraminifera species increases slightly from sample site 1 near the mouth of the estuary (65 species) towards the innermost sample site 4, where 70 species were recorded.

4.2 Species richness and diversity

Species richness values vary between 65–70 among the sample sites (Table 1). The highest value (70 species) was recorded at site 4 and the lowest at site 1 (65 species). Fisher α values range between 24.04–26.60 (Table 2 and Fig. 3), with the highest values at site 4 and the lowest at site 1. As a general trend, species richness and Fisher α values were found to increase from the sampling sites closer to the ocean



Figure 4. (1–3) Quinqueloculina cf. Q. compta Cushman; (4–6) Quinqueloculina cuvierina (d'Orbigny); (7) Quinqueloculina cf. Q. zhengi Parker; (8–10) Quinqueloculina poeyana d'Orbigny; (11–12) Pseudolachlanella eburnea (d'Orbigny); (13–14) Pseudolachlanella bermudezi (Acosta); (15–16) Quinqueloculina moynensis Collins; (17) Quinqueloculina quinquecarinata Collins; (18–21) Quinqueloculina cf. Q. rebeccae Vella; (22–25) Sigmoilinita costata Schlumberger; (26–27) Quinqueloculina samoensis Cushman; (28) Quinqueloculina tantabiddyensis Parker; (29) Quinqueloculina sp. 2; (30–32) Quinqueloculina sp. 1; (33–35) Quinqueloculina sp. 4. The scale bar is 100 and 50 µm for figs. (11)–(12), (16), (21), (24), (26), (29), (30)–(31), and (33).

towards the inner parts of the estuary with the highest values at site 4 (Table 1 and Fig. 2). Dominance, evenness and the Shannon diversity index values recorded revealed only minor variability and were found to be comparatively uniform across all sample sites analyzed (Table 2).

4.3 Distribution patterns

Samples were found to have a comparatively uniform composition without much difference in species richness, species diversity, or community structure. The assemblages analyzed from each sampling site show a striking dominance of *Pararotalia cananeiaensis* (20.7 %–24.2 % of the total assemblages). The second most abundant taxon is *Orbitina carinata*, a hyaline-perforate species with the highest percent abundances distant from the mouth of the estuary. *Ammonia veneta* and *Anomalinulla* sp. 1 are also abundant in all our samples, constituting 3.2%–7.5% and 4.9%–7.5% of the total assemblages, respectively. The porcela-



Figure 5. (1–3) *Quinqueloculina* cf. *Q. carinatastriata* Wiesner; (4–6) *Quinqueloculina* sp. 3; (7) *Quinqueloculina* cf. *Q. bosciana* d'Orbigny; (8–9) *Miliolinella webbiana* d'Orbigy; (10) *Miliolinella* sp. 1; (11) *Hauerina atlantica* Cushman; (12–13) *Inaequalina* sp. 1; (14) *Laevipeneroplis bradyi* Cushman; (15) *Procerolagena oceanica* (Albani); (16–17) *Cushmanina bricei* McCulloch; (18–19) *Fissurina colomboensis* McCulloch; (20) *Fissurina bispinata* Ujiié; (21) *Fissurina* sp. 2; (22–23) *Fissurina semimarginata* Reuss; (24–25) *Fissurina*? sp. 1; (26–28) *Bolivina densipunctata* Sellier de Civrieux; (29–32) *Bolivina ordinaria* Phleger and Parker. The scale bar is 100 and 50 μm for figs. (2), (6)–(7), (17), (20), (22)–(25), (26), and (28)–(32).

neous species *Quinqueloculina moynensis* constitutes between 4.57 %–6.36 % of the total assemblage at individual sites. Species of the genus *Quinqueloculina* are represented in the assemblages by a highly diverse group and constitute 15 species. Among the 93 species recorded, a total of 40 species were present at all four sampling sites including the five most abundant species (*P. cananeiaensis*, *A. veneta*, *Anomalinulla* sp. 1, *O. carinata*, and *Q. moynensis*). Occurrence records of a few species display preferences for particular sites in the estuary. Among these, *Angulogerina* cf. *A. occidentalis, Cribroelphidium mirum,* and *Elongobula parallela* are particularly abundant at site 2, 3, and 4, which correspond to the inner reaches of the estuary. Species recorded exclusively at sites located near the mouth region of the estuary (sites 1 and 2) include *Anomalinulla glabrata, Bolivina densipunctata, Bolivina variabilis, Eoponidella pulchella, Fischerina* sp. 1, *Fissurina*? sp. 1, *Quin-*



Figure 6. (1) Bolivina ordinaria Phleger and Parker; (2–3) Bolivina striatula Cushman; (4–5) Bolivina variabilis Williamson; (6–8) Bolivina cf. B. variabilis (Williamson); (9) Bolivina sp. 1; (10–11) Abditodentrix rhomboidalis (Millett); (12–13) Bolivina brevior Cushman; (14–15) Globocassidulina rossensis Kennett; (16–17) Globocassidulina crassa (d'Orbigny); (18) Lagena tenuis Bornemann; (19) Loxostomina costulata (Cushman); (20–21) Sagrina pulchella d'Orbigny; (22–23) Cassidelina sp. 1; (24) Buliminella elegantissima (d'Orbigny); (25–26) Angulogerina cf. A. occidentalis (Cushman); (27–28) Sigmavirgulina tortuosa (Brady); (29–30) Siphonina reticulata (Czjzek); (31–33) Discorbitina pustulata (Heron-Allen and Earland). The scale bar is 100 and 50 μm for figs. (1), (4)–(5), (12)–(17), (21)–(23), (25)–(26), and (28)–(33).

queloculina poeyana, *Q*. cf. *Q. rebeccae*, and *Q. tantabid-dyensis*. Amphisteginid foraminifera, although present in low abundances, are also restricted to sample site 1 and 2.

5 Discussion

Analyses of foraminiferal assemblages from the Mamanguape mangrove estuary revealed highly diverse, particularly species-rich, and structurally complex biota of benthic taxa. With a total of 93 taxa, the species richness exceeds previous species counts and was found to be the highest among all true mangrove environments studied so far (see below and Fig. 2). In addition, our samples yielded unusual assemblages heavily dominated by calcareous taxa, lacking agglutinated specimens to a great degree, as reflected by the dominance of hyaline-perforate taxa that contribute between 72.3 %–75.1 % to the total population. Porcelaneous miliolids, representatives of shallow marine environments, constitute the



Figure 7. (1–3) Orbitina carinata Sellier de Civrieux; (4–6) Rotorbis auberii (d'Orbigny); (7–9) Neoconorbina sp. 1; (10–11) Neoconorbina radiatogranulata Parker; (12) Neoconorbina terquemi (Rzehak); (13–15) Rosalina bradyi (Cushman); (16) Rosalina sp. 1; (17–19) Rosalina sp. 2; (20–23) Glabratella carinata Seiglie and Bermúdez; (24–27) Glabratella mirabilis Seiglie and Bermúdez; (28–29) Glabratella carinata Seiglie and Bermúdez. The scale bar is 100 and 50 µm for figs. (3)–(5), (7), (10)–(12), (14)–(15), (17), and (20)–(29).

second most abundant group with an abundance of 23.8 %-26.5%. Agglutinated species, which are typically dominant in mangrove environments (Murray, 1991), contribute only 1.7%–2.1% to the total assemblages (Table 2). Species richness values recorded range between 65 and 70 at individual sites, whereas the average species richness was found to be generally much lower in other studies (Fig. 2; Debenay, 1990, 2001; Murray, 2006 and references therein). The atypical composition and highly diverse Mamanguape assemblages raise the question of which driving forces are decisive for the development of such unusual mangrove biota. Because such assemblages rather resemble foraminiferal faunas from shallow coastal settings and are difficult to relate to mangrove habitats, the potential implications for the interpretation of the fossil record are considered.

As outlined above and illustrated in the Fisher α diagram (Fig. 2), the Mamanguape foraminiferal assemblages were found to deviate from usual foraminiferal mangrove biota in species richness, in the composition of wall-structural types, the presence of abundant hyaline-perforate and porcelaneous miliolid taxa, and in particularly low abundances of agglutinated species. While Fisher α value recordings



Figure 8. (1) Glabratella mirabilis Seiglie and Bermúdez; (2–4) *Elongobula parallela* (Cushman and Parker); (5–6) *Eoponidella pulchella* (Parker); (7–8) *Amphistegina lessonii* (d'Orbingy); (9) *Amphistegina radiata* (Fichtel and Moll); (10–12) *Nonionides grateloupii* (d'Orbingy); (13–14) *Svratkina acuta* (Sidebottom); (15) *Anomalinulla glabrata* (Cushman); (16–20) *Anomalinulla* sp. 1.; (21–30) *Pararotalia cananeiaensis* Debenay, Duleba, Bonetti De Melo e Souza & Eichler. The scale bar is 100 and 50 µm for figs. (1), (3)–(4), (6)–(9), (13)–(14), (18)–(20), (24), and (27)–(30).

from previous mangrove studies ranged between 0.3 and 11, the Mamanguape faunas ranged between 24.4 and 26.6 (Fig. 2). The higher numbers are mainly due to the presence of both porcelaneous-miliolid and hyaline-perforate taxa and are more similar to values commonly encountered in coastal nearshore environments at the seaward end of river estuaries or in tropical lagoon or back-reef settings where foraminiferal diversity is much higher than in mangroves (Saunders, 1958; Halicz et al., 1984; Debenay et al., 2001; Langer and Lipps, 2003; Thissen and Langer, 2017; Langer et al., 2013; Fajemila et al., 2020a).

The composition of our benthic foraminifera assemblages also contrasts with the ones found in previous studies on total assemblages of mangrove foraminifera from the southern Atlantic, where benthic foraminifera assemblages are exclusively dominated by agglutinated and hyaline-perforate species and mostly lack porcelaneous taxa (Fig. 2; Murray, 1991, 2006). Agglutinated foraminifera within the Mamanguape mangroves contribute a mere 1% to 2% to the total assemblages, while other studies showed abundances that generally range above 40% (Culver, 1990; Brönniman et al., 1992; Debenay and Guillou, 2002; Murray, 2006).



Figure 9. (1–3) *Ammonia advena* Cushman; (4–10) *Ammonia veneta* (Schultze); (11–14) *Cribroelphidium mirum* Langer and Schmidt-Sinns; (15–16) *Elphidium sagrum* (d'Orbingy); (17–19) *Discorbis willamsoni* Chapman and Parr; (20–21) *Astrononion* gallowayi Loeblich and Tappan; (22–23) Pyrite framboids found in our specimens, (24) *Globigerinoides* sp. 1; (25–26) *Globigerinoides* sp. 2. The scale bar is 100 and 50 µm for figs. (6), (13), (15)–(16), and (18)–(20), 25 µm for figs. (21) and (26), and 10 µm for fig. (23).

Deviations from "typical" mangrove assemblages are also marked by the dominance and numerical abundance of *Pararotalia cananeiaensis* (Debenay et al., 2001), a taxon of a nearshore, shallow-water genus that is commonly not expected in large numbers in mangrove habitats (Geslin et al., 2002; Debenay et al., 2001). Within the Mamanguape mangroves, percent abundances of *P. cananeiaensis* were found to range between 20.1% and 24.2%, making it the most abundant taxon of all species. Similar high abundances were recently reported by Damasio et al. (2020) from the Santos estuary (southeast Brazil), where the species was reported from the bay and along the main channel but absent from the low-saline northernmost mangrove habitats. Eichler et al. (1995) reported the species from the Bay of Trapandé (Brazil) and attributed its inshore occurrence to the marine influence. Similarly, Murray et al. (1982) attributed the upstream presence of small coastal species to be indicative of an upstream transport of sediment of marine origin. Debenay et al. (2001) studied in detail the occurrence and transportation of *P. cananeiaensis* along Brazilian coasts under variable hydrodynamic conditions. They associated the presence of *P. cananeiaensis* in estuary channels with the intensity of

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Table 2. Sample site information including geographical position, salinity (Medeiros et al., 2018), number of species, Fisher α , Shannon's *H*, dominance (SID), evenness $(e^H)/S$, and percent abundances of the agglutinated, miliolid, and perforate specimens as well as the five most abundant species.

	Sample 1	Sample 2	Sample 3	Sample 4
Longitude	6°46′59.61″ S	6°46′57.84″ S	6°47′57.89″ S	6°46′55.93″ S
Latitude	34°56′40.83″ W	34°57′54.95″ W	34°57′49.94″ W	34°58′55.04″ W
Salinity ‰	32 ± 3.9	30.3 ± 3.9	28.3 ± 3.0	28.7 ± 3.1
Fisher α	24.04	24.19	24.89	26.6
Shannon H	3.324	3.439	3.345	3.484
Dominance D	0.08149	0.0696	0.07689	0.06473
Evenness $(e^H)/S$	0.4271	0.4721	0.4297	0.4656
Agglutinated %	1.19	1.73	2.13	1.17
Porcelaneous %	24.78	23.12	23.78	26.53
Hyaline-perforate %	74.02	75.14	73.17	72.30
Ammonia veneta %	7.46	3.18	3.35	5.83
Anomalinulla sp. 1 %	7.46	4.91	5.49	4.96
Orbitina carinata	3.58	7.22	13.16	6.41
Pararotalia cananeiaensis %	24.18	21.97	22.87	20.70
Quinqueloculina moynensis %	5.67	6.36	4.57	4.96

marine intrusion into paralic systems and considered the upstream presence more likely to be a function of transport (allochthonous) than of in situ growth (see also Burone et al., 2006).

