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DANIELE CLAUDINO MACIEL

AVALIAÇÃO DA CONTAMINAÇÃO DO SISTEMA ESTUARINO DO RIO CAPIBARIBE ATRAVÉS DA TRÍADE DA QUALIDADE DO SEDIMENTO

RECIFE, 2015

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Orientadora: Prof^a. Dr^a. Eliete Zanardi-Lamardo
Coorientador: Prof. Dr. José Roberto B. Souza

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Daniele Claudino Maciel

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RESUMO

O sistema Estuarino do Rio Capibaribe (SERC) está localizado na região metropolitana do Recife e trata-se de uma área muito importante do ponto de vista socioeconômico. Apesar disto, recebe intensa carga de efluentes domésticos e industriais, na sua maioria sem tratamento. Vários processos físicos e químicos regem o destino destes contaminantes, incluindo a tendência dos mesmos serem adsorvidos ao material particulado. O regime de circulação do SERC favorece a retenção deste material e, por consequência, os contaminantes acabam se depositando nos sedimentos. O objetivo deste trabalho foi investigar, pela primeira vez, a saúde ambiental do SERC, considerando aspectos químicos e biológicos. Primeiramente, investigou-se a contaminação de sedimentos por Hidrocarbonetos (HCs) e Organoestânicos (OTs), em 9 estações distribuídas dentro do estuário. Também foram analisados HCs em mais 5 pontos da plataforma adjacente ao SERC, com o intuito de se investigar o potencial de transferência de contaminantes do estuário para a área costeira. Os compostos foram determinados através de cromatografia em fase gasosa associada a um detector de ionização de chama (para hidrocarbonetos alifáticos - HAs) e a um detector de espectrometria de massas (para hidrocarbonetos policíclicos aromáticos (HPAs) e OTs). As concentrações dos alifáticos totais no estuário variaram entre 7,5 µg g^{-1} e 190,3 µg g^{-1}. A Mistura Complexa Não Resolvida esteve presente em grande parte das amostras indicando a contaminação dos sedimentos do estuário por óleo. A concentração de HPAs variou desde abaixo do limite de detecção a 497,6 ng g^{-1}, com predominância de fontes pirolíticas, mas, em alguns pontos do estuário, há indicações de contribuição petrogênica, sugerindo uma mistura de fontes. Observou-se uma diminuição na concentração dos HCs em direção a foz do estuário. As concentrações de OTs variaram entre 13,97 e 541,69 ng Sn g^{-1}, e os resultados indicam aporte recente destes contaminantes no ambiente, mesmo após obanimento de seu uso como agente anti-incrustante. A partir da constatação da contaminação dos sedimentos, cinco dos locais mais contaminados e próximos a potenciais fontes de contaminação foram novamente amostrados. O objetivo foi avaliar a qualidade dos sedimentos do estuário através da abordagem clássica “Tríade da Qualidade de Sedimento” (TQS) e o uso de análises multivariadas. Foram investigadas a contaminação por HAs e HPAs, compostos organoclorados (OCs) e metais pesados. Testes ecotoxicológicos e análise da estrutura da comunidade bentônica também foram realizados. Para determinação dos compostos orgânicos foi usada a mesma metodologia descrita anteriormente e, para determinação dos metais, a técnica de ICP-MS. Todas as estações apresentaram contaminação por HCs, OCs e metais pesados, sendo as maiores concentrações na porção superior do estuário e na área portuária. Em alguns casos, alguns compostos excederam os limites de segurança estabelecidos por agências reguladoras internacionais, podendo causar efeitos nocivos à biota. As estações localizadas em frente ao Porto do Recife e na parte mais interna do estuário apresentaram toxicidade para o copépodo *Tisbe biminiensis* e, ainda, na porção mais interna do estuário foram registrados os menores valores de abundância e diversidade de organismos. Os resultados das análises integradas indicaram a presença de um gradiente de diminuição da degradação, do interior do estuário à sua desembocadura. A TQS mostrou-se eficaz para avaliar a qualidade e saúde ambiental da região e pode ser usada em programas de monitoramento de outras regiões estuarinas.

Palavras-Chave: Estuário tropical. Contaminantes. Toxicidade. Estrutura da comunidade bentônica. Degradação.

ABSTRACT

The Capibaribe Estuarine System (CES) is located in the metropolitan region of Recife and, besides its important socio-economic role, it receives high load of domestic and industrial effluents, mostly untreated. Several physical and chemical processes govern the fate of these contaminants, but the hydrophobics tend to be adsorbed to the particulate material. The CES hydrodynamics favors the retention and deposition of this material and, consequently, the contaminants as well. The objective of this study was to investigate, for the first time, the environmental health of CES, considering chemical and biological aspects. Firstly, the sediment contamination by Hydrocarbons (HCs) and Organotins (OTs) was investigated in 9 stations distributed along the estuary. HCs were also analyzed in five stations on the adjacent shelf, in order to investigate the potential transfer of contaminants from estuary to the coastal area. The compounds were analyzed by gas chromatography coupled to a flame ionization detector (aliphatic hydrocarbons - HAs) and a mass spectrometric detector (polycyclic aromatic hydrocarbons - PAHs, and OTs). Concentrations of total aliphatic in the estuary ranged from 7.5 µg g^{-1} to 190.3 µg g^{-1}. The Unresolved Complex Mixture was registered in most samples indicating the contamination of sediments by oil. PAHs concentrations ranged from <limit of detection to 497.6 ng g^{-1}. Pyrolytic compounds predominated in most sites but, in some portions of the estuary, the dominance of petrogenic suggests a mixture of sources. There was a decrease in the HCs concentration from upper to lower estuary. OTs concentrations ranged between 13.97 and 541.69 ng Sn g^{-1}, and the results indicated recent input of this contaminant in the environment, even after its banishment as antifouling agent. Based on the sediments contamination and proximity of potential sources, five stations were again sampled. The objective was to evaluate the quality of the sediments through the classical approach "Sediment Quality Triad" (SQT) and multivariate analyzes. The contamination by PAHs, HAs, organochlorine (OC) and heavy metals were investigated. The OCs were analyzed using the same methodology described above, and for metals, the ICP-MS technique was used. Ecotoxicological bioassays and analysis of benthic community structure were also performed. All stations are contaminated by HCs, OC and heavy metals, and the highest concentrations were recorded in the upper portion of the estuary and in the port area. Some compounds exceeded the safe limits established by international regulatory agencies in a few samples, and they may be harmful to biota. The stations located in front of the Port of Recife and in the inner part of the estuary showed toxicity to the copepod *Tisbe biminiensis*. In addition, the inner portion had the lowest abundance and diversity of organisms. Integrated analyses indicated a decreasing gradient of degradation, from most inner part of the estuary to river mouth. The SQT was effective to assess the quality and environmental health and should be used in monitoring programs of other estuarine areas.

Keywords: Tropical estuary. Contaminants. Toxicity. Benthic community structure. Degradation.

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SUMÁRIO

1. INTRODUÇÃO GERAL

As regiões costeiras possuem características singulares, uma vez que os terrenos próximos ao mar conferem atributos peculiares do ponto de vista econômico, além de apresentarem alta riqueza de espécies e relevância ecológica, qualificando-as como fontes consideráveis de recursos. Dentre estes, pode-se destacar a pesca e aquicultura, a navegação e atracação de embarcações, a instalação de marinas e desenvolvimento do turismo (MORAES, 1999). Desta forma, as regiões costeiras favorecem o desenvolvimento urbano, e a instalação de indústrias e portos. Estes fatores levaram a um crescente uso de vias marítimas para o desenvolvimento do comércio local e internacional.

O Brasil possui uma extensa zona costeira que, de acordo com o Ministério do Meio Ambiente, está inserida em uma faixa que se estende por mais de 8.500 km voltados para o Oceano Atlântico (MMA, 2015). Dentre os diversos ecossistemas presentes na costa brasileira, encontram-se os estuários e manguezais, que estão distribuídos desde o Norte do país, no estado do Amapá, até a região Sul, no estado de Santa Catarina (SCHAEFFER-NOVELLI et al., 1990).

Os estuários são comumente descritos como corpos d’água semi-fechados situados na interface continente e oceano, e caracterizados por serem ecossistemas dinâmicos de alta produtividade e diversidade (BIANCHI, 2006). As áreas estuarinas, normalmente, são ocupadas por portos e indústrias, resultando em aumento populacional e, consequentemente, em uma maior descarga de efluentes industriais e domésticos nesses ambientes (BLABER, 1997). Cerca de 60% das cidades brasileiras se desenvolveram em torno dos estuários, e devido a vulnerabilidade e importância desses ecossistemas, as regiões estuarinas passaram a ser mais intensamente pesquisadas (ZANARDI-LAMARDO et al., 2000; MIRANDA, 2002). A ação antrópica nesses ambientes, especialmente através da introdução de compostos e/ou de energia, podem resultar em efeitos negativos à biota, à saúde e às atividades humanas como navegação, pesca, aquicultura e recreação (VERONEZ JUNIOR et al., 2009).

A maioria dos contaminantes lançados nos corpos hídricos são hidrofóbicos, e devido a sua baixa solubilidade e seu alto coeficiente de partição octanol-água (Kow), são rapidamente adsorvidos às partículas de sedimento, especialmente as finas, como silte e argila e/ou absorvidos pelos organismos (WANG ET AL, 2001; YANG ET AL., 2012). Uma vez depositados nos sedimentos os contaminantes podem sofrer algumas transformações, alterando sua disponibilidade à biota (SARAIVA et al., 2009). Neste sentido, a avaliação da qualidade dos sedimentos torna-se essencial para a compreensão dos processos que ordenam a disponibilidade e o destino destes compostos no ambiente (ALMEIDA e ROCHA, 2006).

1.1 Contaminantes

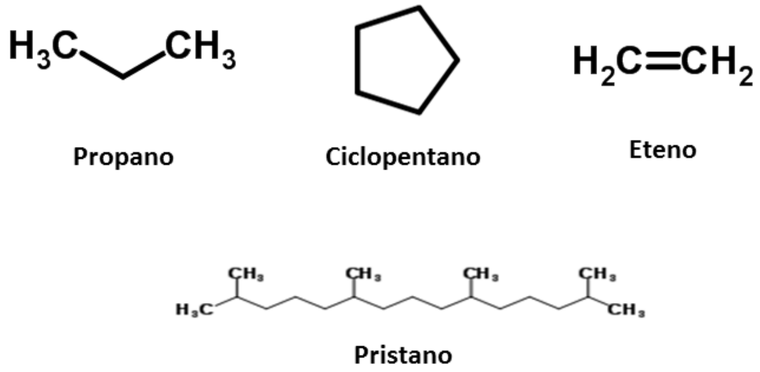
Dentre os diversos compostos introduzidos costantemente nos ambientes estuarinos, sejam através do aporte fluvial, da deposição atmosférica ou até mesmo de forma direta, destacam-se os derivados do petróleo (formados predominantemente por hidrocarbonetos - HCs), compostos orgânicos de estanho (OTs), compostos organoclorados (OCs) e elementos inorgânicos.

1.1.1 Petróleo e seus derivados (Hidrocarbonetos)

Os derivados do petróleo são introduzidos no ambiente através do óleo dos motores das embarcações e descargas operacionais de navios, derramamentos acidentais, efluentes domésticos e industriais, águas pluviais, processos de combustão e emissões veiculares e mineração (ZANARDI et al., 1999; ZHU and WANG, 2003). Cerca de 95% do petróleo é constituído por hidrocarbonetos, que são compostos orgânicos formados por atómos de carbono e hidrogênio e, de acordo com a sua estrutura química, podem ser classificados como hidrocarbonetos alifáticos ou aromáticos (BÍCEGO et al., 2008).

Os hidrocarbonetos alifáticos (HAs) (Figura 1) são constituídos por compostos saturados, que são os alcanos (cadeia normal) e isoprenóides (cadeia ramificada); os cicloalcanos ou naftenos, que apresentam anéis formados por ligações simples; e por compostos insaturados, com uma ligação dupla (alcenos) ou tripla (alcinos) entre os carbonos (UNEP, 1992). Os HAs podem ser introduzidos no ambiente através de diversas fontes, sendo antrópicas, especialmente relacionadas a introdução intencional ou acidental de petróleo e seus derivados, ou de fontes naturais, que podem ser marinhas, oriundas do plâncton e/ou terrestres, derivadas das plantas superiores (VOLKMAN et al., 1992). Contudo, o aporte a partir das ações antrópicas é bastante significativo. Os HAs constituem 80% da composição do petróleo (UNEP, 1992) e, por esta razão, são utilizados como marcadores geoquímicos da contaminação por óleo (ZANARDI et al., 1999).

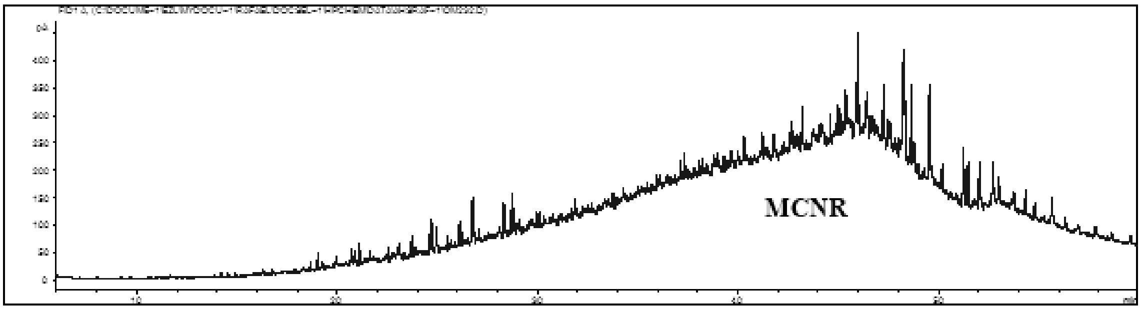
Figura 1. Exemplos de hidrocarbonetos alifáticos.



Fonte: http://www.chemspider.com

Alguns alifáticos, como os terpanos tricíclicos e tetracíclicos, hopanos e esteranos, também são usados como biomarcadores da contaminação por óleo (BÍCEGO et al., 2008). Além destes, a presença da Mistura Complexa Não-Resolvida (MCNR) (Figura 2) também é um indicativo muito forte da contaminação dos sedimentos por óleo (VOLKMAN, 1992). A MCNR é considerada uma mistura de vários isômeros homólogos e estruturalmente complexos de hidrocarbonetos ramificados e cíclicos que não podem ser resolvidos pelas colunas capilares cromatográficas (BOULOUBASSI E SALIOT, 1993). De maneira geral, estes isômeros são resistentes à biodegradação e, consequentemente, se acumulam no sedimento (READMAN ET AL., 2002). A análise da distribuição dos n-alcanos também é uma importante ferramenta para avaliar a contaminação por petróleo, uma vez que em ambientes contaminados ocorre uma distribuição regular entre as cadeias com números ímpares e pares de carbono (VOLKMAN, 1992). Em contrapartida, n-alcanos derivados de fontes biogênicas apresentam preferencialmente cadeias com número ímpar de carbono (BÍCEGO et al., 2008).