Seven different lines of evidence, however, strongly argue for an in situ deposition of assemblages and autochthonous presence of P. cananeiaensis, at least within the Mamanguape estuary. (i) Numerical abundances of P. cananeiaensis, Shannon's H, evenness, Fisher α , and dominance index values do not vary substantially along the sampled transect and are largely uniform across the sample sites. Evidence for decreasing percent abundances upstream or features of successive filtering through the dense tributary mangrove channels is thus lacking. (ii) The Mamanguape River estuary is partially dammed by a natural sand barrier (Fig. 1) and acts as a hindrance in terms of water exchange and transport of coastal species deep into the estuary. (iii) Except for two individuals, planktic foraminifera are absent, thus arguing against a large-scale transport of open-ocean taxa upstream. (iv) The preservation of the taxa recovered ranges from good to excellent and includes both fragile, robust, smaller, and larger species. Features of abrasion, transport, or rolling over large distances were not observed. (v) The sample sites investigated all revealed a particularly high species richness of benthic foraminifera. (vi) The lack of both peneroplid and soritid foraminifera, two groups that are widely present in shallowwater nearshore biofacies in the region (Weinmann, 2009; Disaró et al., 2014), argues against a transport of species from nearshore to estuarine mangrove habitats. (vii) Pyrite framboids were frequently observed within the chambers in the majority of species, reflecting the presence of anoxic conditions at the site of deposition (see Fig. 9, figs. 22–23). Pyrite formation is tightly intertwined with the presence of organic matter (Berner, 1970, 1984; Thiel et al., 2019), suggesting that (a) foraminifera cell cytoplasm was present during deposition; (b) autolytic, bacterially, or prey-mediated protoplasm decay in foraminifera has been largely prevented (Murray and Bowser, 2000); and (c) foraminiferal assemblages are largely autochthonous. The time required for pyrite framboids to form the largest crystals, like the ones found in our assemblages, was previously estimated to be around 35 d (Rickard, 2019).

If a large-scale upstream transport of coastal species would indeed have taken place, a successive filtering and numerical reduction effect of coastal species through the dense mangrove stands would be expected. However, this has not been recorded in the assemblages. Instead, the number of benthic species was found to increase towards the inner parts of the estuary and the assemblages are therefore considered to be largely autochthonous.

Marine conditions supporting such diverse assemblages are manifested in high salinity values in our sampling area during both dry and wet seasons and are interpreted as the result of a longer residence time of marine waters in the estuary (Fig. 2; Dolbeth et al., 2016; Medeiros et al., 2018). Prevailing marine conditions within the dense Mamanguape mangrove estuary were also considered a major factor contributing to fish diversity in previous studies (MacDonald et al., 2008; Blaber, 2013).

The results obtained revealed unprecedentedly high species richness values and assemblages dominated by calcareous species instead of agglutinated forms and represent an atypical example for a mangrove benthic foraminiferal fauna when compared to previous studies. While these findings may seem puzzling at first sight, they are considered to mainly result from high salinity values in the upstream areas of the Mamanguape River estuary (Table 2). The resemblance to shallow-water nearshore foraminiferal communities is both indicated by the presence of a large number of porcelaneous miliolid and hyaline-perforate species and in the abundance of *P. cananeiaensis*. In addition, it should be noted that the low number of agglutinated specimens and species found in our assemblages is not an artifact of preservation, as samples were carefully treated and the material was picked immediately after transportation to the laboratory.

Salinity has long been recognized as a major driving force governing the species richness, composition of wall structural types, and diversity of foraminiferal assemblages (Murray, 1991; Debenay, 1990). A distinct separation between agglutinated and calcareous taxa along a pronounced salinity gradient has recently been reported from Lagos Lagoon, where mangrove and low salinity environments are dominated by agglutinated foraminifera (Fajemila et al., 2020b). While the majority of mangrove assemblages from along the Atlantic coast of South America were reported to be dominated by agglutinated taxa, the Mamanguape mangrove estuary was found to contain abundant calcareous wall types and to be almost devoid of agglutinated taxa. Among the environmental framework conditions promoting such atypical mangrove assemblages of foraminifera, salinity stands out as a prominent control factor. Salinity recordings along the sampling traverse revealed values ranging between $\sim 25\%$ and 35%, indicating mostly euhaline to brackish water conditions. The prevalence of marine conditions within the studied area of the Mamanguape estuary is supported by comparatively long residence times of marine waters, high tidal amplitudes, and semi-diurnal tidal flushing of the estuarine environment. The resemblance of the atypical mangrove assemblages to shallow-water nearshore biota may thus be attributed to the intensity of marine conditions, where calcareous foraminifera dominate over agglutinated taxa (Boltovskoy, 1954; Todd and Brönnimann, 1957; Boltovskoy and Hincapié de Martínez, 1983; Scott et al., 1990; Debenay, 2001; Debenay et al., 2002, 2004; Moreno et al., 2005; Eichler et al., 2007; Camacho et al., 2015; Shaw et al., 2016; Passos et al., 2017). The penetration of calcareous species into the Mamanguape estuary has also been reported from the neighboring Paraíba estuary (Debenay et al., 2002). However, unlike the Mamanguape estuary, the Paraíba estuary receives freshwater discharge from more rivers and invasions of marine foraminifera only occur during the dry season and completely disappear during the rainy season (Debenay et al., 2002).

Independent of whether the foraminiferal assemblages are allochthonous or autochthonous, the atypical Mamanguape mangrove faunas raise questions concerning implications for the interpretations of the fossil record. Unlike previous studies on foraminifera from South American and many other mangrove environments around the world, where specialized agglutinated taxa dominated mangrove environments, exceptionally species-rich and diverse assemblages of foraminifera prevail within the dense mangroves at Mamanguape. Both Debenay et al. (2001, 2002) and Woodroffe et al. (2005) demonstrated that a range of environmental factors jointly govern both the composition, distribution, and preservation of foraminiferal biota in mangrove environments. Besides salinity, these include the elevation, grain size, organic content, and taphonomic processes related to low pH excursions resulting in the removal of agglutinated and calcareous taxa. Post-mortem disaggregation and taphonomic loss may thus bias the fossil record and constitute a serious constraint regarding paleoenvironmental reconstructions.

Mangrove ecosystems have existed at least since the Late Cretaceous and fossil evidence used for paleoecological reconstructions is mostly based on organic remains (fruit; flowers; wood or leaves; or microfossils, particularly pollen; Ellison et al., 1999) and sedimentological features (Augustinus, 1995). Sedimentation of typical fine-grained mangrove deposits results from the reduction in current velocity where tree trunks, prop roots and pneumatophores exercise a filter function and result in typical mangrove mud sediments. Within the Mamanguape estuary, the accumulation and vertical accretion of mangrove mud is a mixture of river-born clastic sediments, organic material produced by mangrove trees and associated fauna, and suspension-rich ocean floating debris penetrating mangrove swamps. In high precipitation areas and under low salinity conditions, the production of organic material is generally promoted but reduces pH conditions within the sediment and limits carbonate availability and thus reproduction of calcareous foraminifera. On the other hand, agglutinated tests are known to resist low pH conditions and dissolution while they are removed through oxidation-reduction reactions (Ellison and Nichols, 1976; Scott and Medioli, 1978; Boltovskoy, 1984; Goldstein, 1988 and the references therein; Thomas and Varekamp, 1991; Debenay et al., 2002, 2004). However, in the absence of considerable rainfall and during the warm periods, penetration of coastal waters into the estuarine system occurs. Based on the intensity of the marine influence and tides, calcareous foraminifera start to dominate and get preserved in the otherwise brackish or freshwater realm (Boltovskoy, 1954; Todd and Brönnimann, 1957; Scott et al., 1990; Debenay, 2001; Debenay et al., 2002, 2004; Moreno et al., 2005; Eichler et al., 2007; Camacho et al., 2015; Shaw et al., 2016; Passos et al., 2017; Woodroffe et al., 2005). Normally, salinity tends to be higher in the downstream areas in estuaries, yet the upstream areas of the Mamanguape are characterized by high salinity (Table 2), indicative of a consistent influence of oceanic waters penetrating even further upstream of the sampling sites.

6 Conclusions

Mangrove environments of the Mamanguape River estuary are home to diverse assemblages of benthic foraminifera including 33 porcelaneous, 53 hyaline-perforate, and 7 agglutinated species and represent an extraordinary ecosystem characterized by atypical, highly diverse, and species-rich benthic foraminiferal biota. The atypical population structure features prominent components of hyaline-perforate and miliolid-porcelaneous benthic foraminifera and are unprecedented in previous mangrove studies. The assemblages resemble coastal nearshore biota that are traditionally not classified as mangrove foraminiferal faunas and are here considered to be the result of highly saline ocean waters consistently penetrating deep into the estuary and promoting the presence of compositionally diverse and species-rich biota. When preserved in the fossil record, such assemblages are not readily related to mangrove ecosystems and would most likely be interpreted as a coastal, nearshore, shallow-water environment. Our findings thus have implications for inferring environmental conditions of past mangrove ecosystems. Salinity was found to be the main controlling factor structuring benthic foraminiferal assemblages, where both the comparatively long residence time of marine waters and the low freshwater inflow support prevailing euhaline to brackish water conditions and the presence of calcareous-dominated, species-rich foraminiferal assemblages.

Code availability. The PAST software of Hammer et al. (2001) is available at: https://www.nhm.uio.no/english/research/infrastructure/past/.

Data availability. All data used in the present study are provided in Tables 1 and 2.

Author contributions. MRL conceptualized the study and collected the material. NS and MRL analyzed the data and prepared and wrote the manuscript.

Competing interests. The authors declare that they have no conflict of interest.

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Acknowledgements. We are grateful to the reviewers and the editor for their constructive comments and suggestions. We also thank Stephanie Pietsch for assistance with the collection of the material and Georg Oleschinski for the SEM pictures of the framboidal pyrite. The analysis and study of the material was supported by a grant from the German Federal Foreign Office and the University of Bonn.

Financial support. This open-access publication was funded by the University of Bonn.

Review statement. This paper was edited by Hiroshi Kitazato and reviewed by Johann Hohenegger and one anonymous referee.

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Chapter 4: Publication



Article



Contamination Levels of Potentially Toxic Elements and Foraminiferal Distribution Patterns in Lagos Lagoon: A Correlation Analysis

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Abstract: The ecological response of benthic foraminifera to bioavailable Potentially Toxic Elements (PTEs) was evaluated in Lagos Lagoon (Nigeria). We sampled and analyzed PTEs across Lagos Lagoon with the aim to investigate the extent of contaminated sediments, to document their distribution, and to explore the relationship between PTE concentration and the spatial distribution, composition, abundance, and species richness of benthic foraminifera biotas. PTE's recordings showed a wide range reflecting a diffuse contamination, where Contamination and Enrichment Factor suggest low to extremely polluted sediments. Findings of a previous survey of the benthic foraminifera inhabiting Lagos Lagoon revealed diverse assemblages of benthic taxa, species-specific distribution patterns, gradients of species richness and abundance, and a disjunct distribution of agglutinated and hyaline-perforate/porcelaneous taxa along a pronounced salinity gradient. Correlation matrix analysis shows that except for Selenium, all PTE total concentrations positively correlate with mud and Total Organic Carbon (TOC) and two of the most abundant agglutinated taxa, Ammotium salsum, and Trochammina sp. 1. Moreover, both species display significant positive correlations with CrF4-CoF2-F3-F4-total-CuF4-total-NiF3-F4-total-AlF4-total-FeF3-F4-total-ZnF3-F4-total. On the other hand, both foraminifers correlate negatively with PbF4-SeF3-Setotal. The overall significant positive correlation of these PTEs suggests that they behave as micronutrients when complexed with organic matter. No significant positive correlation with none of the PTEs in any fraction was found for neither species richness nor for the most abundant hyaline perforate species (Ammonia aoteana). Some PTE fractions were found to correlate either positively or negatively with individual species, suggesting that they function as either micronutrients and/or stressors. The resulting Contamination Factor of the PTE total concentrations shows that only a few sample sites can be classified as "moderately" polluted for chromium, zinc, and copper and that all sampled sites are classified as "highly polluted" for selenium. The highest concentrations for Cr, Cu, Ni, and Zn were found towards the industrialized western part, an area that is characterized by moderate to high diversity but low abundances.

Keywords: benthic foraminifera; toxic elements; pollution; Lagos Lagoon; Nigeria

1. Introduction

Estuaries with limited exchange with the open ocean are among the most vulnerable marine environments to human disturbance (e.g., [1–3]). By providing shelter and access further inland, they provide ideal settings for harbors, shipyards, commercial infrastructure, and industrial areas, where human activities are concentrated. Estuaries are, however, also direct recipients of municipal waste coupled with urban, industrial, and agricultural



Citation: Fajemila, O.T.; Martínez-Colón, M.; Sariaslan, N.; Council, I.S.; Kolawole, T.O.; Langer, M.R. Contamination Levels of Potentially Toxic Elements and Foraminiferal Distribution Patterns in Lagos Lagoon: A Correlation Analysis. *Water* 2022, *14*, 37. https://doi.org/10.3390/w14010037

Academic Editor: Arantza Iriarte

Received: 24 November 2021 Accepted: 21 December 2021 Published: 24 December 2021

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run-offs. The anthropogenic pressure directly impacts the sediment and water quality and leads to environmental degradation.