Figura 2**.** Cromatograma com elevação da linha de base característica da Mistura Complexa Não Resolvida (MCNR).



Os hidrocarbonetos policíclicos aromáticos (HPAs) são compostos formados por dois ou mais anéis benzênicos condensados (Figura 3), e perfazem aproximadamente 15% dos HCs do petróleo, (UNEP, 2002; MEIRE et al., 2007). Apesar de uma proporção menor relativa aos alifáticos, estes compostos têm recebido especial atenção devido a seu alto potencial carcinogênico e mutagênico (ATSDR, 1995). Existem centenas de HPAs no ambiente mas, de acordo com a Agência de Proteção Ambiental Americana (EPA – US), dezesseis são considerados poluentes prioritários, baseados principalmente no seu potencial tóxico e devem ser monitorados nos ambientes sujeitos à contaminação por petróleo. São eles: naftaleno, acenaftileno, acenafteno, fluoreno, fenantreno, antraceno, fluoranteno, pireno, benzo(a)antraceno, criseno, benzo[b]fluoranteno, benzo[k]fluoranteno, benzo[a]pireno, indeno[1,2,3-c,d]pireno, dibenzo[a,h]antraceno, benzo[g,h,i]perileno (Figura 3).

Além da introdução direta do petróleo e seu derivados, os HPAs encontrados no ambiente também podem ser derivados de fontes pirolíticas, os quais são formados sob condições de elevada temperatura, e resultam da combustão incompleta da matéria orgânica e/ou combustíveis fósseis (BOEHM, 2005, MEIRE et al., 2007). Há ainda aqueles que são sintetizados por organismos como plantas, bactérias e fungos, em misturas relativamente simples e em concentrações menores quando comparadas com aquelas geradas pela introdução antrópica (BOEHM, 2005; BÍCEGO et al., 2008).

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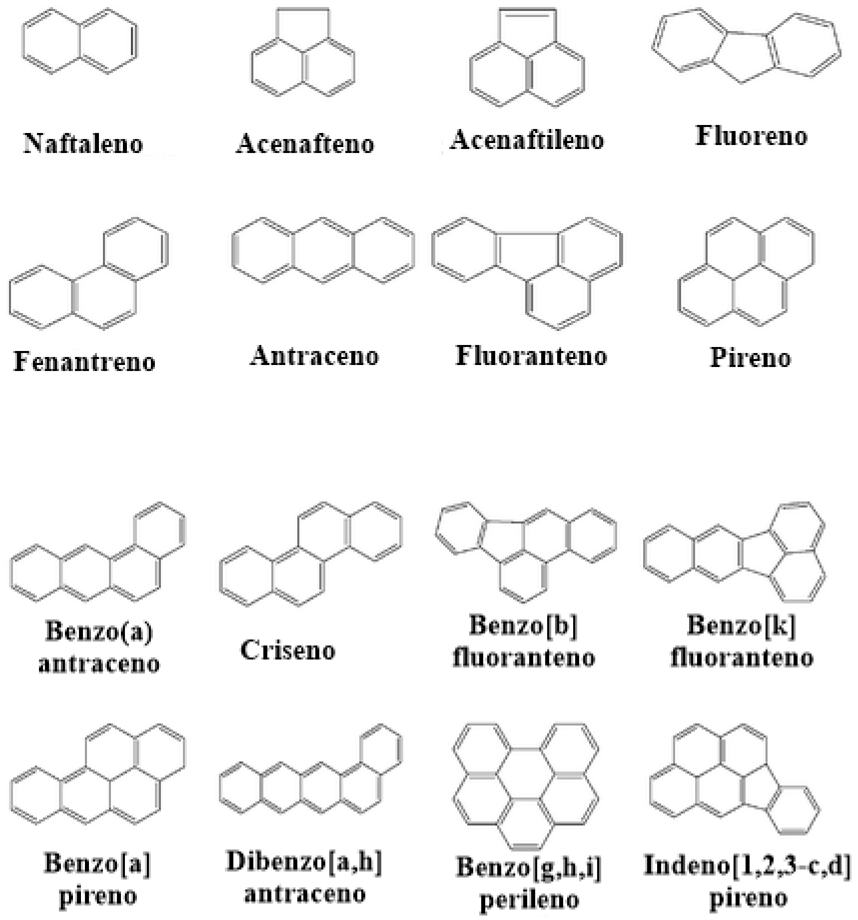
Figura 3. Estrutura molecular dos dezesseis HPAs considerados como poluentes prioritários em estudos ambientais, de acordo com a Agência de Proteção Ambiental Americana (US-EPA).



Adaptado de: BRUZZONITI et al., (2010)

1.1.2. Compostos orgânicos de estanho

Os compostos orgânicos de estanho (OTs), têm sido amplamente utilizados no desenvolvimento de inseticidas, fungicidas, bactericidas, preservativos de madeira, além de suas aplicações como estabilizadores de cloreto de polivinila (PVC), catalisadores de poliuretano e elastômeros de silicone (HOCK, 2001; GODOI et al., 2003). O uso destes como biocidas em tintas anti-incrustantes tem recebido uma atenção especial e se destacado no cenário ambiental, pois estão entre os poluentes mais abundantes nos ambientes marinhos e estuarinos, além dos efeitos nocivos que alguns OTs, especialmente o Tributilestanho (TBT), exercem sobre a biota.

O TBT foi usado extensivamente em tintas antivegetativas a partir da segunda metade do século XX para proteger os cascos de embarcações e outras estruturas submersas (MARTÍNEZ-LLADÓ et al., 2007). Em 2001, o seu uso para este fim foi proibido pela Organização Marítima Internacional, devido a sua elevada toxicidade aos organismos aquáticos (MINKYU CHOI HYO-BANG MOON & GUI-YOUNG KIM, 2009). Entretanto, no Brasil, apenas em 2010 foi gerada a norma jurídica DLG - 797 de 20/12/2010, aprovando o texto da Convenção Internacional sobre Sistemas Anti-incrustantes Danosos em Navios, adotada pela Organização Marítima Internacional, em Londres, em 05 de outubro de 2001.

Em diversas partes do mundo, vários estudos têm mostrado os efeitos do TBT sobre os organismos (THAIN, 1986; RUIZ et al., 1995; MCALLISTER; KIME, 2003; WU et al., 2010). Dentre estes, os mais significativos estão relacionados à sua ação como desregulador endócrino, afetando a reprodução de organismos marinhos, especialmente moluscos gastrópodes. A contaminação por TBT nestes animais pode resultar em um fenômeno denominado imposex, caracterizado pelo surgimento de órgãos sexuais masculinos não funcionais, sobretudo pênis e vaso deferente, em fêmeas (CASTRO; FILLMANN, 2012). O imposex vem sendo utilizado como um excelente marcador da contaminação por OTs no Brasil e no mundo (CAETANO; ABSALÃO, 2002; CAMILLO et al., 2004; CASTRO et al., 2007; PAVONI et al., 2007; BIGATTI et al., 2009). Além do imposex, outros efeitos do TBT têm sido reportados, como alterações morfológicas em conchas de bivales (ALZIEU, 2000), influência na reprodução e desenvolvimento embrionário de moluscos, crustáceos e peixes (RUIZ et al. 1995; McALLISTER; KIME, 2003; AONO; TAKEUCHI, 2008;) e redução significativa na espermatogênese em mamíferos (CHEN et al., 2008).

Apesar da proibição do uso do TBT na formulação de tintas anti-incrustantes, estudos têm apontado a contaminação recente de sedimentos e organismos em diferentes locais da costa brasileira (DORNELES et al., 2008, FELIZZOLA et al., 2008; SANTOS et al., 2010; CASTRO et al., 2012) (Figura 4). Estes dados refletem a fragilidade das autoridades locais na fiscalização do uso indevido deste composto, assim como ressalta a necessidade de estudos que possibilitem o monitoramento contínuo desses ambientes para avaliar o grau de contaminação dos mesmos por OTs.

Figura 4. Imposex e contaminação por organoestânicos de sedimentos e mamíferos marinhos em diferentes locais da costa brasileira. (Harbor = Porto; Bay = Baía; River = Rio; estuary = estuário; coast = costa).

Adaptado de: Castro et al., (2012).

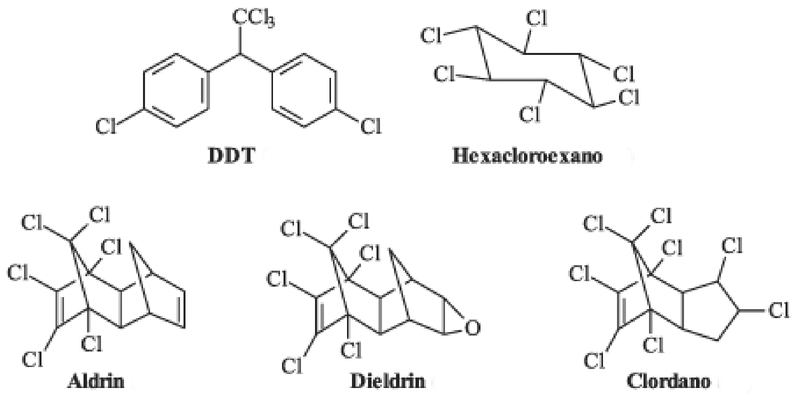
1.1.3. *Organoclorados*

Os compostos organoclorados (OCs) apresentam estruturas de elevado peso molecular e são utilizados extensivamente em práticas agrícolas, no controle de vetores e atividades industrais (MIRANDA FILHO et al., 2008). Os OCs compreendem uma importante classe de poluentes orgânicos persistentes (POPs) e, por esta razão, uma vez no ambiente, a degradação destes compostos ocorre de forma muito lenta (FLORES et al., 2004). Devido a suas características lipofílicas, eles podem se bioacumular e biomagnificar na cadeia alimentar, além de possuírem grande estabilidade físico-química e elevada toxicidade (ALVES; FILHO, 2010).

Dentre os OCs, um grupo de contaminantes que tem recebido bastante atenção devido aos danos causados a diversos organismos são os pesticidas organoclorados

(POCs). Estes compostos são introduzidos no ambiente especialmente através da ação antrópica, uma vez que os POCs são sintetizados desde o século XIX. Além disso, algumas bactérias, algas marinhas e fungos podem produzir pesticidas organoclorados (MIRANDA FILHO et al., 2008). Vários POCs foram produzidos industrialmente (Figura 5), mas dentre estes destacam-se o diclorodifeniltricloroetano (DDT) e seus derivados, o hexaclorobenzeno (HCB), os hexaclorocicloexanos (α-HCH, β-HCH, γ-HCH e δ-HCH), os ciclodienos (aldrin, dieldrin, endrin, clordano, nonacloro, endosulfan, heptacloro e heptacloro-epóxido) e os hidrocarbonetos clorados (mirex, toxafeno e clorodecona) (D'AMATO et al., 2002). Estes pesticidas foram largamente utilizados, principalmente depois da 2ª Guerra Mundial, quando se comprovou a eficácia do DDT contra o mosquito vetor da malária e de outras doenças. Com o uso prolongado, alguns impactos negativos sobre a biota começaram a ser observados, principalmente em algumas espécies de pássaros nos Estados Unidos, cujos sintomas foram relatados, de forma poética, no livro Primavera Silenciosa, de Rachel Carson (1962). Este relato despertou um grande interesse em pesquisadores e agências ambientais de forma que o DDT foi um dos compostos mais estudados no século passado. Devido à comprovação da sua alta toxicidade, seu uso foi banido em diversos países a partir da década de 1970 (MIRANDA FILHO et al., 2008). No Brasil, o uso de pesticidas organoclorados na agricultura foi proibido em 1985, mas a aplicação ilegal continuou por muitos anos (CALDAS et al., 1999). Dentres os principais efeitos que estes contaminantes podem ocasionar sobre os organismos, destacam-se os relacionados a desregulação endócrina, além de efeitos nocivos sobre o sistema reprodutivo e neurológico (ALVES; FILHO, 2010).

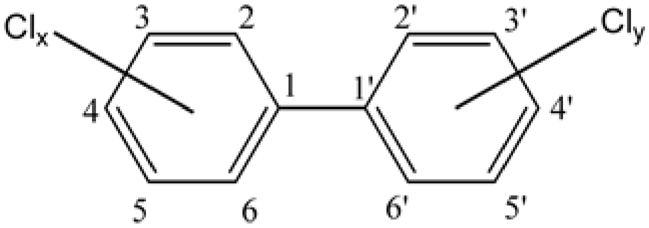
Figura 5. Exemplos de pesticidas sintéticos.



Fonte: Santos et al., (2007).

Outro grupo importante de compostos organoclorados são as bifenilas policloradas (PCBs) (Figura 6). Devido as suas propriedades físico-químicas, tais como, alta constante dielétrica e elevada estabilidade térmica, estes compostos foram extensivamente usados em diversos setores industriais, em capacitores e transformadores elétricos, em fluídos hidráulicos, bombas de vácuo, entre outros (PENTEADO; VAZ, 2001). Alguns desastres ambientais com vazamento de ascarel, óleo contendo uma mistura de hidrocarbonetos de petróleo, Alocloro 124, e PCBs, ocorreram no Brasil (Paraná, Santa Catarina e Rio de Janeiro), resultando na introdução elevada destes poluentes no meio ambiente e a intoxicação de moradores das regiões atingidas (PENTEADO; VAZ, 2001). Por suas características, como lipofilicidade e a persistência, estes compostos apresentam alto potencial de bioacumulação, podendo ser encontrados nos níveis mais altos da cadeia alimentar (DA SILVA; FERREIRA, 2003). Desta forma, apesar da proibição do uso dos mesmos a partir do início da década de 1980, os PCBs ainda são encontrados em diversas matrizes tais como água, solo, sedimento e organismos (DE AZEVEDO, 2007; POLICARPO, 2008; FERREIRA & WERMELINGER, 2013). Os efeitos nocivos dos PCBs sobre a saúde humana têm sido associados principalmente a alterações dermatológicas em adultos e alterações neurocomportamentais e imunológicas em crianças, além de elevado potencial carcinogênico para os animais (ATSDR, 2000).

Figura 6. Estrutura molecular geral das Bifenilas Policloradas (PCBs).



Fonte: http://www. clu-in.org.com

1.1.4 Metais

A poluição por compostos inorgânicos também tem causado preocupação aos agentes governamentais/ambientais e tem sido foco de diversas pesquisas, especialmente em relação à contaminação dos ambientes aquáticos por metais. Estes compostos podem ser introduzidos aos corpos hídricos naturalmente, através de processos geoquímicos e do intemperismo. Entretanto, a contribuição antrópica tem se tornado cada vez mais expressiva, sendo um reflexo da ampla utilização destes recursos pela atividade industrial (NIENCHESKI et al., 2008). Dentre as principais atividades humanas que contribuem para introdução de metais no ambiente, pode-se destacar a mineração; a agricultura; a combustão de combustível fóssil; a indústria metalúrgica, eletrônica e química; pigmentos e tintas e disposição de resíduos (SILVA, 2010).

Vários destes metais são considerados como tóxicos para os organismos, tais como Hg, Cd, Cu, Pb, Mn, Tl, Cr, Ni, Se, Sb, Be, Co, Mo, Sn, W e V, dos quais, os dez primeiros são os de maior utilização industrial e, do ponto de vista toxicológico, os mais estudados (TAVARES e CARVALHO, 1992). Uma grande preocupação em relação à introdução de metais no ambiente deve-se ao fato de que os mesmos podem se bioacumular e causar efeitos tóxicos para diferentes espécies da biota e para o homem (Tabela 1) (VIRGA et al., 2007). A exposição a estes compostos pode causar efeitos adversos, afetando principalmente o sistema neurológico (efeitos neurotóxicos), sistema reprodutivo (efeitos na reprodução e teratogênicos), sistema imunológico e hematológico, entre outros, além dos efeitos carcinogênicos (JÄRUP, 2003).

Tabela 1. Exemplos de metais usados em diferentes atividades antrópicas, e os principais efeitos sobre a saúde humana.

Metais	Aplicações industriais	Principais efeitos
Alumínio	Produção de artefatos de alumínio, soldagem de medicamentos, tratamento da água	Anemia, intoxicação crônica
Arsênio	Metalurgia, manufatura de vidros e fundição	Câncer
Cádmio	Soldas, tabaco, baterias e pilhas	Câncer de pulmões e próstata, lesão renal, alterações no sistema ósseo
Chumbo	Fabricação e reciclagem de baterias, indústria de tintas, pintura em cerâmica, soldagem	Transtornos comportamentais, cefaléia, , perda de apetite e dores abdominais, complicações gastrointestinais
Cobalto	Fabricação de ferramentas de corte e furadoras	Fibrose pulmonar
Cromo	Indústrias de corantes, de esmaltes, de tintas, de ligas de aço e níquel, cromagem de metais	Asma e câncer
Mercúrio	Moldes industriais, indústrias de cloro-solda, garimpo de ouro, lâmpadas fluorescentes	Alterações no sistema nervoso central
Níquel	Baterias, aramados, fundição e niquelagem de metais, refinarias	Câncer de pulmão e seios paranasais

Fonte: http://www.ambientebrasil.com.br

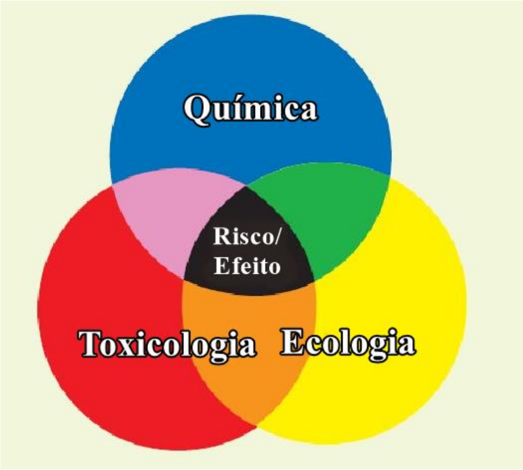
1.2 Avaliação da qualidade dos sedimentos

A maior parte dos contaminantes que chegam aos estuários é rapidamente adsorvida ao material particulado e depositada no sedimento, tornando este compartimento uma fonte secundária de contaminantes para a coluna d'água e para a biota (CHAPMAN, 1989; KIM et al., 2011). Como consequência, esta contaminação pode afetar os organismos vivos causando efeitos agudos, como mortalidade, e crônicos, tais como deficiência reprodutiva, alterações fisiológicas e comportamentais e defeitos congênitos (CHAPMAN, 1990; ZABETOGLOU et al., 2002). Estes efeitos podem, a longo prazo, alterar a estrutura da comunidade bentônica presente nos sedimentos, uma vez que proporcionam habitat, alimentação e local de reprodução para muitos organismos (CHAPMAN, 1989). Ao serem depositados, alguns processos físicos, químicos e/ou biológicos podem levar à transformação dos contaminantes em outros compostos que não são, necessariamente, menos prejudiciais à biota.

Desta forma, a avaliação da qualidade dos sedimentos torna-se imprescindível para conhecer e entender os processos que influenciam a disponibilidade e o destino dos diversos contaminantes nos corpos hídricos. Várias metodologias têm sido desenvolvidas para investigar a qualidade relativa dos sedimentos e algumas propõem estudos de forma conjunta, isto é, através de abordagens integradas, como por exemplo, uma avaliação simultânea da contaminação química, da estrutura da comunidade bentônica e de parâmetros toxicológicos (CHAPMAN et al., 1997).

A avaliação da qualidade dos sedimentos através da ‘Tríade de Qualidade de Sedimento’ tem sido apontada como a melhor abordagem para a investigação da saúde ambiental de um determinado local (CHAPMAN; HOLLERT, 2006). Isto se deve ao fato de que a mesma consiste na avaliação de três vértices (Figura 7): análises químicas para investigar a concentração dos contaminantes nos sedimentos; bioensaios para determinar a toxicidade dos mesmos; e análise da estrutura da comunidade bentônica para avaliar os efeitos da contaminação a nível ecológico (CHAPMAN; HOLLERT, op cit.).

Figura 7. Representação dos três vértices utilizados na abordagem da Tríade da Qualidade do Sedimento.



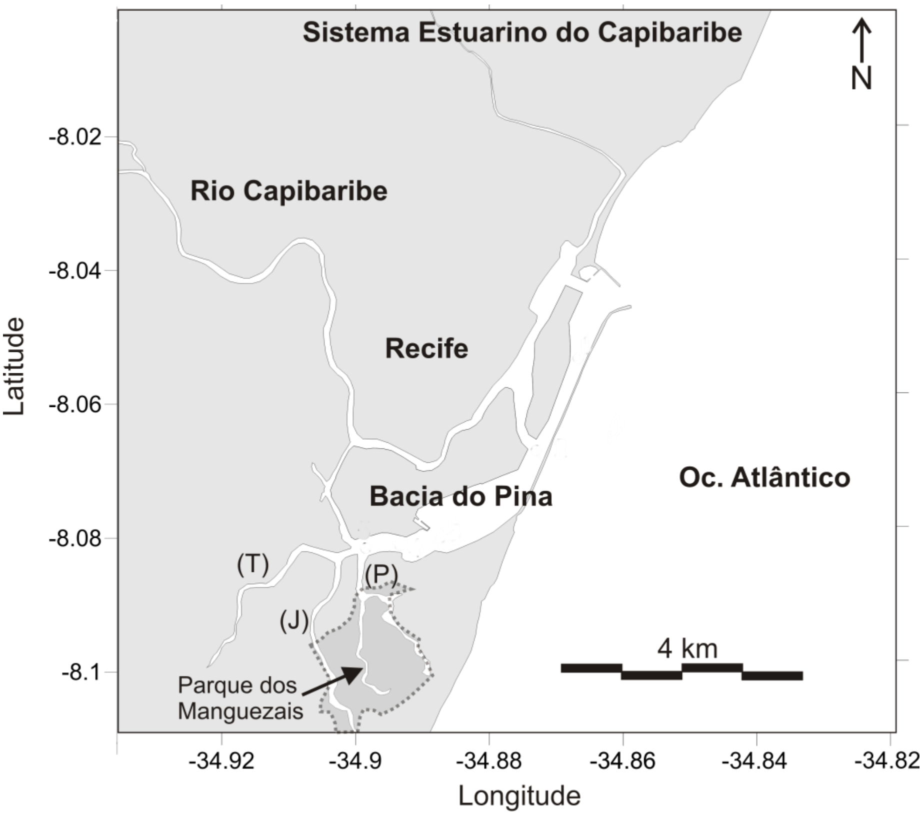
Adaptado de MUHAMMAD et al., (2014).

As análises químicas são imprescindíveis caracterizar as concentrações dos contaminantes presentes no sedimento. Os testes de toxicidade são empregados para avaliar se os contaminantes presentes no ambiente estão biodisponíveis e se causam efeitos na sobrevivência, desenvolvimento e crescimento, reprodução, comportamento e outros aspectos dos organismos. Finalmente, a integridade das populações e estrutura da comunidade bentônica refletem as alterações a médio e a longo prazo nos sedimentos. Os dados ecológicos fornecem evidência direta dos efeitos de contaminantes sobre comunidades naturais, que tipicamente decorrem de períodos mais prolongados de exposição aos contaminantes biodisponíveis no sedimento (CHAPMAN, 1990).

1.3 Sistema Estuarino do Rio Capibaribe (SERC)

O litoral de Pernambuco pode ser classificado como uma grande planície sedimentar, em sua maior parte ao nível do mar, tendo alguns pontos abaixo desse nível. (SILVA et al., 2011). Ao longo desse litoral existem quinze estuários e, dentre estes, encontra-se o Sistema Estuarino do Rio Capibaribe (SERC). O SERC compreende o estuário do Rio Capibaribe, que representa o principal aporte de água doce com uma bacia de drenagem de 7.500 km^2, e a Bacia do Pina, que compreende um corpo de água aproximadamente paralelo ao canal do estuário, e recebe o aporte de vários afluentes, dos quais os rios mais importantes são Jordão, Pina e Tejipió (Figura 8) (SANTOS et al., 2009). Estes pequenos rios consistem basicamente da drenagem urbana da Região Metropolitana do Recife (RMR), fazendo do SERC o corpo receptor de efluentes industriais e domésticos não tratados. O canal do Rio Capibaribe e a Bacia do Pina são separados pelas ilhas Joana Bezerra e Santo Antônio. O canal do estuário é estreito e com profundidade da ordem de 5 m, enquanto que a Bacia do Pina é uma área rasa e larga, com dimensões aproximadas de 3 km de extensão e 500 m de largura (Feitosa et al., 1999). O estuário e Bacia do Pina confluem para o baixo estuário do Rio Capibaribe, onde ocorre a zona portuária, com largura variando entre 200 e 500 m, e profundidade entre 6 e 12 m. A maior profundidade nesta área advém da realização de obras de dragagem para viabilizar as operações portuárias. A circulação é determinada pelo regime de marés e pelo aporte de água doce, sendo este último modulado sazonalmente. As marés locais são do tipo semi-diurnas com altura variando entre 1,5 e 3,0 m nos períodos de quadratura e sizígia, respectivamente. O aporte de água doce é determinado pelo regime hidrológico anual, com chuvas concentradas entre março e agosto, e um período seco entre setembro e fevereiro (ARAÚJO; PIRES, 1998).

Figura 8. Sistema Estuarino do Capibaribe (SERC), Pernambuco, Brasil, e os seus rios fomardores: Rio Capibaribe, Rio Tejipió (T), Rio Jordão (J) e Rio Pina (P).



O SERC abriga o Porto do Recife, um dos mais importantes do país, uma vez que recebe navios para importação e exportação de cargas nacionais e estrangeiras, além de navios de cruzeiro (PORTO DO RECIFE, 2015). Em seu entorno também estão situadas algumas marinas e locais de atracação para mautenção e reparos de barcos de pesca e de recreação, resultando em um constante tráfego de embarcações. As áreas, antes ocupadas por extensos manguezais, hoje estão completamente urbanizadas, o que torna o complexo um verdadeiro reservatório para descarga de indústrias e esgotos domésticos (SANTOS et al., 2009). Apesar da intensa ação antrópica e degradação, ainda é uma região muito importante do ponto de vista econômico, ecológico e social, visto que é utilizada para navegação e atracação de embarcações, lazer (turismo), pesca de subsistência e/ou comercial, além de funcionar como berçário para diversas espécies marinhas.

1.4 Proposta da Tese

A região costeira de Pernambuco tem experimentado um crescimento relevante nas últimas décadas. O SERC, em particular, tem sofrido a influência das ações antrópicas de forma acentuada, especialmente por estar inserido em uma zona urbana densamente povoada, com uma população estimada de 1.608.488 habitantes na cidade do Recife (IBGE, 2014). Apesar da importância ecológica e socioeconômica deste ecossistema estuarino, poucos estudos foram realizados para avaliar a contaminação da área e os efeitos da mesma sobre o ambiente (SERICANO et al. 1995; FERNANDES et al. 1999; LIMA et al. 2002; MACEDO et al. 2007; FAVROD, 2012; CABRAL et al.; 2014; OLIVEIRA et al. 2014). Desta forma, a realização de estudos com enfoque na investigação da contaminação do SERC tornou-se essencial. Com intuito de melhor compreender esta dinâmica entre contaminantes e seus efeitos, este estudo traz, pela primeira vez, dados referentes à contaminação dos sedimentos por vários compostos orgânicos e metais, associados aos efeitos medidos em campo e em laboratório. Numa avaliação prévia, a contaminação por hidrocarbonetos alifáticos, hidrocarbonetos policíclicos aromáticos e compostos orgânicos de estanho foi investigada. Uma vez comprovado que os sedimentos estavam contaminados, um estudo mais abrangente, envolvendo diversas abordagens, foi conduzido. Para tal, novas coletas foram realizadas nos locais mais contaminados e próximos à potenciais fontes de contaminação. Uma investigação da qualidade dos sedimentos do SERC foi realizada, utilizando a abordagem da “Tríade da Qualidade do Sedimento”: uma avaliação integrada de parâmetros químicos, toxicológicos e ecológicos.

Os resultados obtidos durante esta pesquisa foram discutidos e geraram um artigo publicado, um manuscrito aceito para publicação e outros a serem submetidos. Adicionalmente alguns resumos foram apresentados em congressos nacionais e internacionais. A produção científica gerada durante o desenvolvimento desta tese pode ser vizualizada abaixo.