Coastal pollution is alarming in Nigeria, especially in areas where hydrocarbon exploration and other industrial activities are common. The Lagos Lagoon area, with an estimated population of 20 million people, has long been under enormous environmental pressure caused by deforestation, industrial effluents, alteration of the natural landscape, sand mining, and waste disposal. The industrial complex around Lagos Lagoon includes textile, brewery, petrochemical factories, logging and metal industry, power plants, paper mills, and sawmills from which untreated effluents drain into the lagoon through creeks and underground canals releasing potentially toxic elements (PTEs) [4–7]. Past studies revealed that Lagos Lagoon is impacted by PTEs arising from industrial and domestic waste disposal [4,7]. High numbers of PTEs, Polycyclic Aromatic Hydrocarbons (PAHs), as well as agricultural run-offs, were found in the western corridor of the lagoon [4,5,7,8].

Benthic foraminifera are known to bioaccumulate PTEs and have been widely utilized as bioindicators of pollution [9–12]. Our previous, large-scale survey of the benthic foraminifera inhabiting Lagos Lagoon revealed diverse assemblages of benthic taxa, speciesspecific distribution patterns, gradients of species richness and abundance, and a disjunct distribution of agglutinated and hyaline-perforate/porcelaneous taxa along a pronounced salinity gradient [13]. Analysis of foraminiferal distribution patterns also suggested that effluents from the oil industry, PAHs, urban sewage, and high concentrations of PTEs are among the agents driving the abundance, composition, species richness, and spatial distribution of benthic foraminifera in the polluted western section of the lagoon. However, to what extent PTEs impact benthic foraminiferal biotas has not been fully resolved. We sampled and analyzed PTEs across Lagos Lagoon with the aim to investigate the extent to which sediments are contaminated, to document their distribution, and to explore the relationship between PTE concentration and the spatial distribution, composition, abundance, and species richness of benthic foraminiferal biotas. The purpose of this study is to assess the ecological impacts of selected PTEs on the assemblage structures and spatial distribution of benthic foraminifera and to provide guidance on the use of foraminifera as bioindicators of PTE pollution in the coastal areas of the Gulf of Guinea (GoG).

2. Study Area

Lagos Lagoon is located between longitudes $3^{\circ}23'$ and $3^{\circ}40'$ E and latitudes $6^{\circ}22'$ and $6^{\circ}38'$ N (Figure 1) and has a total surface area of 6354.7 km². It is connected to the Atlantic Ocean through the Commodore Channel, which allows for the mixing of lagoon waters with seawater. The depth of the lagoon is relatively shallow and ranges from 5 to 25 m [6]. The salinity of the surface waters is highly variable and strongly impacted by seasonality; 0 to 16‰ in the wet season and up to 35‰ in the dry season especially at the lagoon entrance through the Commodore Channel [7,14–16]. The dissolved oxygen varies between 4–5.5 mg/L [15–17]. Land conversion for urban development has been mainly conducted along the western lagoon shores, with the remaining marshlands being fringed by the mangrove tree *Rhizophora racemosa* [18]. Both the Ogun and Osun rivers serve as fresh water sources, but most hinterland sediments and waste water come through the Ogun River (Figure 1). Seawater enters the lagoon during periods of high tide through the channel, but during low tide and especially the wet season, the diluting effect of waters from both rain and hinterland keep the salinity of the lagoon surface waters at comparatively low levels [19].



Figure 1. Sample sites within Lagos Lagoon, Nigeria (modified from Google map).

3. Methods

3.1. Processing of Sediment Samples

Twenty-six stations were sampled within Lagos Lagoon in May 2019 with support from the Nigerian Institute of Oceanography and Marine Research (Figure 1). The top 2 cm of the bottom sediments were collected, transported to the laboratory, and dried at 50 °C for 48 h to ensure complete water loss. The physico-chemical parameters (temperature, salinity, and pH) were also measured at the lagoon's water surface using a multi-parameter sensor probe-device (HI 9813-6N, Hanna Instruments, Smithfield, RI, USA) (see [13]).

In the laboratory, sub-samples were taken for percent total organic carbon (TOC), percent calcium carbonate (%CaCO₃), grain size, and PTE analyses. The Loss-on-Ignition (LOI) method was used to analyze for TOC and %CaCO₃ using a muffle furnace (Lindberg Blue M, Thermo Scientific, Columbia, MD, USA). This widely used method combusts a sample at two different temperatures to estimate the organic matter and carbonate mass loss (e.g., [20,21]). Each sub-sample was oven-dried at 105 °C for 24 h, and after cooling to room temperature the sub-samples were combusted at 550 °C for 4 h for TOC determination. To assess the %CaCO₃ content, the left-over material from the TOC combustion was baked at 1000 °C for one hour. For grain size analysis all dry sub-samples were wet-sieved (63 µm) and oven-dried at 50 °C for 24 h to determine the mud percent content (silt + clay) by weight difference. Following the methods of Martínez-Colón et al. [1], all size fractions were converted to phi (Φ) units (-1 = gravel; 0 = very coarse sand; 1 = coarse sand; 2 = medium sand; 3 = fine sand; 4 = very fine sand; >4 = mud).

For PTE analysis each sub-sample (one gram) was crushed, powdered, and homogenized using an agate mortar and pestle. The residues were then dry-sieved using a 63 μ m plastic sieve with a Teflon mesh, and the <63 μ m size was used for chemical extractions of PTEs. The sequential extraction analytical methods of Tessier et al. [22] were implemented to assess the concentration of the 11 contaminants of concern (Cr, Co, Cu, Ni, Cd, Pb, Se, As, Al, Fe, Zn). In summary, the <63 μ m sediment sub-samples were each placed in 50 mL Teflon centrifuge tubes, with the residue from each extraction being used as the stock for the subsequent extraction. Constant agitation was achieved by placing samples into an incubator-shaker (TSSWB15 Shaking Water Bath, Thomas Scientific, Columbia, MD, USA) at 150 rpm and 25 °C. After each extraction step, the sub-samples were centrifuged for 30 min at 10,000 rpm and 25 °C in a centrifuge (Sorvall RC6+, Thermo Scientific, Columbia, MD, USA) using an SS-34 rotor attachment. The supernatants were extracted, stored in 50 mL Falcon tubes, and diluted to 30 mL with 2% HNO₃ until ready for analysis. The sediments after each step were then washed with 8 mL DI water and agitated continuously for 5 min before being centrifuged for 30 min as described above. Wash residue supernatants were discarded, and the remaining residual sediments were then ready to advance to the next step in the extraction process.

All PTE concentrations are expressed in mg/kg except for Al, and Fe which are expressed as percentages. Of the five fractions described by Tessier et al. [22], the exchangeable (F1), acid-soluble (F2), reducible (F3), and oxidizable (F4) were analyzed. The residual (F5) fraction was not analyzed because the PTEs found in this chemical fraction will not be bioavailable since they are found within the crystalline structure of silicate minerals. Total concentration, as referred to in this article, represents the summation of all the extracted fractions (F1 + F2 + F3 + F4) of each PTE in each of the sub-samples. Prior to PTE analysis using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), all aliquots were filtered (0.25 μ m) after acidification with 2% HNO₃ to ensure and eliminate any sediment residue. For PTE concentrations that are below the detection limit of the ICP-OES (0.001 mg/L), we recorded the concentration as half of the detection limit based on Parker and Arnold's [23] recommendation. This approach rejects the potential of false zeroes and gives weight to the actual presence of the PTE. For cross-correlation, heat maps illustrating the concentration and spatial distribution were plotted for raw PTEs, mud, TOC, %CaCO₃, and the three most abundant benthic species of foraminifera using the Surfer[®] software ver. 22.1.151 (www.goldensoftware.com, accessed on 10 September 2021).

3.2. Evaluation of Sediment Contamination

The PTE spatial distribution and the level of pollution could provide information that explains the distribution and diversity of foraminifera in the lagoon. To do this, single element and multi-element pollution indices were employed to assess the quality of the sediments. The single element pollution indices, which include the contamination factor (CF) and the enrichment factor (EF), give information on the relationship of the concentration of a PTE at a given location to their corresponding background values. Because of the lack of baseline and historical PTE data in the study area, the average shale composition values as proposed by Turekian and Wedepohl [24] were employed. The CF is defined as the ratio between the concentrations of PTEs at sampling location and their background values (CF = $C_{metal}/C_{background value}$, where C_{metal} = PTE concentration in the sample, and $C_{background value}$ = PTE background value in shale). Four levels of contamination are associated with CF, ranging from un- to highly polluted (Table 1).

Similarly, the EF for all PTEs were also determined by comparing the concentration of individual PTEs relative to a reference concentration. This EF provides information on possible sources, i.e., crustal/geogenic/lithologic or anthropogenic in a given sample. In this study, Al was used as the normalization element because it is considered not to have an anthropogenic origin in the lagoon [25,26]. The EF was calculated according to the following equation:

$$EF = \frac{\left(\frac{C_i}{C_{ref}}\right)Sample}{\left(\frac{C_i}{C_{ref}}\right)Crust}$$
(1)

where C_i is the concentration of the PTE of interest and C_{ref} is the concentration of the normalization element (Al). Generally, five contamination categories are associated with EF (Table 1).

The ecological Risk Index (RI) is a multi-elemental pollution index of a sample location that considers the cumulative Ecological risk factors (Er) of PTEs in a given sediment sample (Table 1). The Er was calculated according to the following equation: $Er = Trf \times CF$, where for a given PTE, Trf is the toxic-response factor and CF is the contamination factor [25].

Qualification	Contamination Factor	Enrichment Factor	Ecological Risk Index
Unpolluted/Slightly	CF < 1	EF < 2	<150 (low)
Moderately	1 < CF < 3	2 < EF < 5	150 < RI < 300 (moderate)
Severely	3 < CF < 6	5 < EF < 20	300 < RI < 600 (considerable)
High	CF > 6	20 < EF < 40	RI > 600 (very high)
Extreme	-	EF > 40	-

Table 1. Thresholds for sediment quality classification for single and multi-element indices (adapted from [27]).

3.3. Multivariate Analyses

Cluster analyses (CA) were carried out using the 13 most frequent and abundant benthic foraminifera (representing 98.3% of the total population). The focus on the 13 most abundant taxa reduces background noise and reveals the underlying signatures of the assemblages [13]. The paired group algorithm using the Bray Curtis matrix was applied to generate R- and Q-mode clusters. Cluster and principal component analyses (PCA), as well as numerical and statistical grain-size analyses involving ternary plot, were computed using the Paleontological Statistics Software Package for Education and Data Analysis (PAST 3.13; [28]).

For cross-correlation and assessment of the significance of individual factors, a Pearson correlation matrix was calculated by (i) using the log-transformation of TOC, %mud, %CaCO₃, S, D, H(S), foraminiferal number (FN), wall texture (% hyaline, % porcelaneous, % agglutinated), total and F1–F4 PTE concentrations and (ii) for the remaining 10 bioavailable PTEs, including the three most abundant species of benthic foraminifera (Ammonia aoteana, Ammotium salsum, and Trochammina sp. 1).

4. Results

4.1. Lagos Lagoon Water Characteristics

Due to the limited exchange with marine waters, the Lagos Lagoon system experiences restricted marine and mainly low salinity, brackish and freshwater conditions [13]. In general, the western sector of the lagoon experiences higher salinity because of its interactions with the Atlantic Ocean (16–34‰). Towards the northern and eastern portions of the lagoon, low salinity (0–10‰) and freshwater conditions are predominant for most of the year. Towards the middle and southwestern portions of the lagoon (e.g., Commodore Channel) the range in salinities fluctuates between 10-16% in the wet season and from 16-34% during the dry season. Surface water pH values are low and range from 5.8–6.9. The surface water temperature varies between 24.1–29.8 °C with a decreasing SW to NE trend.

4.2. Sediment Texture and Characteristics

The TOC values range from 0.32–21.63%. An overall 65–fold increase is observed in a SW (estuarine mouth) to NE trend (Figure 2; Table 2), with 35% of the sampled stations having TOC values > 4%. The %CaCO₃ has an almost 200-fold increase ranging from 0.44–86.16% (Figure 2; Table 2). For sediment texture, medium sand is the most abundant (Φ = 2; 44% of stations) grain size followed by coarse sand (Φ = 1; 16% of stations) and mud ($\Phi > 4$; 16% of stations) (Figure 2; Table 2). Unlike TOC, medium sand increased 20-fold in a NE to SW trend ranging from 2.2-44.13%, with 54% of the sample stations with values > 20%. A ternary diagram (Figure 3) shows the relationship between the sediment texture and TOC. Many stations in the eastern part of the lagoon revealed that TOC > 4%are associated with muddy sediments while those with <4% are associated with sandy sediments in the western and central parts of the lagoon.

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Figure 2. Spatial distribution and % concentration of (**A**) Total organic carbon (%), (**B**) Carbonate (%), (**C**) mud (silt + clay) (%) and percent abundances of the three most abundant species of foraminifera; (**D**) *Trochammina* sp.1, (**E**) *Ammonia aoteana* and (**F**) *Ammotium salsum* in the Lagos Lagoon. LH (Lagos Harbour).

Table 2. Sediment and water characteristics in the Lagos lagoon in May 2019: Depth (m), pH, Temperature (°C), Total Organic Carbon (TOC), Calcium Carbonate (%CaCO₃), mud content (% silt and clay), and Median Grain Size (Φ); (N/D = No Data).