- **Maciel, D. C.,** Costa, B. V. M., Santos, L. P. S., Souza, J. R. B., Zanardi-Lamardo, E. Avaliação da toxicidade dos sedimentos do Sistema Estuarino do Rio Capibaribe (Pernambuco, Brasil) utilizando o copépodo bentônico *Tisbe biminiensis* Volkmann-Rocco (1973). Tropical Oceanography (ISSN: 1679-3013), 43(1): 26-37, 2015. D.O.I:10.5914/1679-3013.2015.0119 (Apêndice 1).
- **Maciel, D. C.,** Souza, J. R. B., Taniguchi, S., Bícego, M. C. e Zanardi-Lamardo, E. Sources and distribution of polycyclic aromatic hydrocarbons in a an urbanized tropical estuary and adjacent shelf, Northeast of Brazil. Aceito pela revista Marine Pollution Bulletin em 30 de Setembro de 2015. D.O.I: 10.1016/j.marpolbul.2015.09.051
- **Maciel, D. C.,** Souza, J. R. B., Taniguchi, S., Bícego, M. C., Schettini, C. A. F e Zanardi-Lamardo, E. Hydrocarbons in sediments in an Estuary-Shelf Transition Area: Sources and Spatial distribution. A ser submetido à Environmental Monitoring and Assessment.
- **Maciel, D. C.,** Castro, I. B., Souza, J. R. B., Yogui, G. T., Fillmann, G. Bícego, M. C., Schettini, C. A. F e Zanardi-Lamardo, E. Butyltin residues and imposex reduction in two tropical harbor areas after TBT ban. A ser submetido à Environmental Science and Pollution Research.
- **Maciel, D. C.,** Costa, B. V. M., Santos, L. P. S., Souza, J. R. B., Zanardi-Lamardo, E. Assessment of sediment quality in a tropical estuary using the Triad approach. A ser submetido à Science of the Total Environment.
- **Maciel, D. C.,** Souza, J. R. B., Taniguchi, S., Bicego, M. C., Schettini, C. A. F., Zanardi-lamardo, E., Miranda, D. A. 2014. Hidrocarbonetos em sedimentos do Sistema Estuarino do Rio Capibaribe e o potencial de transferência para plataforma adjacente. In: XIII Congresso Brasileiro de Oceanografia. 2014, Itajaí, p. 418-419.
- **Maciel, D. C.,** Castro, Í., Fillmann, G., Yogui, G. T., Zanardi-Lamardo, E. 2014. Contaminação por organoestânicos em sedimentos do Sistema Estuarino do Rio Capibaribe, Pernambuco. In: XIII Congresso Brasileiro de Ecotoxicologia. 2014. Guarapari, p. 1824-1825.
- **Maciel, D. C.,** Costa, B. V. M., Santos, L. P. S., Souza, J. R. B., Zanardi-Lamardo, E. 2014. Avaliação da toxicidade subletal dos sedimentos do Sistema Estuarino do Rio Capibaribe (Pernambuco) utilizando o copépodo bentônico

Tisbe biminiensis. In: XIII Congresso Brasileiro de Ecotoxicologia. 2014, Guarapari, p. 679-680.

- Zanardi-Lamardo, E., **Maciel, D. C.,** Lemos, R. T., Taniguchi, S., Bícego, M. C., Souza, J. R. B. 2014. Hidrocarbonetos Policíclicos Aromáticos em sedimentos do Sistema Estuarino do Rio Capibaribe, Pernambuco. In: XIII Congresso Brasileiro de Ecotoxicologia. 2014, Guarapari, p. 418-419.
- **Maciel, D. C.,** Zanardi-Lamardo, E. 2012. Organotin in Antifouling Paints in Brazil: A Review and a Proposal. In: Brazil-TAMU: Science and Education Internationalization, 2012, Ipojuca.

Desta forma, optou-se por apresentar os resultados na forma de manuscritos, com uma conclusão geral.

Manuscrito 1: Hidrocarbonetos em sedimentos numa área de transição estuário-plataforma: fontes e distribuição espacial.

Neste manuscrito são apresentados dados inéditos da contaminação dos sedimentos do SERC por hidrocarbonetos alifáticos, e as principais fontes destes compostos, se naturais ou antrópicas, assim como também é discutido o papel do sistema estuarino na retenção/exportação de contaminantes para a plataforma adjacente.

Manuscrito 2: Fontes e distribuição de hidrocarbonetos policíclicos aromáticos em um estuário tropical urbanizado e plataforma adjacente, Nordeste do Brasil.

Nesta seção da tese é realizada uma avaliação da distribuição espacial dos HPAs em sedimentos do SERC no gradiente estuário-plataforma. Também foi realizada uma análise para se estimar as principais fontes destes compostos, se petrogênicas e/ou pirolíticas. Devido à elevada toxicidade dos HPAs e conhecido potencial carcinogênico, uma avaliação de risco foi feita comparando os resultados obtidos com os limites de segurança estabelecidos por agências reguladoras internacionais.

Manuscrito 3: Butilestânicos e redução de imposex em duas áreas portuárias tropicais após o banimento do TBT.

Este manuscrito apresenta pela primeira vez resultados da avaliação da contaminação do SERC e do estuário de Suape por organoestânicos. Além da avaliação química, foi realizada uma avaliação biológica através da investigação de imposex em gastrópodes distribuídos em áreas adjacentes ao Porto do Recife e ao Porto de Suape. Estes dados fazem parte de um projeto de âmbito nacional (Impacto da utilização de tintas anti-incrustantes nos principais terminais portuários brasileiros - AIBRASIL - FINEP, No. 0404/10), que teve como objetivo investigar a contaminação de vários portos da costa brasileira por BTs, utilizando gastrópodes como bioindicadores.

Manuscrito 4: Avaliação da qualidade dos sedimentos de um estuário tropical usando a abordagem da Tríade

Este manuscrito apresenta uma avaliação da qualidade dos sedimentos do SERC através da aborgadem integrada de dados químicos, ecotoxicológicos e ecológicos, através da abordagem clássica da “Tríade da Qualidade de Sedimentos” e análises multivaridas. Esta é a primeira vez que este tipo de abordagem é empregada para avaliar a saúde ambiental de um ecossitema no estado de Pernambuco.

2. OBJETIVOS

2.1 Objetivo geral

O objetivo deste estudo foi avaliar a contaminação do Sistema Estuarino do Rio Capibaribe através da tríade da qualidade do sedimento, que inclui uma caracterização química e biológica do sedimento desta região.

2.1.1 Objetivos específicos

- Avaliar a distribuição granulométrica e os teores de matéria orgânica em 14 pontos distribuídos ao longo do SERC e plataforma adjacente;
- Investigar a contaminação química através da determinação das concentrações de hidrocarbonetos alifáticos, policíclicos aromáticos e organoestânicos em 14 pontos distribuídos ao longo do SERC e plataforma adjacente;
- Identificar e selecionar locais criticamente impactados no SERC em termos de contaminação química, para realizar uma avaliação integrada de parâmetros químicos e biológicos;
- Investigar a contaminação química através da determinação das concentrações de hidrocarbonetos, organoclorados e metais nos locais selecionados;
- Avaliar a estrutura de comunidade da macrofauna bentônica presente nos sedimentos através da análise de diversidade: riqueza e equitabilidade;
- Avaliar a toxicidade dos sedimentos através de ensaios de toxicidade com o cópepodo *Tisbe biminiensis* Volkmann-Rocco 1973;
- Integrar os dados químicos, toxicológicos e ecológicos através de análises multivariadas.

3. **MANUSCRITO 1:** Hidrocarbonetos em sedimentos numa área de transição estuário-plataforma: fontes e distribuição espacial

Este manuscrito será submetido à Environmental Monitoring and Assessment (Anexo I)

Hydrocarbons in sediments in an Estuary-Shelf Transition Area: Sources and Spatial distribution

Abstract

Estuaries are important ecosystems due to its economic, social and ecological role. As a result of its physical and hydrodynamics characteristics, these environments generally act as sediment traps and may retain a range of contaminants, acting as an important filter to the adjacent areas. The distribution and sources of aliphatic hydrocarbons (AHs) were investigated in Capibaribe Estuarine System (CES) and adjacent shelf, Northeast of Brazil, to evaluate the contamination degree and the results were used to understand better the functionality of this system related to the adjacent coast. Sediment samples from 14 stations were analyzed, using a gas chromatography with flame ionization detection. The results showed higher concentrations of hydrocarbons in the upper portion of estuary, which receives discharge of domestic and industrial effluents. Concentrations in dry weight of total aliphatic ranged from 7.5 to 190.3 µg g^{-1} and n-alkanes, mainly from biogenic sources, ranged from <DL to 9.47 µg g^{-1}. The UCM ranged from <DL to 163.5 µg g^{-1} and, in addition to other geochemical markers, indicated contamination by petroleum hydrocarbons. The hydrocarbons distribution clearly shows a decrease in the concentrations towards the adjacent shelf, evidencing the high retention capacity of this tropical estuary. Nevertheless, sediments from the adjacent shelf presented characteristics of AHs similar to that of samples collected

inside the estuary, pointing out to its potential transfering the contaminants to the adjacent shelf.

Key words: tropical estuary, aliphatic hydrocarbons, sediments, hydrocarbons sources, petroleum contamination.

Introduction

Estuaries are important ecological ecosystems, and its high biological productivity provides support for feeding and reproduction for several species (Day et al. 2012). These coastal environments encourage the development of economic centers, urban expansion, in addition to harbors and industries installation (Ridgway and Shimmield 2002). Consequently, these ecosystems receive different kind of contaminants from anthropogenic discharges and the hydrophobic ones has particulate material or sediments as their final fate. Due to its physical and hydrodynamics characteristics the estuaries generally act as sediment traps and, therefore, these compounds would be retained, making them important filter of contaminants before reaching the adjacent areas (Schubel and Carter 1984). However, under some hydrodynamic conditions, such as floods and storms, estuaries can also act as a source of contaminants to the water column and adjacent shelf, exporting contaminated sediments (Ridgway and Shimmield, 2002).

Among the various contaminants in coastal regions, petroleum has become a great environmental concern. It can reach ecosystems from several sources, such as operational discharges, ships' operations, accidental spills, domestic and industrial effluents, etc (Zanardi et al. 1999; Nishigima et al. 2001; Oliveira and Augusto 2011; Lemos et al. 2014). The aliphatic hydrocarbons (AHs) consists of about eighty percent (80%) of the petroleum and, for this reason, they are widely used as geochemical markers of oil contamination (Volkman et al. 1992; Bícego et al. 2008). Due to their hydrophobic characteristics the AHs tend to partition to particulate material and are investigated in sediments, becoming an important tool to study the fate of pollutants in the environment (Guo et al. 2011; Maioli et al. 2010; Zanardi-Lamardo et al. 2013).

The Capibaribe Estuarine System (CES) crosses the Metropolitan Region of Recife (MRR), which has a population of about 3.7 million inhabitants (IBGE, 2010), one of the most urbanized areas in Brazil. It is estimated that about 55 industries are installed in Recife, and 86% of these showed a medium to high potential of pollution (Nóbrega 2011). In addition, in the lower portion of the Capibaribe River Estuary is located the Port of Recife, providing services for import and export of domestic and foreign cargo ships (Porto do Recife, 2015). The urban development and the disorderly occupation of the riverbanks resulted in a completely altered system regarding its baseline characteristics. Fortunately, some mangrove fringes are still observed in the estuary margins. Despite its social, economic and environmental importance to local and international demand, few studies concerning the contamination level have been accomplished in this area (Sericano et al. 1995; Fernandes et al. 1999; Lima et al. 2002; Macedo et al. 2007; Oliveira et al. 2014). This study investigated for the first time the spatial distribution of AHs in sediments of the CES and adjacent shelf, identifying the major sources and the fate of these compounds in this region.

Materials and methods

Study area

The Capibaribe Estuarine System (CES) comprises the Capibaribe Estuary (the main freshwater inflow) with a drainage basin of 7,500 km^2, and the Pina Sound, which is a body of water close to the channel of the estuary and receives input from several smaller tributaries. These small streams along with Capibaribe River receive most of the urban drainage of the MRR, making the CES the final destination of untreated industrial and domestic effluents. The channel of the Capibaribe Estuary is narrow and has a depth of approximately 5 m, while the Pina Sound is a shallow and wide area with approximately 3 km long and 500 m wide (Feitosa et al. 1999). The Estuary and the Pina Sound flow to the low Capibaribe River Estuary, where the Port of Recife is installed, and it is between 200 to 500 m wide and 6 to 12 m depth. The deepest part of this area results from dredging activities.

The local circulation is determined by the tidal regime and freshwater inflow, with the later being modulated seasonally. Local tides are semi-diurnal, with heights ranging between 1.5 and 3.0 m during neap periods and spring tides. The freshwater inflow is determined by the annual hydrological regime, with rainfall concentrated between March and August and a dry season between September and February. The average discharge of the Capibaribe River (measured by a fluviometric station in São Lourenço da Mata, 22 km inland) represents 90% of the drainage basin, and it is in the order of 11 m^3s^{-1}. During the wet season it can reach a few hundred m^3s^{-1}, and can be significantly reduced in dry season (Schettini, pers. comm.). Besides the river inflow there is also freshwater afflux from domestic and industrial sewage from MRR, which may be equivalent to the river discharge during the dry season.

Despite the intense degradation observed in the area, it is still possible to observe the presence of remaining mangroves on its margins. In addition, near the upper portion of the estuary is located the mangrove forest patch, considered the largest urban mangrove area in the world and the last mangrove remnants preserved in Recife (Silva 2010).

Sampling

Sediments were sampled using a stainless steel "van Veen" grab sampler in 14 stations distributed along the Pina Sound (#1-6), the lower course of the estuary (#7-10) and on the adjacent shelf (#11-14) (Fig. 1). The sampling was done in September and December of 2011, during the low river discharge period. For each site a 2 cm superficial sediment layer was sampled, immediately stored in aluminum containers previously calcined (450° C for 4h) and transferred to the laboratory in a refrigerated case. Samples were kept at -18° C until the laboratory analysis.

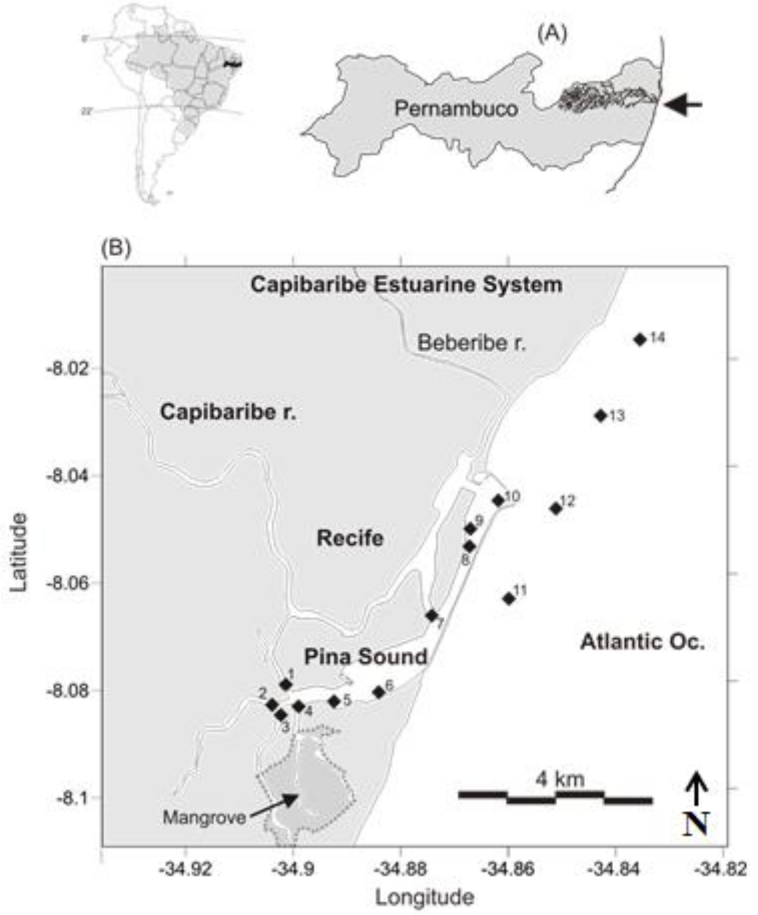


Fig. 1 (A) Capibaribe Estuarine System in South America and the state of Pernambuco. (B) Sampling stations within the Capibaribe Estuarine System (1 to 10) and adjacent shelf (11 to 14).

Organic matter determination and grain size distribution

The percentage of organic matter (OM) was determined by gravimetric method, weighting before and after the oxidation of an aliquot of dry sediment with hydrogen peroxide 10% (Schumacher 2002). Grain size distribution was determined according to Suguio (1973) and the results were interpreted using Sysgran 3.0 software. Sediments were classified into different categories based on the classification adopted by Flemming (2000).

Hydrocarbon analysis

Sediments were freeze-dried and an aliquot of 20g was Soxhlet extracted to analyze the aliphatic hydrocarbons (AHs) with a mixture of hexane / dichloromethane (1:1, v/v), according to the method described in UNEP (1992) and Bícego et al. (2009). All samples, blanks and reference material certificates were spiked with surrogate standards hexadecene and eicosene 12h before extraction. The extracts were purified and fractionated by silica/alumina gel chromatography (5% deactivated) and eluted with n-hexane. The AHs were analyzed in a gas chromatograph (Agilent Technologies 6890) equipped with a flame ionization detector (GC-FID). The temperatures of the injector and detector GC-FID were 280°C and 300°C, respectively. The carrier gas was H_2 ultra-pure (UP 5.0) and the auxiliary gas was N_2.

The chromatographic column used was a DB-5 from J & W Scientific, which is 30m long, 0.25mm internal diameter and a 0.25mm thick film (5% phenyl methylpolysiloxane). The oven temperature was programmed to ramp from 40°C to 60°C at 20°C min^{-1}, 60°C to 290°C at 5°C min^{-1}, holding for 5 min and finally 290°C to 300°C at 10°C min^{-1}, remaining constant for 10 min. The n-alkanes, pristane and phytane isoprenoids, the unresolved complex mixture (UCM) and the total aliphatic were identified and calculated. The identification was done by comparison with the retention times of reference standards. Quantification was done by the ratio between the responses of surrogates and the compounds of interest, considering the analytical curve, with at least five different concentrations for each analyte.

Quality control was based on the analysis of procedural blanks, blank spikes, matrix spikes and duplicates of the sediment sample. Procedural blanks revealed no contamination. Standard reference material (IAEA-417, the International Atomic Energy Agency) was analyzed and the results fell within the standard deviation for all compounds analyzed and the recovery ranged between 65% and 100%. The recovery of the surrogate standard varied between 51% and 94%. The detection limits were calculated as three times the standard deviation of seven replicates of sediment and they can be seen in Table 1. More information and details of the analytical procedure are described in UNEP (1992) and Bícego et al. (2009).

Data analysis

Data were tested for normality using the Komogorov–Smirnov test. The Pearson correlation coefficient was performed between the size of the grains, OM, total aliphatic (ΣAliph) and n-alkanes for each station studied and used the Student's t-test to assess the significance of the same ($\alpha=0.05$). Variations between concentrations of n-alkanes and individual location of stations were evaluated through non-parametric analysis of nonmetric multidimensional scaling (MDS). Data were normalized and used the Euclidean distance as the coefficient association. To test the significance of the groups defined, Analysis of Similarity (ANOSIM) was used.

Results and discussion

Grain size distribution and organic matter

The sediment grain size distributions for all stations can be seen in the ternary diagram of Flemming (2000) (Fig. 2). The samples had a dominance of muddy fraction with a higher contribution of silt relative to clay. It was possible to observe a general pattern of sand predominance in the adjacent shelf and higher mud content at the Pina Sound and Lower estuary sediments.

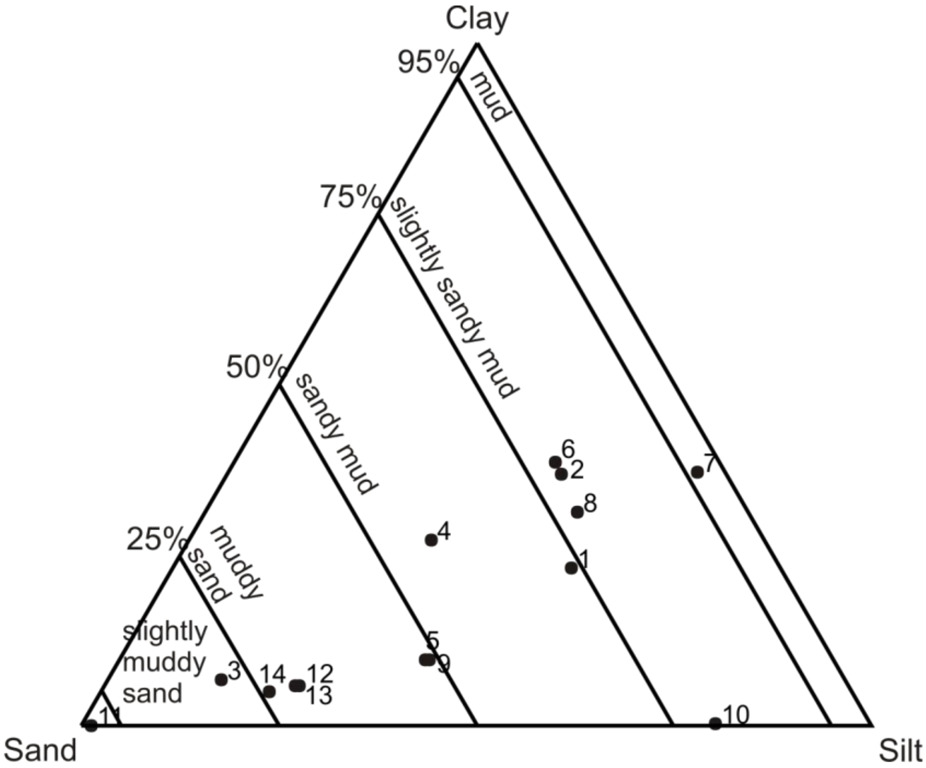


Fig. 2 Ternary diagram (Flemming, 2000) showing the grain size distribution of the sediment samples from Capibaribe Estuarine System (1-10) and adjacent shelf (11-14).

The percentage of organic matter in sediments ranged from 3.0% (#11) to 14.1% (#1), with the highest levels recorded at stations 1, 6, 7, 9 and 12 (above 10%, Fig. 3). There was no significant correlation between the percentages of OM and the size fractions (sand, silt, clay and silt + clay) ($p > 0.05$), but it is possible to observe a general decreasing pattern of OM along lower estuary toward adjacent shelf stations (Fig. 3). Most of the samples located at Pina Sound had an average content of OM smaller than those recorded in the lower estuary.

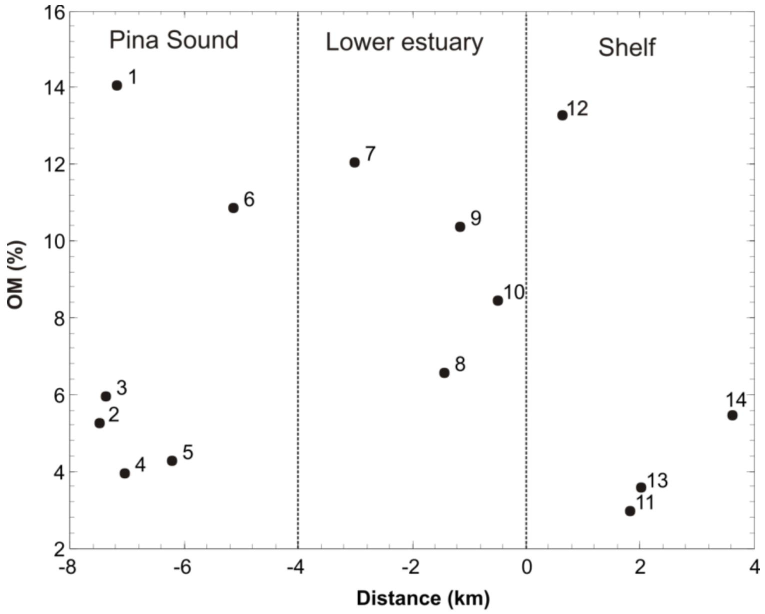


Fig. 3 Organic matter content (%) of the samples along the estuary-shelf gradient (Capibaribe Estuarine System) . The distance reference is the estuarine mouth (0 km).

Spatial distribution of Aliphatic Hydrocarbons

Concentrations of total aliphatic (Σaliph) at Pina Sound and the lower estuary ranged from 43.9 µg g^{-1} (#10) to 190.3 µg g^{-1} (#1), and concentrations higher than 100 µg g^{-1} were observed at samples # 1, 2, 3, 4, 6 and 7 (Table 1, Fig. 4). According to Volkman et al. (1992), concentrations higher than 100 µg g^{-1} of Σaliph in sediments rich in organic matter are an indication of oil contamination. Samples #1-4 are located in the confluence zone of the tidal channels, and the sample #6 is located close to some marinas hosting anchoring area for fishing and recreation vessels. Additionally, #6 is close to Brasilia Teimosa village that throws their effluents directly into the estuary. Sample #7 is located in the lower estuary at the intersection between Pina Sound and the main channel of the Capibaribe River, which carries considerable amount of untreated industrial and domestic effluents. Moreover, there are wharfs for fishing vessels and tugs operating in harbor, as well as boats that transport cargo and fuel to outside of the State. Samples from the adjacent shelf had Σaliph concentrations ranging from 7.1 µg g^{-1} (# 11) to 36.6 µg g^{-1} at the mouth of the estuary (# 12).

Table 1. Aliphatic hydrocarbons concentrations (μg g^{-1} dry weight) and diagnostic indices determined by sediments from Capibaribe Estuarine System, Pernambuco.

	Sampling stations													
AHs	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Pris	0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.05	0.02	<0.01	0.01	<0.01	0.3
Phy	0.4	0.5	0.4	0.2	0.2	0.2	0.1	0.1	0.05	0.02	<0.00	0.01	<0.00	0.3
Σaliph	190.3	145.7	163.3	119.5	92.4	104.6	109.1	92.5	63.1	43.9	7.1	36.6	15.7	10.6
Σn-alk	7.9	3.0	5.6	5.8	5.9	9.5	7.0	5.5	4.5	2.7	<DL	2.0	0.1	7.2
R	26.9	42.3	22.6	32.0	16.7	25.9	19.2	14.7	9.9	5.7	<0.76	4.3	<0.76	8.9
UCM	163.5	103.3	140.7	87.5	75.7	78.7	89.9	77.8	53.2	38.3	<3.41	32.2	15.6	<3.41
ΣLMW	1.2	0.8	1.3	0.2	0.8	0.9	0.4	0.4	0.3	0.1	<0.01	0.1	<0.01	2.3
ΣHMW	6.7	2.2	4.3	5.6	5.2	8.6	6.6	5.1	4.2	2.6	---	1.9	0.1	4.9
LMW/HMW	0.2	0.4	0.3	0.04	0.1	0.1	0.1	0.1	0.1	0.04	---	0.03	---	0.5
CPI	2.2	13.2	1.5	1.8	3.1	3.0	3.0	3.1	4.2	4.3	---	4.8	---	1.1
Pri/Phy	0.6	0.8	0.7	0.6	0.7	0.7	0.7	0.9	1.0	1.0	---	1.0	---	1.0
n-C_{17}/Pris	1.5	0.7	1.3	0.8	2.5	2.3	1.8	1.5	2.5	2.3	0.0	1.5	0.0	1.0
n-C_{18}/Phy	0.3	0.2	0.4	0.1	0.6	0.6	0.4	0.4	0.5	0.6	0.0	0.0	0.0	1.0
UCM/R	6.1	2.4	6.2	2.7	4.5	3.0	4.7	5.3	5.4	6.8	---	7.4	---	---

AHs= aliphatic hydrocarbons **; Pri**= Pristane**; Phy =** Phytane; **Σaliph=** Total aliphatic hydrocarbons; **Σn-alk =** total n-alkanes; **R**= Aliphatics resolved**; UCM** = Unresolved complex mixture; **LMW** = Low molecular weight; **HMW**= High molecular weight; **CPI**= carbon preference index;; **UCM** = Unresolved complex mixture; **R**= Aliphatic resolved, (---) = Not calculated.

CPI = (1/2)[(C25 + C27 + C29 + C31 + C33)/(C24 + C26 + C28 + C30 + C32)+ (C25 + C27 + C29 + C31 + C33)/(C26 + C28 + C30 + C32 + C34)].

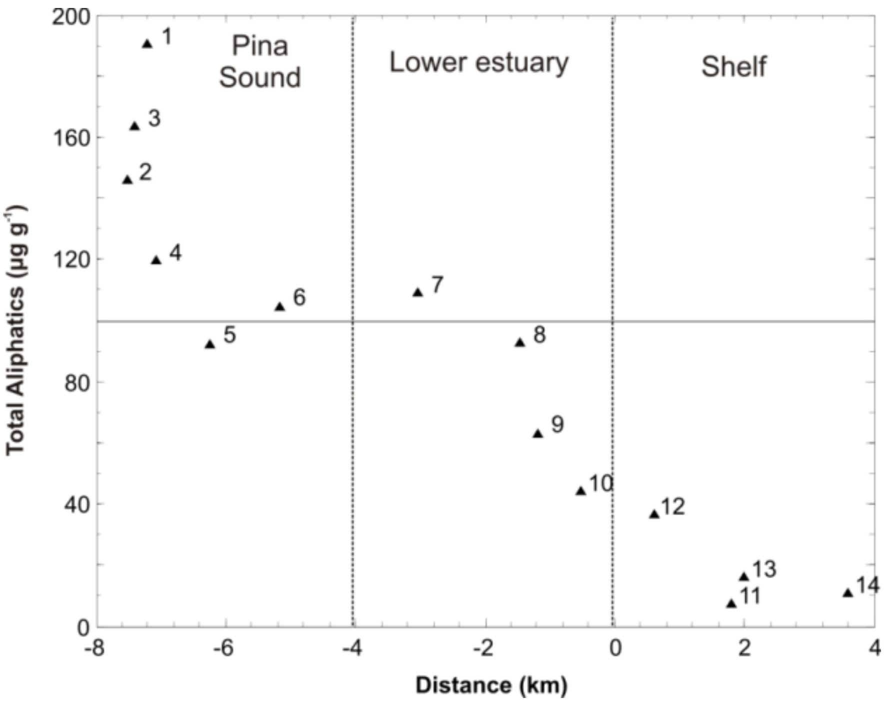


Fig. 4 Total aliphatics concentration (**Σaliph -** µg g^{-1}) in sediment samples along the estuary-shelf gradient. The distance reference is the estuarine mouth (0 km).