Station #	Depth (m)	pН	Temp (°C)	TOC (%)	CaCO ₃ (%)	Mud (%)	Φ
1	13	6.9	29.8	0.86	2.01	0.18	2
2	12	6.8	29.2	0.51	0.93	0.22	2
3	13	6.5	29.1	2.05	3.87	22.96	2
4	10	6.4	29.0	1.94	86.16	20.55	2
5	5	6.9	29.2	7.91	6.31	84.53	>4
6	7.5	6.6	29.2	9.38	10.90	72.38	>4
7	3	6.6	27.5	4.79	10.01	44.51	4
8	4	6.5	27.3	0.76	2.27	0.00	1
9	6	6.5	26.2	0.32	2.96	0.17	1
10	7	6.6	27.8	6.31	5.76	63.27	>4
11	7	6.4	27.7	6.55	6.17	74.75	>4
12	3	6.7	26.8	0.43	0.44	0	3
13	4	6.8	26.7	1.68	0.55	0	3
14	12	6.5	27.7	1.78	1.60	0.22	2
15	12	5.8	26.3	1.38	1.50	12	2
16	5	6.8	25.9	3.25	2.85	0	1
17	4	6.7	25.1	5.25	6.23	0.12	2
18	3	6.8	24.5	4.46	6.07	0.16	1
19	4	6.7	24.9	2.18	6.43	0.18	2
20	3	6.8	24.1	4.44	3.01	0	2
21	3	6.6	24.2	2.74	1.16	0	2
22	3	6.8	25.1	8.52	20.32	1.74	2
23	4	6.6	24.3	11.09	6.88	60.47	4
24	2	6.5	24.3	14.79	5.03	32.69	3
25	3	6.8	24.5	21.63	8.24	N/	D
26	5	6.7	24.2	16.05	5.69	60.78	4



Figure 3. Ternary diagram of grain-size analysis distribution and organic matter content (TOC).

4.3. Spatial Distribution of Foraminiferal Assemblages

Q-mode cluster analysis revealed that foraminiferal assemblages recorded across the lagoon exhibit a two-part pattern that is separated along the lines of wall structural types (Figure 4). The clusters occupy different sectors of Lagos Lagoon, and their spatial separation was found to be largely driven by salinity (see [13]). Agglutinated foraminifera strongly dominate in the low saline eastern and northwestern portions of the lagoon and foraminifera with a hyaline-perforate or porcelaneous test are mainly present in the marine-influenced areas. Cluster Q1 (including subclusters I, II, and III) is dominated by agglutinated taxa (*Ammotium, Trochammina,* and *Ammobaculites*) and contains those samples sites with TOC > 4%, while samples with TOC < 4% and dominated by hyaline-perforate species (*Ammonia*) are clustered in Q2 (Figure 4).



Figure 4. Cluster analysis dendrograms (Bray Curtis coefficient matrix). Q-mode: Q1 = TOC > 4% and Q2 = TOC < 4% (modified after [13]).

4.4. Potentially Toxic Elements (PTEs)

A total of 11 PTEs (Cr, Co, Cu, Ni, Cd, Pb, Se, As, Al, Fe, Zn) were analyzed in this study (see Appendix A—Table A1). Some PTEs were found to be below the detection limit in certain sediment chemical fractions and were not used in the statistical analysis. These include Cr, Ni, and Pb in the F1 fraction and Se in the F4 fraction. Cd and As were completely excluded from this study due to having concentrations below the detection limits in almost all the sampled stations. All of the total PTE concentrations cross-correlate positively amongst themselves except for Cr-Cu and Cr-Al pairs, which have no significant correlations at the 95% confidence interval. Selenium negatively cross-correlates with the rest of the PTEs. All the PTEs have relative even distributions related to their total concentrations with the following range values: Cr (2.34–96.42 mg/kg), Co (0.38–16.02 mg/kg), Cu (1.11–130.32 mg/kg), Ni (0.51–13.58 mg/kg), Pb (3.51–916.47 mg/kg), Se (31.82–120.92 mg/kg), Al (0.02–0.31%), Fe (0.11–2.20%), and Zn (11.07–183.63 mg/kg) (Figure 5A–I). The highest concentrations for Cr, Cu, Ni, and Zn were found towards the west of the lagoon.



Figure 5. Heat maps showing the spatial distribution and total concentration of PTEs across the Lagos Lagoon. (**A**) Chromium (Cr), (**B**) Cobalt (Co), (**C**) Copper (Cu), (**D**) Nickel (Ni), (**E**) Lead (Pb), (**F**) Selenium (Se), (**G**) Aluminum (Al), (**H**) Iron (Fe), (**I**) Zinc (Zn). All concentrations are in mg/kg except for Al and Fe which are in percent. LH (Lagos Harbour).

The percent recovery of each PTE across the sequential fractions is illustrated in Figure 6; while their actual concentrations are provided in Appendix A (Table A1). Most PTEs were found dominant in the F3 fraction except for Cu and Al, which dominate the F4 fraction, respectively. The spatial distribution maps of the bioavailable PTE fractions (F1–F4) are shown in Appendix B (Figures A1–A4). For Cr, the following concentrations (mg/kg) were found in each fraction: 0.06-0.21 (Cr_{F2}), 1.5-93 (Cr_{F3}), and 0.48-7.23 (Cr_{F4}). For Co, the following concentrations (mg/kg) were found in each fraction: 0.06-0.21 (Cr_{F2}), 1.5-93 (Cr_{F3}), and 0.48-7.23 (Cr_{F4}). For Co, the following concentrations (mg/kg) were found in each fraction: 0-0.39 (Co_{F1}), 0-1.47 (Co_{F2}), 0.3-10.2 (Co_{F3}), and 0-4.38 (Co_{F4}). The Cu_{F1} through Cu_{F4} concentrations (mg/kg) ranged from 0.06-3, 0-0.24, 0-3.9, and 0.18-129.51 respectively. For Ni, the following concentrations (mg/kg) are found in each fraction: 0-0.33 (Ni_{F2}), 0.30-11.4 (Ni_{F3}), and 0.12-3.36 (Ni_{F4}). In the case of Pb, the concentrations (mg/kg) ranged from 0.27-3.18 (Pb_{F2}), 1.80-15.6 (Pb_{F3}), and 0.02-0.96 (Pb_{F4}). For Se, the following concentrations (mg/kg) were found in each fraction:

0–0.57 (Se_{F1}), 0.9–19.8 (Se_{F2}), and 30–111.3 (Se_{F3}). For the Al_{F1} through Al_{F4} chemical fractions, the Al values ranged (%) from 6.3×10^{-5} – 3.88×10^{-3} , 9.9×10^{-5} – 7.53×10^{-4} , 0.01–0.04, and 4×10^{-3} –0.28 respectively. For Fe, the following values (%) were found in each fraction: 1.4×10^{-4} – 7.6×10^{-3} (Fe_{F1}), 2.9×10^{-4} – 7.4×10^{-3} (Fe_{F2}), 0.1–1.62 (Fe_{F3}), and 5.4×10^{-3} –0.73 (Fe_{F4}). Finally, for Zn, the following concentrations (mg/kg) were found in each fraction: 0.06–0.87 (Zn_{F1}), 0.48–21 (Zn_{F2}), 9.9–103.8 (Zn_{F3}), and 0–137.31 (Zn_{F4}). Most of the bioavailable PTEs have the same relative even distribution as the total distribution. However, in the western and eastern sections of the lagoon, Co_{F1}, Ni_{F4}, and Fe_{F4} have the highest concentrations, while Se_{F3} and Pb_{F4}, are found in the central part.



Figure 6. PTEs recovery percentage. (A) Aluminum (Al), (B) Cobalt (Co), (C) Chromium (Cr), (D) Copper (Cu), (E) Iron (Fe), (F) Nickel (Ni), (G) Lead (Pb), (H) Selenium (Se), (I) Zinc (Zn). All concentrations are in mg/kg except for Al, and Fe which are in (%). F1 = exchangeable; F2 = acid-soluble; F3 = reducible; and F4 = oxidizable.

Principal component analysis (Figure 7) confirms the separation of the sample stations based on the amount of TOC in each sample (>4% or <4%) and on the total PTE concentrations of Fe, Al, Zn, Se, and Pb as revealed in ellipsoids A-F. All stations with <4% TOC seem not to be affected by PTEs given their low concentrations (e.g., PCA vectors at the origin). However, the sampled stations with >4% TOC found in the western and eastern parts of the lagoon seem to be influenced more by Fe (ST7, ST10, ST24, ST25) and Al (ST11, ST22, ST23).

Correlation Matrix Analysis

The Pearson correlation matrix (Table 3) shows that the PTE total concentrations positively correlate with mud and TOC except for Se, which correlates negatively. Similarly, most of the sequentially extracted PTEs correlate positively with mud and TOC except for Pb_{F4} and Se_{F3}, which correlate negatively. Only Al_{F1} and Fe_{F1} correlate negatively with mud. The fractions Cr_{F2}, Cu_{F1}-Cu_{F3}, Pb_{F2}, Se_{F1}-Se_{F2}, Al_{F2}-Al_{F3}, Fe_{F2} show no significant correlation at the 95% confidence interval with mud and TOC. Overall, most PTEs show no significant correlation with %CaCO₃ except for Co, Ni, Fe, and Zn, whose total concentra-



tions correlated positively. Similarly, Cr_{F4}-Co_{F3-F4}-Ni_{F3-F4}-Al_{F1}-Fe_{F3-F4}-Zn_{F3-F4-total} have a positive correlation with %CaCO₃.

Figure 7. Principal components showing the dominance of PTEs with respect to TOC percentages. Ellipsoids A–F show sample stations with similar concentrations of TOC and dominant PTEs.

Among the three most abundant species in Lagos lagoon, *A. salsum*, and *Trochammina* sp. 1 positively correlate with mud and TOC. In addition, *A. salsum* correlates positively with Cr_{F4} - Co_{F2} -F3-F4-total- Cu_{F3} -F4-total- Ni_{F3} -F4-total- Pb_{F3} -total- Al_{F4} -total- Fe_{F3} -F4-total and negatively with the Pb_{F4} - Se_{F3} -total. For *Trochammina* sp. 1, the following significant correlations were recorded: (1) positively with Cr_{F2} -F3-F4-total- Cu_{F2} -F4-total- Ni_{F3} -F4-total- Cu_{F2} -F4-total- Ni_{F3} -F4-total- Pb_{F3} -total- Al_{F2} -F4-total- Fe_{F3} -F4-total- Zn_{F3} -F4-total- Cu_{F2} -F4-total- Ni_{F3} -F4-total- Pb_{F3} -total- Ni_{F3} -F4-total- Zn_{F3} -F4-total-Si is a negative one with Co_{F1} . No significant correlation is observed between species richness (S) and the PTEs coupled with TOC and %CaCO_3. Similarly, H(S) show no correlation with PTEs except negatively with Co_{F2} -F4-total- Ni_{F4} -total- Pb_{F3} -total- Fe_{F3} -F4-total- Zn_{F3} -F4-total and with TOC. On the other hand, dominance (D) and FN have very similar positive correlations with Co_{F2} -F3-F4-total- Al_{F4} -total- Fe_{F3} -F4-total- Zn_{F3} -F4-total and TOC while negatively with Cu_{F1} in the case of D and with Pb_{F4} - Se_{F3} -total regarding FN.

Based on wall texture, the percentage of agglutinated foraminifera (AGL) correlates positively with TOC, D, FN, and A. Similar to the agglutinated species *A. salsum*, the AGL also correlates positively with the Cr_{F4} - $Co_{F1-F2-F3-F4-total}$ - $Cu_{F2-total}$ - $PB_{F3-total}$ - $Al_{F4-total}$ - $Fe_{F3-F4-total}$ - $Zn_{F3-F4-total}$ and negatively with PbF4-Se_{F3-total}. The percentage of porcelaneous species (P) correlates positively with S-H(S) and negatively with TOC-CaCO₃-D. However, given the very small number of porcelaneous individuals per sample (1–14), the correlations found with PTEs are considered false positives/negatives. The percentage of hyaline taxa (HP) correlates negatively with TOC and FN. Unlike *A. aoetana*, however, the HP mostly correlates negatively with Cr_{F4} - $Co_{F1-F2-F3-F4-total}$ - $Cu_{F2-F3-total}$ - $Al_{F4-total}$ - $Fe_{F3-total}$ - $Zn_{F3-total}$ and positively with PbF4 and Se_{F3-total}.

Table 3. Pearson correlation matrix of PTEs of interest (F1–F4 fractions), total organic matter (%TOC), mud (%mud), species richness (S), dominance (D), Shannon [H(S)], foraminiferal number (FN), A = *Ammonia aoteana*; B = *Ammotium salsum*; C = *Trochammina*, and percent abundances of agglutinated (AGL), porcelaneous (P), and hyaline-perforate (HP) benthic foraminifera (n = 26; grey = significant positive correlation; red = significant negative correlation; black = correlation not considered; p < 0.05 [0.40]).