Σaliph concentrations were higher than those found in estuaries in urban areas such as the Changjiang Estuary in China, 11.82 µg g^{-1} (Bouloubassi et al. 2001); Todos os Santos Bay, Brazil, 8.53 µg g^{-1} (Medeiros and Bícego 2004); Cross River Estuary, Nigeria, 16.84 µg g^{-1} (Oyo-Ita et al. 2010) and in the Estuarine System Mundaú-Maguaba, Brazil, 139.5 µg g^{-1} (Silva et al. 2013). On the other hand, the Σaliph concentrations are relatively low compared to chronically contaminated sites, such as Sfax, Tunisia, with 1729 µg g^{-1} (Zaghden et al. 2007); Kuching, China, 1400 µg g^{-1}(Yusoff et al. 2012), Baku, Azerbaijan, 1820 µg g^{-1} (Tolosa et al. 2004) and Rio de Janeiro, Brazil, 20000 µg g^{-1} (Wagener et al. 2012).

Concentrations of total n-alkanes (Σn-alk) ranged from <DL at the adjacent shelf (#11) to 9.5 µg g^{-1} at Pina Sound (#6), and their individual distribution can be visualized in Fig. 5. The concentration at Station #2 (Pina Sound) was as low as the ones at the shelf, in opposition to Station #14 (shelf) that had a concentration as high as those reported in the Pina Sound sediments.

Sediments from Pina Sound (#1-6) and lower estuary (#7-10) showed the same distribution pattern and all n-alkanes studied were observed, with a prevalence of n-$C_{29,}$ n-C_{31} e n-$C_{33.}$ Exception was observed at sample #2, where some high molecular weight n-alkanes are missing (Fig 5). The n-C_{17} was the most abundant among n-alkanes of low molecular weight (n-C_{12} to n-C_{20}) (Fig. 5). Station #12, on the shelf, had a similar distribution to that observed in samples from the estuarine system. Sample #14 showed a unique pattern and homogeneous distribution of n-alkanes (Fig. 5), with individual concentrations ranging from 0.20 to 0.38 µg g^{-1}.

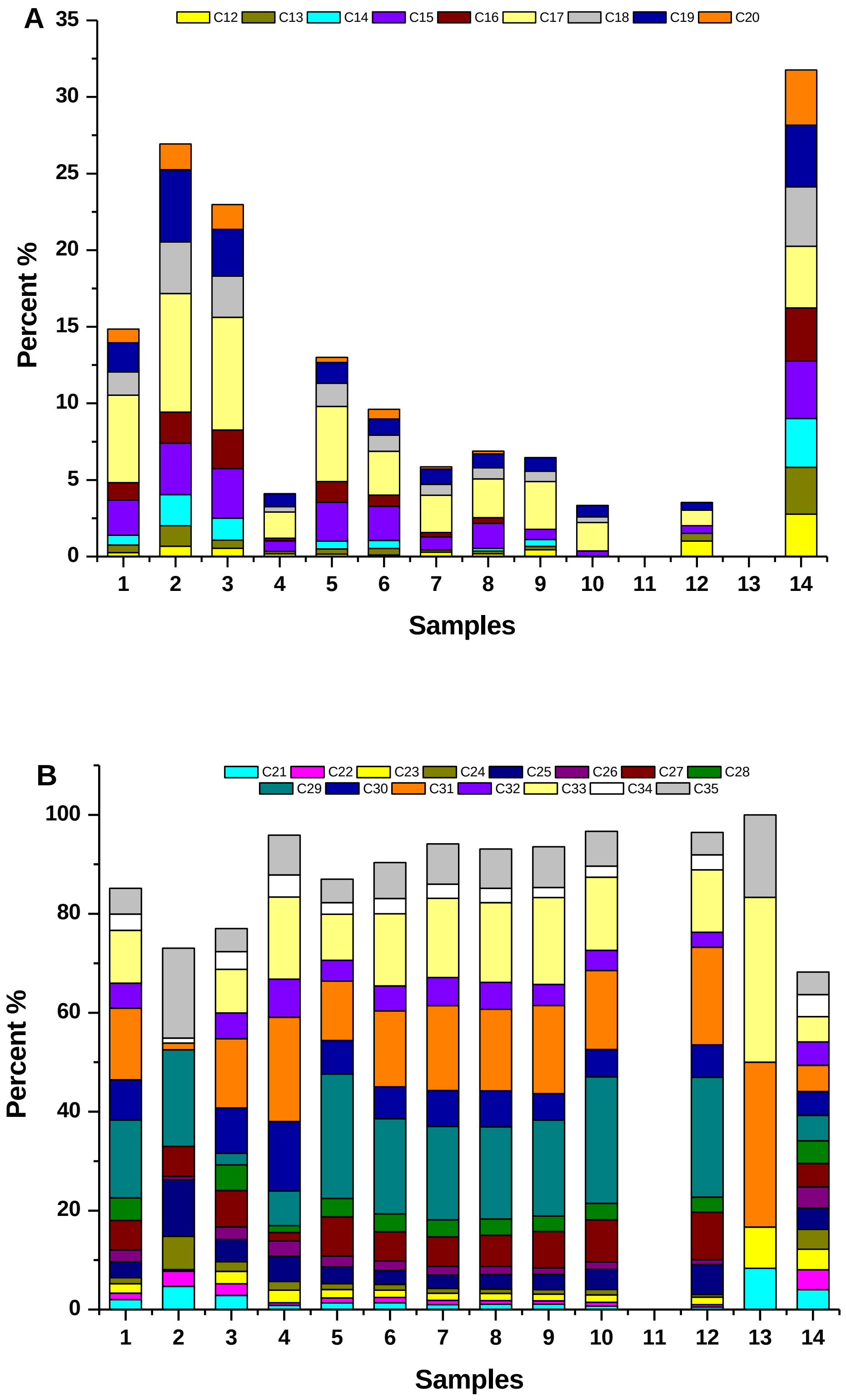


Fig. 5 The distribution of n-alkane in sediment samples from Capibaribe Estuarine System (#1-10) and adjacent shelf (#11-14). A (Low molecular weight: nC_{12}-nC_{20}) and B (High molecular weight nC_{21}-nC_{35}).

The multidimensional scaling analysis (MDS) was performed by grouping the stations in relation to their position and assessing the individual n-alkanes concentrations (Fig. 6): the Pina Sound (#1-6), lower estuary (#7-10) and adjacent shelf (#12-14). The ANOSIM (R global 0.55, p <0.01) was subsequently performed and showed two regions significantly different. One distinct distribution pattern was observed for samples #6 to #10, #12 and #13, which is explained by the decrease in the n-alkanes concentrations from Pina Sound towards the adjacent shelf. These results suggest that the main source of these compounds is in the upper portion of the estuary. A similar pattern of a decrease in the hydrocarbons concentrations towards the sea also was observed in Pearl River Estuary, China (Wang et al. 2002), and estuary of the Chao Phraya River, Thailand (Boonyatumanond et al. 2006). The other group is formed by samples #1-5, and did not present a defined pattern, probably due to the influence of distinct sources, once this area is formed by several tidal creeks confluence in addition to the Waste Water Treatment Plant (WWTP) nearby these stations.

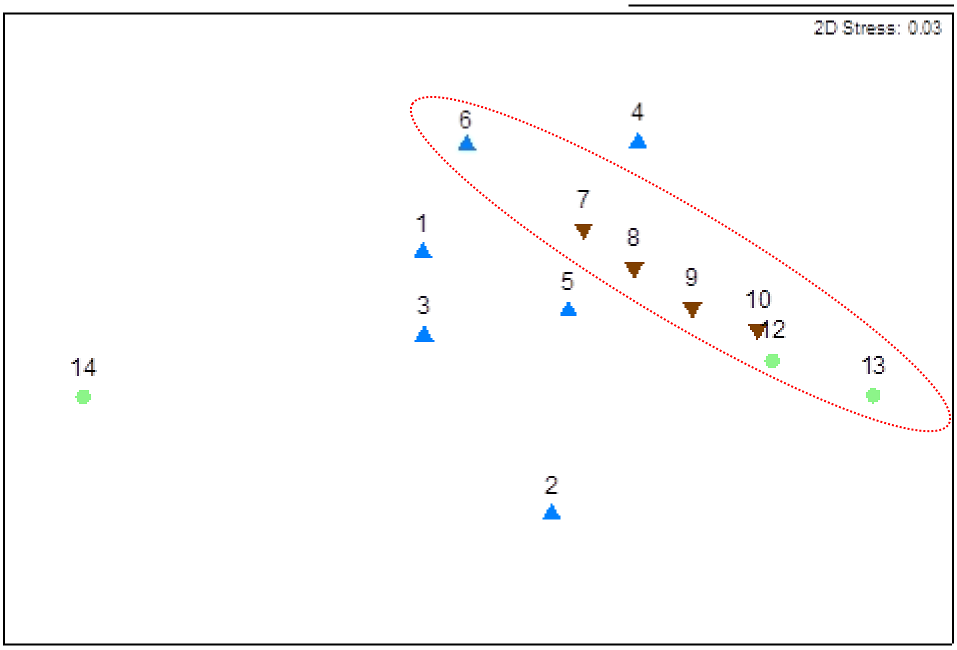


Fig. 6 Non-parametric multidimensional scaling (MDS) analysis performed by grouping the concentrations of n-alkanes and the location of individual stations. ▲= Pina Sound (1-6), ▼= Lower estuary (6-10); ● Adjacent Shelf = (12-14).

Considering the concentration average of stations #1, 2 and 3, 166 µg g^{-1}, as the reference level source, and the concentration average of stations #11 to 14, 17 µg g^{-1}, as the reference level of concentration in the shelf, we obtain a decay rate of 90% of Σaliph. Although relative, this value indicates a high trapping capacity of HCs by the estuary, as well as a high dilution rate towards the adjacent shelf. The linear decrease suggests a gradual dilutive process in the transfer of these compounds. Studies focused on transport of hydrocarbons in an urban estuary revealed that most of these compounds (> 90%) were retained in the estuarine sediments and did not flush out to the sea (Bates et al. 1987; Murphy et al. 1988). Considering a long term accumulation trend, the estuarine sediments might be a threat in the case of remobilization making the pollutants available for the biota (Bates et al. 1987; Murphy et al. 1988).

Due to the low aqueous solubility and high hydrophobicity of hydrocarbons, these compounds tend to be associated with suspended particulate material (SPM), which may be ultimately accumulated in the sediment (Tolosa et al. 2004). This was corroborated by the fact that Σaliph correlated significantly with clay ($r=0.6081$, $p=0.021$). Thus, it is reasonable to infer a direct relationship between the dynamics of HCs with that of the fine sediments (Salomons and Förstner 1984; Dyer 1995; Dhivert et al. 2015).

In general estuaries act as filters (Shubel and Carter, 1984), trapping sediments from the river source, and even importing sediments from adjacent shelf (Schettini et al. 2013). During periods of low discharge the processes of erosion, transport and deposition of sediment are ruled mainly by the tidal currents. However, during high discharge events the current speed can increase relative to those observed during periods dominated by the tides. In these conditions the erosion of the surface layer of sediments and transportation downstream may occur (Schettini, 2002). The samples used in the present study were collected during the period of low river discharge when the tide is the principal agent controller of the hydrodynamic system. Under such conditions this estuary shows a partially mixed circulation pattern (Schettini, pers. comm.). In this case, the gravitational circulation is well developed, and together with the tidal pumping effects, prevent the SPM escape to the adjacent shelf (Schettini et al. 2006).

Although the hydrodynamic system favors sediment retention most of the time due to the predominance of tidal processes, during the wet season high discharge floods may occur and promote material exportation. The occurrence of heavy rainfall events in the watershed can produce elevated river discharge of up to two orders of magnitude for short periods of time (e.g. days). Adsorbed materials might be transported together with the sediment, which may explain the concentrations of aliphatic, organic matter and fine sediment observed in the external shelf, particularly at sample #12. Once in the shelf dilution, dispersion and degradation of these materials is expected. In this eventuality, it is expected that there is a preferential northward transport according to the prevailing currents regime (Lira et al. 2010).

Source diagnoses

Some diagnostic indices are commonly used to assess the origin of hydrocarbons, if natural or anthropogenic (Wang et al. 2007; Gao and Chen 2008; Guo et al. 2011; Martins et al. 2012). The carbon preference index (CPI) was calculated to assess the origin of n-alkanes. CPI values greater than 1 indicate biogenic and terrestrial input, while values less than 1 indicate anthropogenic sources, such as oil pollution (Aboul-Kassim and Simoneit 1996). CPI values were greater than 1 for all samples, except #14, showing a predominant input from higher plants (Table 1). Compounds with 25 to 35 carbons are characteristic of the waxes mangrove leaves (Kristensen et al. 2008). There is a reduced fringe of mangroves along the Capibaribe Estuary, in addition to a mangrove reserve of 2.5 km^2 at the Pina Sound southern area, which justify the higher concentrations of n-alkanes derived from terrestrial plants. The St #14 had a homogeneous distribution of n-alkanes and the CPI was 1.1, suggesting a petrogenic contribution. The ratio of low molecular weight (LMW) (n-C_{12} to n-C_{20}) to high molecular weight (HMW) (n-C_{21} to n-C_{35}) is also used as an indicator of hydrocarbons source. Ratios greater than 1 indicate a petrogenic contribution and less than 1 suggest hydrocarbons from biogenic origin (Gao and Chen, 2008). All calculated ratios ranged from 0.04 (#4 and #10) to 0.5 (#14) (Table 1), confirming the results revealed by the CPI.

Two ratios between some pairs of n-alkanes were applied to identify if the dominance of natural contribution are from planktonic marine or terrestrial: n-C_{29}/n-C_{17} ratio <1 indicates a planktonic marine source and >1 is terrestrial (Le Dréau et al. 1997); the n-C_{31}/n-C_{19} ratio <0.4 indicates autochthonous sources (planktonic) and values > 0.4 allochthonous of terrestrial origin (Yusoff et al. 2012). The n-C_{29}/n-C_{17} ratio showed values greater than 1 for all samples, except for #3, and the n-C_{31}/n-C_{19} ratios were higher than 0.4 in all samples, except for #2. These results suggest that the organic matter present in the system is mainly from allochthonous source. High levels of chlorophyll-a were recorded in Pina Sound, close to Stations #2 and #3 in previous studies (Feitosa et al. 1999; Santos et al. 2009) that corroborate the predominance of marine input at Stations #2 and #3.

In spite of the evident contribution of natural n-alkanes, it is interesting to note that the Σn-alc concentrations at almost all stations were less than 10% of Σaliph, suggesting other sources of aliphatic hydrocarbons to the sediments. The whole series of n-alkanes was observed in sediments, except at station #2, and it is an indicator of petrogenic contributions to the formation of sedimentary organic matter.

The isoprenoids pristane (C_{19}) and phytane (C_{20}) are common components of crude oil but pristane is usually originated from natural sources (Steinhauer and Boehm 1992). The ratio between these isoprenoids is used to determine the relative contribution of oil to sediment contamination, where values <1 indicate oil contamination, and values between 3 and 5 are characteristic of non-contaminated sediments (Steinhauer and Boehm, 1992). The pristane/phytane ratio varied between 0.6 and 1.0 for all samples (Table 2), including those from the adjacent shelf, pointing out an oil contamination. Similar results were reported at oil contaminated sites such as the São Vicente Estuary (Brazil) (Bícego et al. 2006), Sfax (Tunisia) (Zaghden et al. 2007), Daya Bay (China) (Gao and Chen, 2008), Ushuaia Bay (Argentina) (Commendatore et al. 2012) and at the São Sebastião Channel (Zanardi-Lamardo et al. 2013).

The degree of oil degradation in the environment was evaluated by the ratios between n-C_{17}/pristane and C_{18}/phytane, since the n-alkanes chains degrade more quickly than the branched ones (Leahy and Colwell 1990). Values less than 2 indicate the presence of degraded oil while values greater or equal to 2 suggest recent oil introduction (Colombo et al. 1989; Weiner 2000). The values for the C_{18}/phytane were smaller than 2 in all samples. The n-C_{17}/pristane ratio presented similar results except for samples #5, #6, #9 and #10, which had 2.3-2.5, and suggests a recent contribution of oil in these stations (Table 2). These results suggest that there is a mixture of degraded and recently introduced oil at these stations.

Sample #5 was collected in front of a wastewater treatment plant (WWTP) and in a confluence region of the tidal creeks that forms the estuarine system. These rivers continually receive the discharge of domestic and industrial effluents that are mostly untreated. Station #6 receives a direct introduction of sewage from Brasilia Teimosa Village, as well as from the oil discharge of vessels docked in the marinas located in this area. Samples #9 and #10 were collected close to the Port of Recife and continuously receive oil input from vessels and port activities.

The presence of the Unresolved Complex Mixture (UCM) (Fig. 7) is an important indicator of sediment contamination by oil (Volkman et al. 1992). UCM in samples ranged from <DL (# 11 and #14) to 163.5 µg g^{-1} (#1) (Table 1), and represented more than 70% of Σaliph in all samples, except at #11, #14, indicating contamination by oil (Readman et al. 2002). The ratio between UCM and resolved aliphatics (R) is used as a diagnostic criteria of anthropogenic contributions (Silva et al. 2013). Values lower than 4 indicate the presence of petroleum residues and higher than 4 can be indicative of recent inputs (Simoneit 1984; Lipiatou and Saliot 1991). The UCM /R ratio was >4 in most samples, except for samples #2, #4 and #6, and it was not possible to calculate it for samples #11, #13 and #14. The highest values of UCM were recorded at samples #1, 2 and 3, located in the upper portion of the Pina Sound where high concentrations of dissolved and/or dispersed petroleum hydrocarbons (DDPHs) were also recorded (Favrod, 2012).

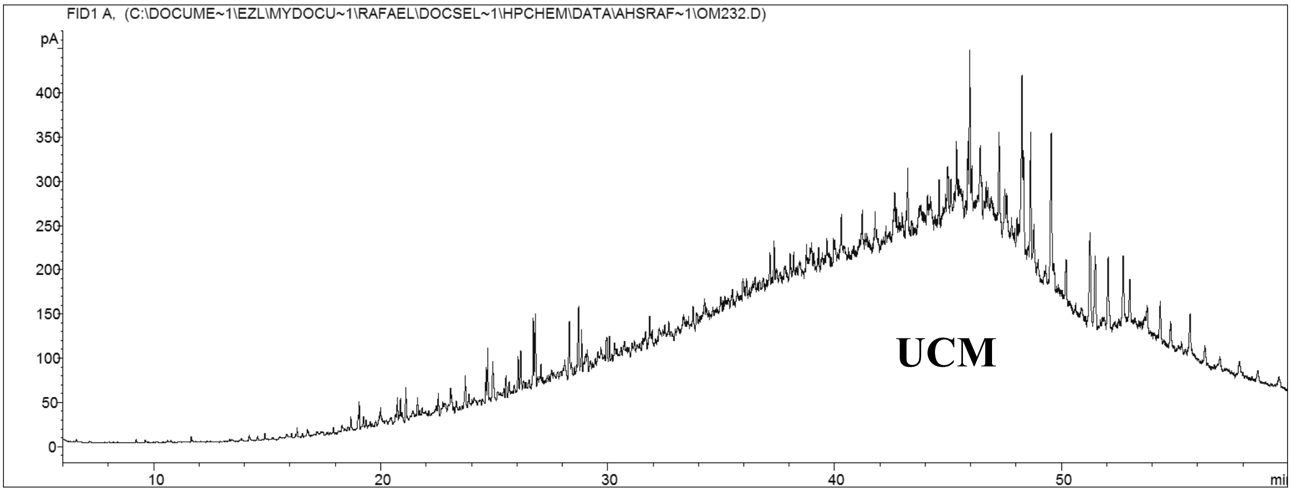


Fig. 7 Chromatogram of sample from station #2 (Capibaribe Estuarine System) using GC-MS, highlighting the prominent unresolved complex mixture (UCM).

Conclusion

Sediments from the Capibaribe Estuarine System are chronically contaminated by recently introduced and/or degraded petroleum. These input are from untreated domestic and industrial effluents, especially at the upper portion of the estuary (Pina Sound). In addition, mangrove leaves and marine plankton contribute to the sedimentary organic material in the area.

The upper portion of the Pina Sound acts as an important area of deposition and accumulation of hydrophobic material. This system showed a high retention capacity, but not enough to prevent contamination of the adjacent shelf.

The present study reinforced that aliphatic hydrocarbons are valuable tools to assess the environmental quality of coastal water bodies, and further contributes to understanding the dynamics of pollutants.

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4. MANUSCRITO 2: Fontes e distribuição de hidrocarbonetos policíclicos aromáticos em um estuário tropical urbanizado e plataforma adjacente, Nordeste do Brasil

Este manuscritos foi submetido à Marine Pollution Bulletin (Anexo II)

Sources and distribution of polycyclic aromatic hydrocarbons in a an urbanized tropical estuary and adjacent shelf, Northeast of Brazil

Abstract

The Capibaribe Estuarine System (Northeast of Brazil) is a important ecosystem that supply food and protection for nursery of several species, of ecological and economic importance. It is located inside an urbanized area, and receives untreated domestic and industrial effluents, houses some marinas and the Port of Recife, very important to national and international community. The distribution and sources of PAHs were investigated in sediments from CES and adjacent shelf. Total PAHs concentrations ranged from non-detectable to 497.6 µg g^{-1}. Pyrolytic sources predominated in most of sites, but the presence of petrogenic PAHs was also recorded. A concentration decrease of PAHs toward adjacent shelf suggests that the main source of these compounds is in the upper portion of the estuary, where there is an intense discharge of sewage, but atmospheric input also seems to be relevant to the area. The results reinforce the important role of an estuary in contaminants retention.

Key words: Sediment, Capibaribe Estuarine System, pyrolytic, petrogenic.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are among the priority organic contaminants studied, especially due to its wide distribution and high toxicity (Baumard et al., 1998; Magi et al., 2002; Boonyatumanond et al., 2006; Chen et al., 2013; Rahmanpoor et al., 2014). These compounds are lipophilic, easily absorbed by organisms tissues (Tuvikene, 1995), and are associated to carcinogenic and teratogenic effects (ATSDR, 1995). PAHs may be originated from incomplete combustion of organic matter under high temperature (pyrolytic), or formed during diagenesis process and formation of fossil fuels under low temperature (petrogenic) (Boehm, 2005; Meire et al., 2007). In addition, some organisms such as plants, bacteria, and fungi can synthesize PAHs, even though in much lower concentrations compared to those processes mentioned above. These compounds may reach the marine environment through several ways, including atmospheric deposition, fluvial runoff, discharge of domestic and industrial effluents and oil and its derivatives spill (Zaghden et al., 2007; Lemos et al., 2014). Among marine ecosystems estuaries are, generally, more susceptible to receive anthropogenic discharges and has been focus of several studies to evaluate their contamination (Budzinski et al., 1997; Viguri et al., 2002; Bícego et al., 2006; Wagener et al., 2012; Sharif et al., 2014). The Capibaribe Estuarine System (CES) is located in the metropolitan area of Recife, Northeastern Brazil, and it is formed by the confluence of several tidal creeks, consisting in a natural drainage system. The CES receives continuously the discharge of domestic and industrial effluents, mostly untreated (Nascimento et al., 2003; Somerfield et al., 2003), in addition to several marinas that provide shelter and maintenance to fishing and recreation boats. The CES houses the Port of Recife that has a key role within the international community, including Mercosul, North America and European countries (Porto do Recife, 2015).

The general cargo handling in 2014 was 1.512.183 million tons (Porto do Recife, 2015). Furthermore, due to its location, the CES receives atmospheric input of chemicals from industrial activities and fossil fuels combustion, as well as from sugar cane field burning, which is a common practice in many cities around Recife. There is an estimate of 620,000 cars circulating every day at the metropolitan area (CTTU, 2015) and, in the past two years, Pernambuco produced approximately 13,351.6 tons of sugar cane (SINDAÇÚCAR, 2015).

Despite its economic, ecological and social role for local and international community, it is visible the degradation, but just few contamination studies were performed in the area (Sericano et al., 1995; Fernandes et al., 1999; Lima et al., 2002; Macedo et al., 2007; Oliveira et al., 2014). This study investigated PAHs in sediments from CES and the adjacent platform, to characterize the spatial distribution and the main sources of these compounds to the area. Due to its physical and hydrodynamics characteristics the estuaries generally act as sediment traps (Wolanski et al., 2006) and, for this reason, this study also evaluated the role of the CES as contaminants retainer.

Materials and method

Fourteen sediments samples were collected in September and December 2011, using a stainless steel "van Veen" grab sampler, distributed along the estuary (#1-10) and adjacent shelf (#11-14). The superficial sediment layer (2 cm) was collected and stored in aluminum containers and kept at -18° C until the laboratory analysis.

The extraction of PAHs was performed according to the method described in UNEP (1992). An aliquot of 20g of sediment freeze-dried was extracted in Soxhlet with a mixture of n-hexane / dichloromethane (1:1, v/v), for eight hours. The extracts were purified and fractionated by silica/alumina gel chromatography (5% deactivated) and eluted with a mixture of dichloromethane and n-hexane (3:7, v/v). The PAHs were analyzed in a gas chromatograph (Agilent Technologies 6890), equipped with an HP-5MS fused silica capillary column (30m, 25 mm i.d. and 0.25 µm film thickness) and coupled to a 5973N Mass Spectrometer (MS), in the selected ion monitoring (SIM) mode. The carrier gas was H_2 ultra-pure (UP 5.0). The 16 priorities PAHs listed by the US Environmental Protection Agency (US-EPA) were identified (Table 1).

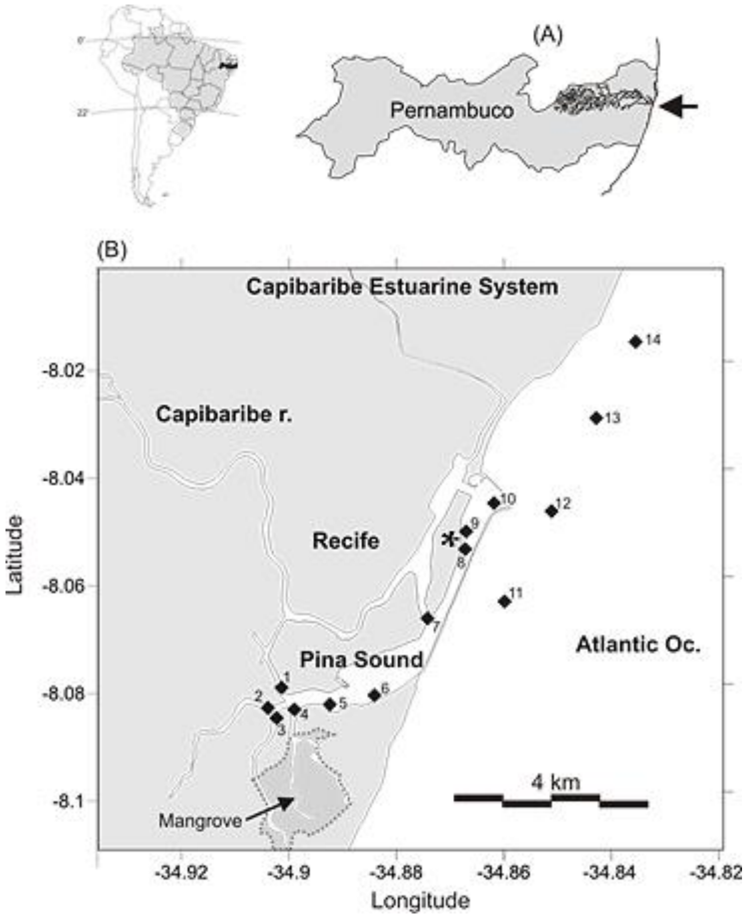

Figure 1. (A) Capibaribe Estuarine System in South America and the state of Pernambuco. (B) Sampling stations within the Capibaribe Estuarine System (1 to 10) and adjacent shelf (11 to 14). (*****) Port of Recife. Capibaribe r.= Capibaribe River.

Replicate samples, certified reference materials (IAEA-417, from International Atomic Energy Agency) and procedural blanks were used as quality control procedures. The recovery of the surrogate standard varied between 45% and 104%. The detection limit was obtained from three times the standard deviation of seven replicates of sediment and it can be seen in Table 1.

The granulometric analysis was performed according to Suguio (1973) and the results interpreted using Sysgran 3.0 software. Organic matter (OM) percentage was determined by gravimetry through the differences between dry weight before and after the oxidation of sediment with hydrogen peroxide 10% (Schumacher, 2002).

Data normality was verified using the Kolmogorov-Smirnov test. Pearson correlation were performed between the grain size, OM, and PAHs for each station studied and the Student's t test was used to assess the significance of the same (α=0.05). Student's t test was also used to evaluate differences between PAHs of different molecular weight. Analyses were performed by BioEstat 5.0 software.