PTEs	S	D	H(S)	FN	Α	В	С	AGL	Р	HP	%TOC	%CaCO ₃	%Mud
Cr _{F2}	-0.07	-0.03	0.00	0.16	-0.30	0.28	0.52	0.33		-0.36	0.27	-0.13	0.33
Cr _{F3}	0.11	-0.01	0.04	0.23	-0.15	0.17	0.41	0.21		-0.17	0.42	0.26	0.57
Cr _{F4}	-0.11	0.38	-0.33	0.55	-0.17	0.66	0.69	0.40		-0.41	0.82	0.40	0.62
Crtotal	0.08	0.03	0.00	0.27	-0.17	0.25	0.49	0.24		-0.21	0.52	0.29	0.63
Co _{F1}	-0.13	-0.06	0.00	0.08	-0.41	0.23	0.47	0.12		-0.13	0.47	0.17	0.58
Co _{F2}	-0.27	0.46	-0.44	0.60	-0.25	0.63	0.69	0.46		-0.47	0.66	0.36	0.65
Co _{F3}	-0.20	0.62	-0.51	0.77	-0.16	0.68	0.68	0.61		-0.59	0.66	0.51	0.57
Co _{F4}	-0.21	0.64	-0.53	0.75	-0.05	0.71	0.74	0.42		-0.43	0.75	0.58	0.74
Cototal	-0.25	0.65	-0.56	0.77	-0.14	0.70	0.71	0.58		-0.56	0.72	0.56	0.65
Cu _{F1}	0.04	-0.40	0.31	-0.35	-0.08	-0.33	-0.13	-0.29		0.28	-0.33	-0.24	-0.17
Cu _{F2}	-0.03	-0.10	0.08	0.29	-0.34	0.34	0.65	0.44		-0.44	0.23	-0.30	0.17
Cu _{F3}	0.15	0.08	-0.03	0.33	-0.33	0.45	0.21	0.39		-0.42	0.19	0.02	0.06
Cu _{F4}	-0.16	0.43	-0.38	0.59	-0.16	0.60	0.68	0.37		-0.39	0.71	0.41	0.71
Cutotal	-0.12	0.37	-0.33	0.58	-0.23	0.61	0.68	0.40		-0.42	0.62	0.31	0.66
Ni _{F2}	-0.24	0.18	-0.21	0.14	0.19	0.09	0.32	-0.15		0.19	0.52	0.33	0.37
N1 _{F3}	-0.16	0.47	-0.39	0.65	-0.09	0.58	0.71	0.39		-0.37	0.76	0.63	0.76
N _{1F4}	-0.22	0.58	-0.50	0.69	-0.01	0.70	0.77	0.34		-0.35	0.82	0.57	0.79
Nitotal	-0.19	0.53	-0.45	0.68	-0.04	0.61	0.73	0.36		-0.35	0.80	0.64	0.78
Pb _{F2}	-0.19	-0.03	-0.08	-0.13	-0.27	-0.08	0.08	0.15		-0.16	-0.10	-0.27	0.13
Pb _{F3}	-0.19	0.56	-0.48	0.68	-0.15	0.63	0.56	0.65		-0.60	0.75	0.32	0.45
PD _{F4}	-0.01	-0.28	0.18	-0.69	0.35	-0.74	-0.85	-0.61		0.63	-0.72	-0.19	-0.82
Pototal	-0.21	0.48	-0.44	0.58	-0.18	0.54	0.52	0.58		-0.54	0.07	0.28	0.44
Se _{F1}	0.21	-0.08	0.12	0.13	0.23	0.08	-0.09	-0.16		0.09	0.07	0.23	-0.09
Se _{F2}	-0.27	0.07	-0.17	-0.22	-0.15	-0.12	-0.11	0.25		-0.29	-0.11	-0.19	-0.15
Sototal	0.00	-0.35	0.29	-0.64	0.12	-0.58	-0.84	-0.43		0.40	-0.58	-0.37	-0.62
	0.04	0.01	0.20	0.05	0.09	0.08	0.04	0.05		0.42	0.18	0.46	0.50
	0.05	-0.07	0.00	0.00	0.14	0.15	0.09	0.05		_0.07	0.10	0.40	0.30
	_0.15	0.07	_0.12	0.20	_0.13	0.10	0.41	0.20		_0.27	0.10	-0.26	_0.10
Al	_0. <u>2</u> 0	0.50	-0.39	0.77	-0.12	0.10	0.00	0.50		-0.52	0.80	0.20	0.62
Altotal	-0.10	0.30	-0.39	0.77	-0.17	0.00	0.73	0.50		-0.52	0.82	0.34	0.69
Feri	-0.11	0.13	-0.16	0.16	-0.18	0.09	0.72	0.23		-0.19	0.32	0.25	0.54
Fera	-0.29	0.23	-0.30	0.20	-0.11	0.26	0.48	0.29		-0.25	0.28	0.07	0.19
Fer2	-0.16	0.48	-0.40	0.68	-0.08	0.61	0.82	0.51		-0.50	0.69	0.42	0.63
Fe _{F4}	-0.11	0.54	-0.45	0.77	-0.19	0.82	0.78	0.50		-0.53	0.86	0.47	0.79
Fetotal	-0.16	0.54	-0.46	0.73	-0.10	0.69	0.82	0.53		-0.52	0.76	0.48	0.71
Zn _{F1}	0.01	-0.15	0.12	0.07	-0.18	0.09	0.36	0.06		-0.04	0.40	0.03	0.52
Zn_{F2}	0.03	0.18	-0.12	0.41	-0.24	0.38	0.13	0.21		-0.25	0.43	0.27	0.51
Zn _{F3}	-0.21	0.52	-0.44	0.70	-0.22	0.64	0.68	0.59		-0.57	0.80	0.45	0.69
Zn _{F4}	-0.08	0.49	-0.38	0.76	-0.16	0.68	0.78	0.48		-0.50	0.72	0.44	0.73
Zntotal	-0.15	0.49	-0.40	0.74	-0.24	0.68	0.74	0.57		-0.57	0.77	0.43	0.74
%TOC	-0.23	0.55	-0.48	0.67	-0.17	0.72	0.67	0.48	-0.49	-0.46			
%CaCO ₃	-0.25	0.58	-0.52	0.40	0.23	0.29	0.32	0.05	-0.47	-0.02			
%Mud	-0.08	0.27	-0.22	0.50	-0.15	0.53	0.73	0.27	-0.31	-0.29			
AGL	-0.06	0.40	-0.30	0.59				_		_			
Р	0.60	-0.74	0.77	-0.33									
HP [–]	0.04	_0.39	0.29	_0.61									

4.5. Sediment Contamination Indices

Based on the CF of the PTE total concentrations, six stations can be classified as "moderately" polluted for chromium (5), copper (10), and zinc (6, 10, 25, 26), and 100% of the sampled stations are classified as "highly polluted" for selenium (Table 4 and Figure 8A). The EF show that 27% (Cr), 46% (Ni), and 4% (Fe) of the sample sites fall in the category "moderately" polluted while 58%, 31%, 89%, 54%, and 58% of samples are classified as "severely" polluted for Cr, Co, Cu, Ni, and Fe, respectively. The category of "high" pollution was represented by 4% (Cr), 38% (Co), 8% (Cu), 27% (Pb), 46% (Zn), and 38% (Fe) of the samples in the lagoon. Furthermore, 12% (Cr), 27% (Co), 4% (Cu), 73% (Pb), 100% (Se), and 54% (Zn) of the samples are categorized as "extremely polluted" (Table 4 and Figure 8B).

Sample Stations		Contamination Factor (CF)								Enrichment Factor (EF)									Ecological Risk Factors (Er)								
	Cr	Со	Cu	Ni	Pb	Se	Zn	Al	Fe	Cr	Со	Cu	Ni	Pb	Se	Zn	Fe	Cr	Со	Cu	Ni	Pb	Se	Zn	Al	Fe	
St 1	0.1	0.0	0.0	0.0	0.3	142.3	0.1	0.0	0.0	24.8	15.1	18.3	4.7	141.6	59,160.3	48.5	9.9	0.1	0.0	0.2	0.0	1.7	142.3	0.1	0.0	0.0	144.4
St 2	0.0	0.0	0.0	0.0	0.2	166.2	0.2	0.0	0.0	6.9	5.2	9.7	4.4	47.2	43,916.1	57.4	10.1	0.1	0.0	0.2	0.0	0.9	166.2	0.2	0.0	0.0	167.6
St 3	0.0	0.1	0.1	0.0	0.2	158.9	0.1	0.0	0.1	9.4	23.7	21.0	9.5	43.9	39,698.2	34.3	19.8	0.1	0.1	0.4	0.0	0.9	158.9	0.1	0.0	0.1	160.6
St 4	0.1	0.3	0.0	0.1	0.4	126.0	0.3	0.0	0.2	9.1	44.3	7.4	10.6	53.5	19,102.9	41.9	23.9	0.1	0.3	0.2	0.1	1.8	126.0	0.3	0.0	0.2	129.0
St 5	1.1	0.5	0.1	0.1	0.8	66.4	0.9	0.0	0.3	88.3	44.1	9.0	7.9	66.0	5473.4	75.2	26.9	2.1	0.5	0.5	0.1	4.0	66.4	0.9	0.0	0.3	74.8
St 6	0.7	0.5	0.2	0.2	0.7	114.4	1.2	0.0	0.2	62.5	49.0	19.4	17.9	61.4	10,261.0	104.4	22.1	1.4	0.5	1.1	0.2	3.4	114.4	1.2	0.0	0.2	122.4
St 7	0.0	0.3	0.1	0.1	0.5	129.7	0.8	0.0	0.2	2.8	27.1	10.6	4.0	35.9	10,099.7	58.5	15.8	0.1	0.3	0.7	0.1	2.3	129.7	0.8	0.0	0.2	134.2
St 8	0.0	0.4	0.0	0.0	0.3	119.1	0.3	0.0	0.1	6.9	81.1	5.2	6.7	68.6	24,908.0	62.6	30.9	0.1	0.4	0.1	0.0	1.6	119.1	0.3	0.0	0.1	121.7
St 9	0.0	0.3	0.0	0.0	0.3	142.6	0.3	0.0	0.1	8.6	86.4	6.5	8.4	70.6	37,594.9	76.2	32.3	0.1	0.3	0.1	0.0	1.3	142.6	0.3	0.0	0.1	144.8
St 10	0.1	0.8	2.9	0.1	0.5	77.2	1.9	0.0	0.4	4.9	43.0	147.6	6.7	23.0	3932.0	98.5	19.2	0.2	0.8	14.5	0.1	2.3	77.2	1.9	0.0	0.4	97.4
St 11	0.1	0.8	0.1	0.1	0.6	53.0	0.7	0.0	0.4	4.3	33.5	5.8	5.2	26.0	2129.9	27.3	16.2	0.2	0.8	0.7	0.1	3.2	53.0	0.7	0.0	0.4	59.1
St 12	0.0	0.1	0.1	0.0	0.5	160.9	0.2	0.0	0.1	9.7	37.6	23.2	2.0	132.6	42,146.7	50.7	22.4	0.1	0.1	0.4	0.0	2.5	160.9	0.2	0.0	0.1	164.3
St 13	0.0	0.2	0.0	0.0	0.5	162.1	0.3	0.0	0.1	7.9	35.5	5.3	3.4	93.3	33,111.7	56.6	22.1	0.1	0.2	0.1	0.0	2.3	162.1	0.3	0.0	0.1	165.2
St 14	0.1	0.1	0.1	0.0	0.6	201.5	0.2	0.0	0.1	7.5	10.1	8.8	2.3	77.7	27,959.9	29.3	12.2	0.1	0.1	0.3	0.0	2.8	201.5	0.2	0.0	0.1	205.1
St 15	0.4	0.1	0.0	0.0	0.6	189.3	0.2	0.0	0.1	64.9	17.9	7.6	3.3	93.7	32,003.0	32.7	14.6	0.8	0.1	0.2	0.0	2.8	189.3	0.2	0.0	0.1	193.5
St 16	0.1	0.1	0.1	0.0	0.3	161.7	0.2	0.0	0.1	9.0	19.5	9.1	6.1	45.8	22,894.2	28.0	15.7	0.1	0.1	0.3	0.0	1.6	161.7	0.2	0.0	0.1	164.1
St 17	0.0	0.2	0.0	0.0	0.5	150.2	0.2	0.0	0.1	7.0	29.6	5.6	3.0	82.7	24,514.2	40.0	18.9	0.1	0.2	0.2	0.0	2.5	150.2	0.2	0.0	0.1	153.5
St 18	0.1	0.3	0.1	0.1	0.5	135.7	0.3	0.0	0.2	5.0	26.6	7.3	5.9	41.9	12,578.6	27.7	15.2	0.1	0.3	0.4	0.1	2.3	135.7	0.3	0.0	0.2	139.4
St 19	0.0	0.3	0.1	0.0	0.4	125.0	0.3	0.0	0.2	6.1	32.5	6.7	5.8	52.3	15,980.6	40.5	21.2	0.1	0.3	0.3	0.0	2.0	125.0	0.3	0.0	0.2	128.2
St 20	0.0	0.1	0.0	0.0	0.4	186.0	0.2	0.0	0.1	6.6	18.8	8.1	3.2	78.2	39,514.4	41.3	14.1	0.1	0.1	0.2	0.0	1.8	186.0	0.2	0.0	0.1	188.5
St 21	0.0	0.1	0.1	0.0	0.4	120.6	0.2	0.0	0.1	5.0	15.7	6.7	4.3	43.3	14,483.3	28.9	17.4	0.1	0.1	0.3	0.0	1.8	120.6	0.2	0.0	0.1	123.2
St 22	0.1	0.6	0.2	0.1	0.7	114.9	0.5	0.0	0.2	10.1	43.4	14.4	6.0	49.5	7740.7	35.7	15.1	0.3	0.6	1.1	0.1	3.7	114.9	0.5	0.0	0.2	121.4
St 23	0.1	0.5	0.3	0.1	0.5	116.6	0.6	0.0	0.3	4.2	21.4	9.9	4.3	21.7	4603.6	23.8	12.2	0.2	0.5	1.3	0.1	2.7	116.6	0.6	0.0	0.3	122.3
St 24	0.1	0.6	0.2	0.1	0.5	92.3	0.6	0.0	0.4	6.ð	37.5	10.1	7.3	31.4	5563.4	35.1	21.3	0.2	0.6	0.8	0.1	2.6	92.3	0.6	0.0	0.4	97.6
St 25 St 26	0.1	0.7	0.2	0.1	0.7	/4./ 120.6	1.4	0.0	0.5	5.ð 2.7	33.U 16.9	7.4 8.6	5.4 2.7	30.0 21.7	3383.9 2185.0	33.Z	21.1	0.3	0.7	0.8	0.1	3.3 4.1	120.6	1.2	0.0	0.5	01.0 129.0
51.26	0.1	0.6	0.3	0.1	0.8	120.6	1.4	0.0	0.2	5.7	10.8	0.0	2.7	21.7	5105.9	57.7	4.0	0.3	0.6	1.0	0.1	4.1	120.6	1.4	0.0	0.2	128.9

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 Table 4. Potentially toxic element pollution indices.



Figure 8. Single element and multi element indices of pollution. (**A**) Contamination Factor (CF), (**B**) Enrichment Factor (EF), and (**C**) Ecological risk index (RI). PTEs: potentially toxic elements.

The RI is an independent ecological assessment tool that considers PTE toxicity in sediments and their Er in the environment with respect to background values (e.g., [29,30]). The Er and RI values are shown in Table 4. Most of the PTEs show low Er values except for Se which is exceptionally high. Moreover, the summative RI index values range between 59 (low) and 205 (moderate) across the sample stations (Table 4 and Figure 8C). Most of the high values of RI are due to the Se concentrations.

5. Discussion

Lagos Lagoon is an extremely dynamic ecosystem and has experienced significant human interference for more than 100 years [31]. This includes a growing population entailing large space demands, a rapid depletion of wetlands with increasing urbanization, and the construction of a harbor mole to facilitate navigation through a permanent lagoonal entrance channel. Driven by growth and industrialization, the lagoon receives enormous amounts of largely untreated industrial and other wastes with significant toxic potential. As described previously, most of the pollution in the lagoon is considered to be of anthropogenic origin [6,18,32–34]. The pollution loads recorded within the lagoon represent a cocktail of environmental contaminants, have a significant toxic potential for humans, and wildlife and include heavy metals such as mercury and cadmium, as well as organic compounds polychlorinated biphenyls (PCBs), phenols, and PAHs [34]. To assess the status of pollution and the effect of individual PTEs on the distribution of previously studied benthic foraminifera, we performed an in-depth cross-correlation analysis across Lagos Lagoon.

5.1. Potentially Toxic Elements in Sediments

Previous studies have demonstrated that most anthropogenic inputs are concentrated along the western shoreline before fanning out into the lagoon [19,35,36]. The east and west spatial distribution of PTEs is related to their affinity to adsorb to clay surfaces present in mud sediments under toxic environmental conditions (F1 fraction; [37]). In this study, we recorded consistently high individual contamination levels along the western lagoon

shores for Co, Al, Fe, and Zn in the F1 fraction, for Ni in the F2 fraction, for Cr and Ni in the F3 fraction, and for Co, Ni, and Fe in the F4 fraction (Figures 5 and 6; Table 4; Appendix A— Table A1). However, given the relative acidic conditions of the lagoon (pH: 5.8–6.9), it is probable that the F2/F3 fractions are not significantly sequestered during carbonate mineralization [1,38]. This may explain the very low concentration found in the respective exchangeable and acid-soluble fractions (Figure 6). Interestingly, most PTEs are found in the F3 fraction as a response to oxygen levels (Figure 6) related to water column mixing during tidal flushing (0.6–1.2 m; [3]). During iron-oxide precipitation, PTEs will be sequestered and/or adsorb to this reducible fraction as evidenced by Se comprising 95.9% of the PTEs in the F3 fraction.

The F4 fraction contains relatively high bioavailable PTE concentrations (Figure 6; Appendix A—Table A1) as a response to complexation due to organic pollution (e.g., sewage) and flocculation of organic matter typical of estuarine environments [39]. Among the PTEs analyzed, Cu has been shown to constitute a proxy for the amount of sewage input (e.g., [40,41]). Since the lagoon receives copious amounts of sewage (e.g., [16,32]), this could explain the high abundance of this PTE in the F4 (oxidizable).

In general, PTEs have shown that 65% and 35% of the sampled stations have "low" and "moderate" levels of ecological risk, respectively. This variation is related to the sample stations (ST15–ST20) under a "moderate" level of ecological risk, consisting of very low TOC with sandy sediments and a high number of calcareous foraminifera in the central portion of the lagoon. The sole fractionation of Se with oxide-hydroxides associated with the F3-reducible fraction further indicates that this portion of the lagoon is more oxygenated. On the contrary, the sample stations with "low" ecological risk are located in the western and eastern sectors of the lagoon and consist of high TOC muddy sediments dominated by agglutinated foraminifera. In this case, the negative correlation of Se with TOC and mud suggests that these portions of the lagoon are less oxygenated, maintaining this PTE in other insoluble forms such as its elemental state or as selenide [42].

In addition, highly polluted conditions are indicated by the amount of Selenium which is consistent with reports of other PTEs (e.g., Zn) being released and transported by artificial canals, streams, and rivers from industrial effluents [35,43,44]. Unfortunately, the sources of the high pollution status of Se in single and multi-element pollution indices (CF, EF, and RI) are not yet known, and there is no previous literature on the analysis of this PTE in Lagos Lagoon. Moreover, the mobilization of Se in aquatic systems could be a result of anthropogenic activities, such as petroleum transport/oil refining, metal smelting, municipal landfills, and paint production amongst others (e.g., [45–47]), which are prevalent around the lagoon. Other studies have also shown that bioaccumulation along food chains can be another factor responsible for Se mobilization and cycling in aquatic ecosystems [48]. Furthermore, calculations of the Enrichment Factor (EF) show that most of the PTEs analyzed indicate a "moderately" to "extremely" polluted environment. This is compatible with the high PTE values found by Don-Pedro et al. [35].

The total Se distribution map suggests that the source of pollution could come from the northern and southern shores. "High" Se concentrations have only been reported by Overah et al. [49] in urban-derived sediments found in gutters along the Lagos Bar Beach (eastern shore of the Commodore Channel), but neither actual concentrations nor the source of the PTE was provided. The Five Cowrie Creek is a narrow tidal channel connecting the Commodore Channel to the central portion of the lagoon [14]. This bypassing of marine waters into the central part of the lagoon provides a pathway for Se, as demonstrated by a "hotspot" located at the central part of the lagoon (Figure 5; Appendix B—Figure A3). Given that PTEs are found as organo-metallic compounds in the oil, another source of Se most likely could come from the Apapa-Badagry Creek in the Lagos Harbor which is known to have high concentrations of hydrocarbons (PAHs) [5]. The levels and detrimental effects of Se in Lagos Lagoon have not been studied so far, which explains how this PTE's enrichment could go unnoticed over such a long period of time.
5.2. Correlation between PTEs and Benthic Foraminiferal Assemblages

Several workers have reported the influence of PTEs on the distribution and diversity of benthic foraminifera especially in estuarine-lagoon environments (e.g., [2,38,50–52]). However, the concept of using total and bioavailable PTE concentrations does not directly apply when used as an assessment of the ecological response of benthic foraminifera to pollutants [1,37,53]. The degree of uncertainty when using total concentrations does not allow for the proper assessment of PTE impacts. For example, *A. salsum, Trochammina* sp. 1, and the percentage values of agglutinated taxa have significant positive correlations with several PTEs in the -F3 bioavailable and total concentrations, while hyaline-perforate mostly had negative correlations (Table 3). However, the PTEs in this fraction are not readily bioavailable to the foraminifera, given that they are sequestered within the crystalline structures of oxides after precipitation. Based on the generalized feeding habits (e.g., detritivory, omnivory) of foraminifera, it is suspected that PTEs found in the F1 and F4 fractions are the most bioavailable and should be only considered when assessing the impacts of these contaminants.

In Lagos Lagoon, the foraminiferal species richness was shown to be comparatively low (42 taxa; [13]), a feature that is common in estuarine and lagoonal habitats [3,54–56]. Species richness, however, did not show any significant correlation with the PTEs in any fraction, but non-significant negative correlations were observed for Co_{F2,F3,F4}, Ni_{F2,F4}; Pb_{total}, Se_{F2}, Al_{F3}, Fe_{F2}, Zn_{F3}, and TOC. A statistically significant positive correlation, however, was recorded between species richness and percent abundances of foraminifera with a porcelaneous test wall (Table 3). As pointed out by Fajemila et al. [13,57], foraminiferal species richness and abundances of porcelaneous taxa are primarily driven by salinity, suggesting that the individual PTEs or fractions thereof have secondary importance for species with a porcelaneous test wall.

Agglutinated taxa are interchangeably abundant with calcareous taxa along different corridors of the lagoon with respect to sediment characteristics and salinity values governed by the interplay between marine and fresh waters [13]. For example, A. salsum, which is widely recognized as a stress-tolerant species (e.g., [52,58–60]), coupled with *Trochammina* sp. 1 occupies most of the easterly and westerly sites in the lagoon (Figure 2). Ammotium salsum and Trochammina sp. 1 were found to have significant positive correlations with CrF4-CoF2-F3-F4-total-CuF4-total-NiF3-F4-total-AlF4-total-FeF3-F4-total-ZnF3-F4-total and correlate negatively with Pb_{F4}-Se_{F3}-Se_{total}. Oxidizable fraction (F4) is directly related to organic matter, and potentially bioavailable along trophic transfer lines [1,37,52]. The overall significant positive correlation of these PTEs suggests that they behave as micronutrients and not as stressors when complexed with organic matter in the GoG. A positive correlation between the % abundances of agglutinated taxa and TOC (Table 3) provides additional support for this hypothesis. In addition, positive correlation records between percent abundances of agglutinated foraminifera, D, and FN (Table 3) show that besides TOC, salinity plays a major role. A similar finding was reported from west African lagoons by Debenay [61], who identified A. salsum as a stress-tolerant species following salinity gradients. Although it is difficult to disentangle which vector gradient (pollution vs. salinity) is responsible for their dominance and distribution patterns, both A. salsum and Trochammina sp. 1 are considered bioindicators of environmental stress in Lagos lagoon given their affinity to PTEs in the organic-bound F4 fraction.

It is important to notice that the percent abundances of agglutinated foraminifera correlate positively with several PTEs in the F3-total fractions. Because these fractions are not readily bioavailable, they may represent false positives. For example, the positive correlation between mud and TOC strongly suggests that organic-rich muddy sediments are dominant in the lagoon. Since organic matter plays an integral role in the level of oxygenation, it comes as no surprise that the percentage of agglutinate foraminifera correlates positively with PTEs in the iron-oxide (F3) fraction.

The significant positive correlation of both *A. salsum* and *Trochammina* sp. 1 with TOC supports the affinity of certain PTEs to organic matter. As shown by Fajemila et al. [13], the

abundance is highest where there are low salinity readings which coincides with higher amounts of TOC with PTEs complexed in the F4 fraction. The single PTE that negatively correlates with *A. salsum* and *Trochammina* sp. 1 is Pb_{F4} , suggesting that it is the only PTE with a detrimental effect on the distribution of the two species.

Among all hyaline-perforate benthic foraminifera present in Lagos Lagoon, members of the genus Ammonia were found to constitute the most abundant. Several species of the genus Ammonia are well-known to be a stress-tolerant (e.g., [62–64]). Ammonia, however, shows no correlation with mud and TOC. Its highest abundances were found to be at sites around the center of the lagoon where TOC values are <4% (Figure 2). This particular Ammonia distribution could be linked to variable pH conditions, and culture experiments have documented that drastic decalcification occurs when pH approaches 7 [65]. Similarly, Pettit et al. [66] found very few specimens of Ammonia in both the living and dead assemblages at pH levels between 7.55-7.88 in the Gulf of California. Acidic lagoon conditions of Lagos Lagoon (pH 5.8–6.9) may therefore limit the abundance of calcareous taxa, a feature that is also supported by the lack of correlation between CaCO₃ and A. aoteana. This hypothesis is consistent with the observations of Dias et al. [67] who studied the long-term biological response of foraminifera to acidification and reported foraminiferal assemblages dominated by agglutinated taxa at pH < 7.6. In addition, the negative correlation between percent abundances of hyaline-perforate taxa with both TOC and FN (Table 3) suggests that the environmental conditions at high TOC values favor agglutinated over hyaline-perforate taxa.

Although *A. aoetana* is the dominant hyaline-perforate taxon with practically no correlation with PTEs, the percentage of the hyaline group shows numerous significant correlations with non-bioavailable $Co_{F2-F3total}-Cu_{F2-F3-total}-Pb_{F3-total}-Se_{Fe-total}-Al_{total}-Fe_{F3-total}-Zn_{F3-total}$. However, this PTE_{F3} fraction is not bioavailable and is considered a false positive as it has no direct effect on this group. Interestingly, the percentage of hyaline-perforate taxa correlates positively with bioavailable Pb_{F4} , suggesting that the latter behaves like a micronutrient. However, TOC shows a strong negative correlation with both Pb_{F4} and the percentage of hyaline-perforate taxa (Table 3).

As pointed by Martinez-Colón et al. [1], PTEs in the exchangeable fraction (F1) could be bioavailable to the foraminifera. A positive correlation was found for Al_{F1} with mud, indicating that the sediment provenance is mostly terrestrial. Co_{F1} also correlates positively with *Trochammina* sp. 1 and negatively with *A. aoteana*, suggesting that it functions both as a micronutrient and a stressor, respectively. No other PTE in the F1 exchangeable fraction correlates significantly with *A. salsum*, *Trochammina* sp. 1, or *A. aoteana*.

The low foraminiferal ecological risk index values recorded are characteristic for stressed environments (e.g., [68,69]). For example, H(S) only correlates negatively with Co-Ni-Pb-Fe in the F4 fraction and with TOC and %CaCO₃. This suggests that a multitude of variables, including low dissolved oxygen, variable water acidification (linked to salinity), and aforementioned bioavailable PTEs are impacting the composition and distribution of assemblages. Similarly, the statistical relationship between foraminiferal dominance (D) values with TOC, CaCO₃, and several PTEs suggests the same effects on the dominance and distribution of *A. salsum*.

Previously recorded data showed that the foraminiferal number (FN) increases westeast across the lagoon with an accompanying decrease in species richness [13]. FN correlates positively with Co-Ni-Al-Fe-Zn and negatively with Pb in the F4 fraction. This suggests that besides salinity, the composition and texture of sediments play a factor in the foraminiferal distribution. In addition, the sediments showing a significant positive correlation with mud and TOC, suggest that several PTEs or fractions thereof play an auxiliary role.

Previous studies reported that under conditions of heavy metal pollution foraminiferal population density tends to decline but that a suite of environmental factors can make parsing the effect of contaminants from other variables difficult [70–75]. Recent studies by Smith and Goldstein [73] showed that exposure to elevated concentrations of Ni and Zn resulted in limited abundances under varying salinity and temperature conditions. The effects on species richness and test deformities, however, remained puzzling and

inconsistent. No significant positive correlation with none of the PTEs was found for neither species richness nor for the most abundant hyaline-perforate species (*A. aoteana*) in Lagos Lagoon. Previously recorded data from Lagos Lagoon showed that the FN in total assemblages increased west-east across the lagoon with an accompanying decrease in species richness and salinity [13]. Total abundances were recorded to correlate positively with Co-Ni-Al-Fe-Zn and negatively with Pb in the F4 fraction. Therefore, the spatial distribution of foraminiferal diversity is best explained by their negative correlation with $Co_{F4}-Ni_{F4}-Fe_{F4}$, as it also follows salinity gradients. On the contrary, positive correlations exist between $Co_{F4}-Cu_{F4}-Ni_{F4}-Fe_{F4}-Zn_{F4}$, FN, and TOC, suggesting that these PTEs behave as micronutrients.

Except for Se_{F4} and Pb_{F4}, no significant and consistent negative correlation between the abundance, species richness, and spatial distribution of benthic foraminifera and PTEs was found in Lagos Lagoon. We acknowledge that the PTEs analyzed in this study represent only a fraction of the pollution cocktail accumulating within Lagos Lagoon. Especially in the industrialized western part of the lagoon, other toxic substances, including PAHs, PCBs, and high concentrations of organic compounds, were reported to have detrimental and lethal effects on marine life [1,2,76,77].

6. Conclusions

Foraminiferal assemblages collected across the highly polluted Lagos Lagoon display a distinct separation of agglutinated and hyaline-perforate/porcelaneous taxa. The spatial separation is largely oriented along the salinity contour lines. Superimposed on the salinity-driven distributional differences are a multitude of stressors related to increasing anthropogenic influences. Our in-depth cross-correlation analysis on the spatial distribution foraminifera and a selection of Potentially Toxic Elements (PTEs) yields the following major conclusions:

- 1. The results of our study do not show a significant negative correlation between benthic foraminiferal species and PTEs except for Pb_{F4} and Se_{F4}.
- 2. The spatial distribution of foraminiferal species richness and diversity negatively correlates with Co_{F4}-Ni_{F4}-Fe_{F4}, a feature that tracks the salinity gradients. On the contrary, the positive correlation of Co_{F4}-Cu_{F4}-Ni_{F4}-Al_{F4}-Fe_{F4}-Zn_{F4} with the foraminiferal number distribution suggests that these PTEs behave as micronutrients since it also correlates favorably with TOC.
- 3. This study provides new information on the bioavailability of PTEs, especially of Se in Lagos Lagoon. The relatively high concentrations of Se suggest that it has been accumulated unnoticed in the lagoon over the years. Lagos Lagoon will continue to act as a sink for Se and other PTEs, therefore, their potential impact on the lagoon's ecosystem must be monitored and assessed for proper management and control to minimize further impacts of all these pollutants on coastal activities.
- Shannon diversity values H(S) show significant negative correlations with various bioavailable PTEs in the F4 fraction (Co-Ni-Fe).
- 5. An interesting finding is Se being the only PTE with the highest total concentration (32–120 mg/kg) within the central portion of the lagoon. The Ecological Risk Index and Enrichment Factor also suggest that this part of the lagoon is experiencing moderate to severely polluted environmental conditions. It is uncertain what the extent of its effects on the foraminiferal assemblages is, since the abundance of *A. salsum* and *Trochammina* sp. 1, and the FN are the only parameters showing significant negative correlations with the non-bioavailable fractions of Se.
- 6. Our study serves as a baseline for future studies investigating the environmental impact of pollution on benthic foraminifera, species richness, and within the Lagos Lagoon environment. Because the PTEs analyzed in this study represent only a portion of the pollution cocktail accumulating within Lagos Lagoon, future analysis of PAHs, PCBs, and OCs would provide a more comprehensive view on the status of pollution and their effects on foraminiferal bioindicators. Consistent with previous work, our

results identify the problems associated with using total concentrations of selected PTEs alone as tools for biomonitoring, but support the usefulness of foraminiferal abundance and species richness as tools for environmental analysis.

Author Contributions: Conceptualization, O.T.F. and M.R.L.; methodology, O.T.F., M.M.-C. and M.R.L.; investigation, O.T.F., M.M.-C. and I.S.C.; resources, M.M.-C. and M.R.L.; formal analysis, O.T.F., M.M.-C., N.S., I.S.C. and T.O.K.; writing—original draft preparation, O.T.F., M.M.-C., N.S., I.S.C., T.O.K. and M.R.L.; writing—review and editing, O.T.F., M.M.-C., N.S. and M.R.L.; visualization, O.T.F., M.M.-C. and M.R.L.; supervision, M.R.L.; funding acquisition, O.T.F., M.M.-C. and M.R.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from the Tertiary Education Tax Fund Nigeria (www. tetfund.gov.ng, accessed on 20 May 2019) to O.T.F. (TETFund/16b/0014), the German Science Foundation (DFG; www.dfg.de, accessed on 23 November 2021) to MRL (LA 884/10-1, LA 884/5-1), the National Science Foundation Geography and Spatial Sciences Program to M.M.-C. (grant number 1853794), and the Early Career Research Fellowship from the Gulf Research Program of the National Academies of Sciences, Engineering, and Medicine to M.M.-C. (grant number 2000009944). The content of this publication is the solely the responsibility of the authors and funders were not involved in the study design, data collection and analysis, interpretations, decision to publish, or preparation of the manuscript.

Data Availability Statement: The datasets generated and/or analyzed during the current study are included in this article.

Acknowledgments: The authors gratefully acknowledge support from the Nigerian Institute of Oceanography and Marine Research (NIOMR) and Sina Adegbie for processing samples.

Conflicts of Interest: The authors declare no conflict of interests.

Appendix A

PTE concentrations for each fraction (F1–F4).

Table A1. PTE concentrations for each fraction. Concentrations are in mg/kg and in percent (*). (BDL = Below Detection Limit).

Samula ID	Fraction #1 (F1: Exchangeable)											
Sample ID	Cr	Со	Cu	Ni	Cd	Pb	K *	Se	As	Al *	Fe *	Zn
1	0.75	0.39	0.87	BDL	2.25	1.11	0.01	0.42	0.15	$1.11 imes 10^{-4}$	$2.76 imes10^{-4}$	0.57
2	0.18	BDL	0.39	BDL	0.18	0.78	0.02	0.39	0.18	$1.35 imes10^{-3}$	$2.34 imes10^{-4}$	0.24
3	BDL	0.33	0.30	BDL	0.06	0.60	0.04	0.24	BDL	$9.3 imes10^{-5}$	$2.01 imes10^{-4}$	0.27
4	BDL	0.00	0.33	BDL	0.00	0.75	0.09	0.48	BDL	$2.4 imes10^{-3}$	$5.49 imes10^{-4}$	0.15
5	0.03	0.18	0.24	BDL	0.12	BDL	0.14	0.00	0.09	$1.65 imes10^{-3}$	$3.16 imes10^{-3}$	0.60
6	0.06	0.24	0.33	BDL	0.42	BDL	0.13	BDL	BDL	$3.88 imes10^{-3}$	$7.55 imes10^{-3}$	0.87
7	0.00	0.09	0.18	BDL	0.00	BDL	0.06	0.15	BDL	$2.13 imes10^{-3}$	$4.24 imes10^{-3}$	0.21
8	BDL	BDL	0.09	BDL	BDL	BDL	0.01	0.18	BDL	$2.55 imes 10^{-4}$	$7.92 imes10^{-4}$	0.06
9	BDL	BDL	0.09	BDL	BDL	BDL	0.01	0.03	0.09	$9.3 imes10^{-5}$	$3.42 imes 10^{-4}$	0.06
10	BDL	0.27	0.09	BDL	0.03	BDL	0.15	0.42	BDL	$2.94 imes10^{-4}$	$1.15 imes10^{-3}$	0.24
11	0.00	0.15	0.12	BDL	0.03	BDL	0.15	0.09	BDL	$1.6 imes10^{-3}$	$3.3 imes10^{-3}$	0.21
12	BDL	0.00	3.00	BDL	BDL	BDL	0.01	0.12	BDL	$6.3 imes10^{-5}$	$1.44 imes10^{-4}$	0.12
13	BDL	0.00	0.12	BDL	BDL	BDL	0.01	0.15	BDL	$9.3 imes10^{-5}$	$4.47 imes10^{-4}$	0.45
14	BDL	0.03	0.09	BDL	BDL	BDL	0.06	0.30	BDL	$5.1 imes 10^{-4}$	$8.22 imes 10^{-4}$	0.18
15	BDL	0.06	0.09	BDL	BDL	BDL	0.04	0.24	BDL	$9.9 imes10^{-4}$	$1.4 imes10^{-3}$	0.27
16	BDL	0.06	0.09	BDL	BDL	BDL	0.05	0.12	0.09	$1.03 imes10^{-3}$	$1.69 imes10^{-3}$	0.18
17	BDL	0.03	0.30	BDL	BDL	BDL	0.02	0.09	BDL	$3.54 imes 10^{-4}$	$8.76 imes10^{-4}$	0.12
18	BDL	0.06	0.06	BDL	0.00	BDL	0.09	0.15	BDL	$2.88 imes 10^{-4}$	$6.15 imes10^{-4}$	0.18
19	BDL	0.06	0.09	BDL	BDL	BDL	0.05	0.21	BDL	$2.46 imes 10^{-4}$	$6.3 imes 10^{-4}$	0.12
20	BDL	0.03	0.09	BDL	BDL	BDL	0.03	0.24	BDL	2.13×10^{-4}	4.41×10^{-4}	0.12
21	BDL	0.00	0.06	BDL	BDL	BDL	0.05	0.33	BDL	$4.65 imes 10^{-4}$	$9.42 imes 10^{-4}$	0.21
22	BDL	0.06	0.09	BDL	0.00	BDL	0.07	0.57	BDL	1.53×10^{-4}	$4.17 imes10^{-4}$	0.12
23	BDL	0.18	0.06	BDL	0.06	BDL	0.18	0.24	BDL	3.54×10^{-4}	7.14×10^{-4}	0.42
24	BDL	0.15	0.06	BDL	0.03	BDL	0.14	0.12	BDL	$4.77 imes 10^{-4}$	$1.46 imes 10^{-3}$	0.27
25	0.45	0.33	0.33	BDL	0.27	BDL	0.14	0.48	BDL	$6.9 imes 10^{-4}$	$4.17 imes 10^{-4}$	0.30
26	BDL	0.18	0.18	BDL	0.39	BDL	0.11	0.36	BDL	$1.5 imes10^{-4}$	$3.6 imes10^{-4}$	0.57

	Fraction #2 (F2: Acid-soluble)												
Sample ID	Cr	Со	Cu	Ni	Cd	Pb	K *	Se	As	A	.1 *	Fe *	Zn
1	0.12	0.00	0.15	0.03	0.03	0.39	0.01	1.350	BDL	4.77	$\times 10^{-4}$	$6.3 imes10^{-4}$	0.60
2	0.06	0.03	0.09	0.03	0.03	0.36	0.02	1.56	BDL	4.02	$ imes 10^{-4}$	$4.71 imes10^{-4}$	6.00
3	0.06	0.21	0.00	0.21	0.00	0.36	0.03	1.74	BDL	9.9 >	$< 10^{-5}$	$2.85 imes 10^{-4}$	1.20
4	0.06	0.18	BDL	0.12	0.03	0.30	0.05	1.59	BDL	5.13	$ imes 10^{-4}$	$1.08 imes10^{-3}$	0.81
5	0.09	0.05	0.12	0.06	0.03	0.66	0.10	1.71	BDL	5.97	$\times 10^{-4}$	$2.1 imes10^{-3}$	2.85
6	0.06	0.66	0.12	0.30	0.18	0.96	0.09	1.68	BDL	3.27	$ imes 10^{-4}$	$1.15 imes 10^{-3}$	6.57
7	0.06	0.51	0.03	0.06	0.06	0.66	0.04	1.74	BDL	4.62	$\times 10^{-4}$	$1.58 imes10^{-3}$	21.00
8	0.06	0.09	0.09	0.06	0.00	0.27	0.02	1.89	BDL	7.53	$\times 10^{-4}$	1.13×10^{-3}	0.66
9	0.06	0.03	0.03	0.00	0.03	0.42	0.02	2.07	BDL	2.76	$\times 10^{-4}$	$6 imes 10^{-4}$	0.51
10	0.09	1.17	0.12	0.21	0.03	0.60	0.10	1.77	BDL	6.78	$\times 10^{-4}$	5.45×10^{-3}	1.38
11	0.15	1.47	0.15	0.06	BDL	2.13	0.09	1.71	BDL	5.76	$\times 10^{-4}$	4.69×10^{-3}	1.41
12	0.09	0.15	0.21	BDL	BDL	1.77	0.01	1.80	BDL	2.43	$\times 10^{-4}$	3.2×10^{-3}	0.57
13	0.06	0.03	0.15	BDL	BDL	0.84	0.02	1.89	BDL	2.46	$\times 10^{-4}$	7.32×10^{-4}	0.48
14	0.15	0.24	0.12	BDL	BDL	2.64	0.03	19.80	BDL	4.53	$\times 10^{-4}$	1.27×10^{-3}	0.87
15	0.15	0.18	0.09	BDL	BDL	3.18	0.02	1.98	BDL	3.48	$\times 10^{-4}$	5.76×10^{-4}	0.84
16	0.06	0.18	0.03	0.15	0.03	0.42	0.03	2.07	BDL	6 ×	10-4	1.08×10^{-3}	0.99
17	0.09	0.27	0.09	0.03	BDL	1.20	0.02	0.90	BDL	4.35	$\times 10^{-4}$	7.42×10^{-3}	1.23
18	0.06	0.21	0.03	0.21	0.00	0.60	0.04	1.77	BDL	4.35	$\times 10^{-4}$	8.28×10^{-4}	0.78
19	0.06	0.30	0.12	0.18	0.03	0.30	0.03	1.86	BDL	3.66	$\times 10^{-4}$	2.07×10^{-6}	1.05
20	0.06	0.15	0.03	0.09	0.00	0.30	0.02	1.86	BDL	5.45	$\times 10^{-4}$	8.7×10^{-3}	1.1/
21	0.06	0.10	0.06	0.10	0.03	0.50	0.05	1.05		5.19	$\times 10^{-4}$	2.29×10^{-3}	1.11
22	0.06	0.51	0.06	0.06	0.03	0.48	0.05	2.04		1.85	× 10 -4	1.22×10^{-3}	2.28
25	0.09	1.02	0.12	0.09	0.05	0.45	0.08	1.09	BDL	4.2 >	$\times 10^{-4}$	1.19×10^{-3}	1.71
24	0.21	0.45	0.24	0.55	0.10	0.45	0.12	2.04	BDL	0.01	$\times 10^{-4}$	5.21×10^{-4}	2.25
25	0.09	0.45	0.12	0.13	0.12	0.30	0.11	2.04	BDL	3.87	$\times 10^{-4}$	1.17×10^{-3}	7 35
	0.112	0.07	0.10	0.12	0.21	Fract	ion #3 (F	3: Reducil	ole)	0.07		1.10 / 10	
Sample ID	Cr Co Cu Ni Cd Ph K*							*	Se	As	Al *	Fe *	Zn
1	2.6	0.2	0.6	0.6	0.2	4.5	2.01	10-3	82.1	RDI	0.02	0.10	0.0
1	1.5	0.3	0.0	0.0	0.3	1.9	2.01 ×	10^{-3}	02.4	BDL	0.02	0.10	9.9 11 /
2	1.5	0.5	0.0	1.5	0.3	1.0	2.32 ~	10^{-3}	92.1	BDL	0.02	0.17	11.4
4	3.9	3.9	0.0	33	BDI	5.1	2.43 ^	10 11	73.5	BDI	0.01	0.22	22.2
5	93.0	8.4	0.6	5.1	BDL	15.3	0.01		38.1	BDL	0.02	1.15	70.5
6	60.0	8.1	0.3	11.4	0.3	12.6	0.02		66.9	BDL	0.03	0.79	89.1
7	2.7	4.5	0.0	2.4	BDL	8.4	0.01		75.9	BDL	0.02	0.56	38.1
8	2.4	6.6	0.6	1.8	BDL	5.7	$1.53 imes 10^{-3}$		66.9	BDL	0.02	0.68	25.8
9	2.1	5.4	0.6	1.8	BDL	4.2	$1.47 \times$	10^{-3}	81.3	BDL	0.01	0.56	24.6
10	5.7	10.2	0.6	5.7	BDL	8.4	0.02		44.1	BDL	0.02	1.24	44.7
11	6.3	10.2	0.6	5.7	BDL	10.8	0.	02	30.0	BDL	0.02	1.25	45.3
12	2.7	2.4	0.6	0.3	BDL	7.8	$7.80 \times$	10^{-4}	92.1	BDL	0.02	0.40	17.1
13	2.7	3.0	0.6	0.9	BDL	7.5	9.00 ×	10-4	92.7	BDL	0.03	0.50	24.3
14	2.7	0.9	0.6	0.6	0.0	7.8	3.15 ×	10^{-3}	100.8	BDL	0.03	0.32	18.3
15	33.0	1.5	0.6	0.9	0.0	7.2	2.25 ×	10-3	111.3	BDL	0.02	0.35	16.5
16	3.3	1.8	0.3	2.1	0.0	5.4	2.73 ×	10^{-3}	94.8	BDL	0.02	0.39	16.5
17	2.7	2.7	0.3	0.9	BDL	8.1	1.56 ×	10^{-3}	88.5	BDL	0.03	0.50	20.7
18	3.0	3.3	0.0	2.4	BDL	7.5	4.95 ×	10^{-3}	79.5	BDL	0.02	0.57	22.2
19	3.0	3.6	0.3	2.1	RDL	6.9	3.15 ×	10^{-3}	72.9 100 F	RDF	0.02	0.68	26.1
∠U 21	1.5	1.2	0.0	0.6	0.0	0.3	1.8×10^{-3}		109.5	DUL	0.02	0.24	10.2
∠1 22	2.4 0.2	1.8	0.3	1.5	00L 27	0.3 12 5	3.48 ×	3.48×10^{-3}			0.03	0.53	19.8 41.4
22	9.0 5 1	7.7 5.7	5.9 1.9	4.0 3.0	2.7 0.6	10.5	0.)1)1	67.8	BDL	0.02	0.01	41.4 37 0
23	5.1	93	1.0	6.0	BDI	99	0.01		53.7	BDL	0.02	1 17	44 7
25	8.1	9.6	0.6	5.4	BDL	12.9	0.03		42.3	BDL	0.02	1.62	88.5
26	5.4	8.1	0.3	4.5	0.3	15.6	0.0	02	69.9	BDL	0.04	0.71	103.8

Table A1. Cont.

	Fraction #4 (F4: Oxidizable)											
Sample ID	Cr	Со	Cu	Ni	Cd	Pb	К *	Se	As	Al *	Fe *	Zn
1	0.90	0.00	0.36	0.12	0.03	0.81	0.03%	0.19	BDL	$3.84 imes 10^{-3}$	0.01	0.00
2	0.60	0.03	0.57	0.18	0.03	0.63	0.04%	5.64	BDL	0.01	0.01	3.00
3	1.80	0.66	3.18	0.87	BDL	0.75	0.08%	BDL	BDL	0.02	0.16	0.18
4	1.44	1.47	1.86	1.32	BDL	0.90	0.12%	BDL	BDL	0.04	0.11	3.12
5	3.30	1.53	3.96	1.38	BDL	0.03	0.04%	BDL	BDL	0.08	0.39	12.63
6	2.58	1.38	8.97	1.86	BDL	0.12	0.02%	BDL	BDL	0.06	0.37	14.04
7	0.48	1.50	5.94	1.05	BDL	0.15	0.08%	BDL	BDL	0.08	0.39	12.03
8	0.51	0.66	0.33	0.30	0.00	0.57	0.04%	2.46	BDL	0.02	0.01	1.89
9	0.75	0.78	0.39	0.36	0.00	0.72	0.05%	2.13	BDL	0.02	0.01	2.28
10	2.91	4.38	129.51	3.06	BDL	BDL	0.19%	BDL	BDL	0.14	0.54	137.31
11	3.18	4.02	5.64	2.97	BDL	BDL	0.10%	BDL	BDL	0.19	0.65	17.70
12	0.54	0.18	0.18	0.18	0.00	0.54	0.03%	2.52	BDL	0.01	0.01	0.60
13	0.72	0.27	0.30	0.21	0.00	0.78	0.06%	2.49	BDL	0.01	0.01	1.08
14	1.98	0.21	2.04	0.51	BDL	0.75	0.08%	BDL	BDL	0.03	0.10	0.72
15	1.38	0.27	1.23	0.39	BDL	0.69	0.07%	0.06	BDL	0.02	0.05	0.75
16	2.34	0.57	2.46	0.66	BDL	0.63	0.11%	BDL	BDL	0.04	0.13	1.14
17	1.05	0.45	0.84	0.30	BDL	0.81	0.07%	0.60	BDL	0.02	0.04	1.23
18	1.80	1.89	3.45	1.74	BDL	0.93	0.18%	BDL	BDL	0.07	0.20	5.19
19	1.23	0.87	1.86	0.78	BDL	0.96	0.10%	BDL	BDL	0.04	0.10	2.79
20	1.23	0.30	1.59	0.33	BDL	0.75	0.06%	BDL	BDL	0.02	0.08	0.96
21	1.26	0.51	2.10	0.72	BDL	0.60	0.10%	BDL	BDL	0.04	0.15	1.71
22	4.05	1.77	5.58	1.38	BDL	0.69	0.21%	BDL	BDL	0.10	0.45	6.51
23	4.29	4.05	9.30	3.36	BDL	0.03	0.05%	BDL	BDL	0.19	0.73	17.94
24	4.23	1.35	6.33	1.92	BDL	0.06	0.23%	BDL	BDL	0.12	0.50	8.64
25	2.85	3.45	6.33	2.58	BDL	BDL	0.19%	BDL	BDL	0.16	0.57	24.66
26	7.23	3.15	13.95	2.40	BDL	BDL	0.25%	BDL	BDL	0.28	ND	23.85

Table A1. Cont.

Appendix B

Spatial heat maps for the bioavailable PTEs. Spatial distribution maps of the bioavailable PTEs.







Figure A2. Acid-soluble fractions heat maps.



Figure A3. Reducible fractions heat maps.



Figure A4. Oxidizable fractions heat maps.

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Declaration of Authorship

Chapter 2

<u>Published as</u>: Fajemila OT, Sariaslan N, Langer MR (2020) Spatial distribution of benthic foraminifera in the Lagos Lagoon (Nigeria): Tracing the impact of environmental perturbations. PLoS ONE 15(12): e0243481. https://doi.org/10.1371/journal. pone.0243481

O.T.F. and M.R.L. designed the study. O.T.F., N.S. and M.R.L. wrote the manuscript. M.R.L. reviewed and edited the final version.

Chapter 3

<u>Published as</u>: Sariaslan, N. and Langer, M. R.: Atypical, high-diversity assemblages of foraminifera in a mangrove estuary in northern Brazil. Biogeosciences 2021; 18, 4073–4090, https://doi.org/10.5194/bg-18-4073-2021, 2021.

N.S. and M.R.L. designed the study. N.S. performed the laboratory analyses, took the SEM pictures, prepared the graphics. N.S. and M.R.L. wrote the manuscript.

Chapter 4

<u>Published as</u>: Fajemila OT, Martínez-Colón M, Sariaslan N, Council IS, Kolawole TO, Langer MR. Contamination Levels of Potentially Toxic Elements and Foraminiferal Distribution Patterns in Lagos Lagoon: A Correlation Analysis. Water 2022; 14(1):37. https://doi.org/10.3390/w14010037

O.T.F., M.R.L. and M.M.-C designed the study including the methodology and performed the investigation. M.M.-C. and I.S.C. acquired the resources. O.T.F., M.M.-C., N.S., I.S.C. and T.O.K. performed the formal analysis. O.T.F., M.M.-C., N.S., I.S.C., T.O.K. and M.R.L. O.T.F., M.M.-C., N.S. and M.R.L. wrote the original manuscript; O.T.F., M.M.-C., N.S. and M.R.L. wrote the original manuscript; O.T.F., M.M.-C., N.S. and M.R.L. prepared the writing through review and editing. O.T.F., M.M.-C. and M.R.L. prepared the visuals. O.T.F., M.M.-C. and M.R.L. acquired the funding and supervised the study.

Chapter 5

<u>Unpublished:</u> Steemans P, Sariaslan N, Cascales-Minana B, Langer M, Meienbrock W, Servais T. A complex Early Devonian palynoflora from the Waxweiler Lagerstätte (Klerf Formation, Rhenish Massif, western Germany): palaeobotanical implications. Palynology 2022

P.S., T.S., M.R.L. and N.S. designed the study including the methodology and performed the investigation. M.R.L., N.S. and T.S. acquired the resources. T.S. and N.S. performed the formal analysis. N.S., P.S., T.S., B.C.M. and M.R.L. wrote the original manuscript. N.S. and T.S. completed the writing through review and editing. N.S. prepared the visuals. P.S. and M.R.L. acquired the funding.