Results and discussion

The samples had a predominance of silt and clay, except at stations 3, 5 and 9, and samples located on the shelf (stations 11-14) (Table 1). The percentage of OM in sediments ranged from 3.0% to 14.1%, with the highest levels recorded at stations 1, 6, 7, 9 and 12 (Table 1).There was no significant correlation between of OM and size fractions (sand, silt, clay, and silt + clay) ($p > 0.05$).

The sum of the 16 PAHs (∑16PAHs) ranged between < detection limit (<DL) and 497.6 ng g^{-1}dry weight (Table 1). Based on ∑16PAHs concentrations, sediments from the estuary and station 13, located in the adjacent shelf, may be classified as moderately contaminated (100 – 1000 ng g^{-1} de Σ 16HPAs), and stations 11, 12 and 14 (adjacent shelf) as slightly contaminated (0 – 100 ng g^{-1} de Σ 16HPAs) (Baumard et al., 1998).

Table 1. Organic matter (OM), sand, silt and clay (%), and PAHs concentrations (ng g^{-1} dry weight) in sediments from CES and adjacent shelf.

		Sampling stations															
	DL	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	11	12	13	14	**TEL**	**PEL**
OM		14.1	5.3	6	4	4.3	10.9	12.2	6.7	10.4	8.5	3	13.36	3.7	5.6		
Sand		26.6	20.9	78.9	42.4	51.9	20.8	3.6	21.7	51.3	19.7	98.8	69.6	70.2	73.9		
Silt		50.2	41.9	14.0	30.3	38.4	40.5	59.0	46.9	38.9	79.7	0.9	24.3	23.9	20.8		
Clay		23.2	37.0	7.0	27.3	9.8	38.7	37.4	31.3	9.7	0.58	0.2	6.1	5.9	5.2		
Naphthalene	0.7	10.0	23.6	9.2	21.4	6.0	12.6	11.1	9.8	7.7	5.2	<DL	2.7	1.0	1.1	34.6	391
Acenaphtylene	0.7	2.1	3.5	0.7	1.6	1.1	3.0	2.7	2.7	1.7	1.6	<DL	1.1	<DL	<DL	5.87	128
Acenaphthene	0.2	0.7	0.7	1.1	0.8	1.0	0.7	0.5	0.5	0.2	0.7	<DL	0.4	<DL	<DL	6.71	88.9
Fluorene	0.2	5.1	10.0	6.0	11.2	3.3	5.0	3.5	2.4	1.4	1.0	<DL	0.7	<DL	<DL	21.2	144
Phenanthrene	4.7	21.9	31.4	23.8	37.4	13.7	31.2	14.4	14.6	11.4	11.1	<DL	<DL	<DL	<DL	86.7	544
Anthracene	1.1	2.6	5.8	2.5	5.3	1.3	5.6	14.1	2.5	1.8	2.1	<DL	3.1	<DL	1.5	46.9	245
Fluoranthene	15.7	38.9	61.5	54.4	45.3	26.4	59.2	34.1	23.3	<DL	35.7	<DL	<DL	26.6	<DL	113	1494
Pyrene	15.5	34.8	91.1	43.6	44.4	24.6	56.8	31.4	25.3	17.7	27.5	<DL	<DL	21.5	<DL	153	1398
Benzo[a]anthracene	8.6	16.4	28.6	16.9	15.3	<DL	30.2	14.8	10.8	<DL	17.7	<DL	<DL	16.7	<DL	74.8	693
Chrysene	7.8	26.7	58.5	36.3	24.5	16.2	38.9	21.2	14.9	11.1	18.3	<DL	<DL	16.3	<DL	108	846
Benzo[b]fluoranthene	3.5	19.8	24.6	13.7	20.0	11.8	22.7	18.6	12.9	7.8	11.1	<DL	<DL	11.9	<DL		
Benzo[k]fluoranthene	6.3	16.8	22.2	18.6	18.1	9.1	26.0	12.2	9.0	<DL	10.8	<DL	<DL	9.7	<DL		
Benzo[a]pyrene	7.3	21.1	24.1	20.7	19.9	10.1	32.6	15.4	13.5	10.6	16.7	<DL	<DL	19.3	<DL	88.8	763
Indeno[1,2,3-cd]pyrene	5.9	20.1	37.9	24.0	25.1	<DL	38.0	23.1	<DL	13.2	17.4	<DL	<DL	18.1	<DL		
Dibenzo[a,.h]anthracene	1.5	5.8	12.1	6.4	6.5	3.4	10.8	5.4	3.9	3.6	4.1	<DL	<DL	5.0	<DL	6.22	135
Benzo[ghi]perylene	4.6	32.5	61.9	33.3	37.0	17.7	44.1	28.8	22.7	20.0	19.6	<DL	7.9	20.9	<DL		
Σ16PAHs		275.4	497.6	311.3	333.6	145.9	417.4	251.3	168.7	108.0	200.6	<DL	16.0	166.9	2.6		
∑HPAs LMW		42.5	75.1	43.3	77.6	26.5	58.1	46.4	32.4	24.1	21.7	---	8.1	1.0	2.6		
∑HPAs HMW		233.0	422.5	268.0	256.0	119.4	359.3	204.9	136.3	83.9	178.9	---	7.9	165.9	---		
LMW/HMW		0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.3	0.1	---	1	---	---		
Ant/178		0.11	0.16	0.09	0.12	0.09	0.15	0.49	0.15	0.14	0.16	---	1	---	1		
Fl/Fl+Py		0.53	0.4	0.56	0.51	0.52	0.51	0.52	0.48	---	0.57	---	---	0.55	---		

DL (Detection limit); TEL (ThresholdEffectLevel); PEL (ProbableEffectLevel);LMW (Low molecular weight); HMW (High molecular weight);Ant/178= Anthracene/ Anthracene+Phenanthrene; Fl/Fl+Py= Fluoranthene/Fluoranthene+Pyrene.

Some studies in coastal areas worldwide also reported similar concentrations, within the moderate contamination range, as São Sebastião Channel, Brazil, (257.4 ng g^{-1},Zanardi et al., 1999), Chioggia, Italy (501.1 ng g^{-1},Magi et al., 2002), Mai Po, China (373.0 ng g^{-1}, Liang et al., 2007), Gorgan Bay, Iran (516.18 ng g^{-1},Araghi et al., 2014) and Suape Bay, Brazil (888.42 ng.g^{-1}, Lemos, 2013). On the other hand, these concentrations are lower than that reported to chronically contaminated regions, such as Santos Estuary, Brazil (68,130 ng g^{-1}, Bícego et al, 2006), Guanabara Bay, Brazil (78,520 ng g^{-1}, Wagener et al, 2012), Boston Harbor, United States (358,000 ng g^{-1}, Wang et al., 2001) and Gijón, Spain (475,280 ng g^{-1}, Viñas et al., 2010).

The highest concentrations (>300 ng g^{-1}) were recorded at stations 2, 3, 4 and 6, located in the upper portion of the estuary. At this point, there is a confluence of four tidal creeks that carry untreated sewage from the metropolitan area of Recife, an intense traffic of fishing and recreation boats, as well as some marinas. According to the hydrodynamic of this estuary, the tide regime tend to trap water at this local, favoring the sediment deposition (Araújo et al., 2011). Due to the low aqueous solubility and high hydrophobicity of PAHs, these compounds tend to associate with suspended particulate matter, and are incorporated to the sediments, especially fine particles (Wang et al, 2001; Tolosa et al., 2004). This was confirmed through the positive correlation between ∑16PAHs and clay (r=0.73; p=0.002). There was no significant correlation between of ∑16HPAs and OM (r=0,0568, p=0,8472). The smallest concentrations were recorded at sediments from adjacent shelf, at stations #11, #12 and #14 predominantly sandy, where the currents are strong.

The identification of major sources of PAHs is very important to better understand the processes occurring in the environment and the contribution that urbanization and hydrodynamics has upon estuary contamination. In addition, it may point out directions to the controlling agencies, in order to them take actions avoiding further contamination (Jamhari et al., 2014). Pyrolytic processes produce high molecular weight (HMW) PAHs with 4 or more aromatic rings, while two and three rings PAHs (low molecular weight - LMW) are mostly derived from petrogenic sources (Boehm, 2005). Compounds of HMW are commonly detected in urban and industrial areas, and from sugar cane soot (Simpson et al., 1996; Zamperlini et al., 2000;Yunker et al., 2002; Sprovieri et al., 2007; Zhang et al., 2011). The most abundant PAHs in the samples were fluoranthene (4 rings), pyrene (4 rings), and benzo[g,h,i]perylene (6 rings) (Table 1) representing, together, 30 to 50% of ∑16PAHs.In all samples, the sum of HMW concentrations were higher than LMW (LMW/HMW <1, Table 1), with significant differences (t=2.6277; p=0.0147), indicating pyrolytic origin predominance (Magi et al., 2002; Acquavita et al., 2014).

To estimate the sources of PAHs in the environment, the ratios between the concentrations of some pairs of isomers are commonly applied (Tam et al., 2001; Yunker et al., 2002; Yunker et al., 2014). The ratios between anthracene and anthracene more phenanthrene (Ant/178) and between fluoranthene and fluoranthene more pyrene (Fl/Fl+Py) were calculated. Ratios for Ant/178 greater than 0.1 are indicative of pyrolytic sources and less than 0.1 are indicative of petrogenic sources (Budzinski et al., 1997, Yunker et al., 2002). For Fl/Fl+Py, ratios greater than 0.5 are indicative of pyrolytic sources, and less than 0.5, petrogenic sources (Yunker et al., 2002). Based on these ratios, there was a predominance of pyrolytic contribution, corroborating to the previous results. Exceptions were observed at Stations #2 and #8 that indicated petrogenic prevalence based on the Fl/Fl+Py ratio (<0.4), as well as at Sts #3 and #5, based on the Ant/178 (<0.1), suggesting that, at these sites, there is a mixture of sources (Table 1 and Fig. 2). Sts #2, #3 and #5 are located in the upper portion of the estuary, at the confluence of the tidal creeks, and receives a huge load of domestic and industrial effluents mostly untreated. In addition, St# 5 is nearby a sewage treatment plant, which is not working properly. Sediments from St# 8 were sampled in front of the Port of Recife, which receives oil directly from vessels and daily harbor activities.

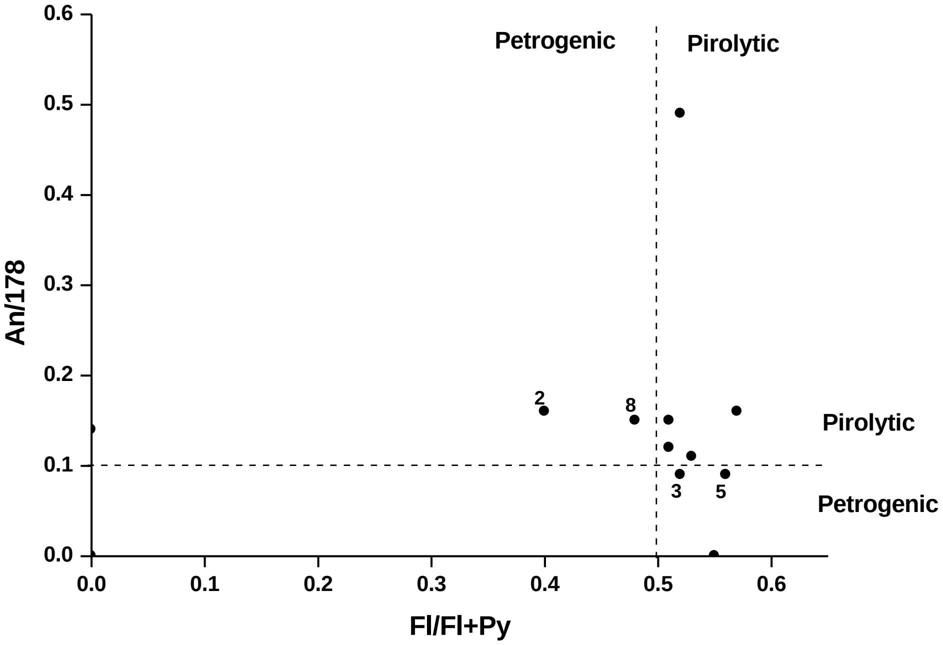

Figure 2. Plot Ant/178 versus Fl/Fl+Py ratios in sediments from Capibaribe Estuarine System and adjacent shelf .

A distinct distribution pattern was observed along the gradient estuary - platform: there is a LMW decrease from Sts #2 and #4 to St #6, and then, successively to #10,located in the lower estuary, toward to the adjacent shelf (Figure 3A). No pattern was observed at Sts #1, #3 and #5 in the upper portion of estuary. A detailed circulation pattern in this portion of this estuary could better explain the differences between Sts #1 to # 5. Unfortunately, a micro scale circulation study in the area does not exist. The grain size distribution is associated to the adsorptive capacity, since fine particles retain contaminants more efficiently than thicker ones (Dyer, 1995). The sediments from Sts #1, #3 and #5 had lower % clay compared to Sts #2 and #4, which suggest lower organic compounds retention at these sediments. These results point out the upper portion of the estuary as the main source of petrogenic PAHs to the CES and revealed the retention capacity of this estuary, acting as an important filter to the adjacent area. Similar results were observed in the same area for aliphatic hydrocarbons in sediments and dissolved and dispersed petroleum hydrocarbons in water, in previous studies (Zanardi-Lamardo, Pers. Com.). The tide regime is the main controlling agent of the CES hydrodynamics (Araújo et al., 2011) and may be the main reason for the retention effect.

A similar decrease pattern was also observed for HMW PAHs, except for St#4, that was not in the decay line, and Sts #10 and #13 that presented higher relative concentrations compared to nearby stations. As mentioned above HMW PAHs are typically associated whit airborne particles, thus are originated from different sources and spread through the air and waterways (Gao et al., 2007). Therefore, these results were already expected to CES sediments because of the likely contribution from gas, diesel, coal combustion and sugar cane soot from the Harbor of Recife, urban area and sugarcane field, surrounding the estuary and Recife metropolitan area.

Individual concentration of PAHs was compared to limits of potential toxic effect established by the Canadian Environmental Agency, the TEL (Threshold Effect Level) and the PEL (Probable Effect Level). Concentrations below the TEL usually do not cause toxic effects in organisms, while concentrations above the PEL are associated with harmful effects (CCME, 2008). None of the studied PAH presented concentration higher than the corresponding PEL (Table 1), but the dibenzo[a,h]anthracene concentration was higher than the TEL, at Sts#2, #3, #4, and #6 (Table 1). This compound has a high carcinogenic and teratogenic potential (IARC, 1983) and, at reported concentrations, could result in harmful effects to local biota.

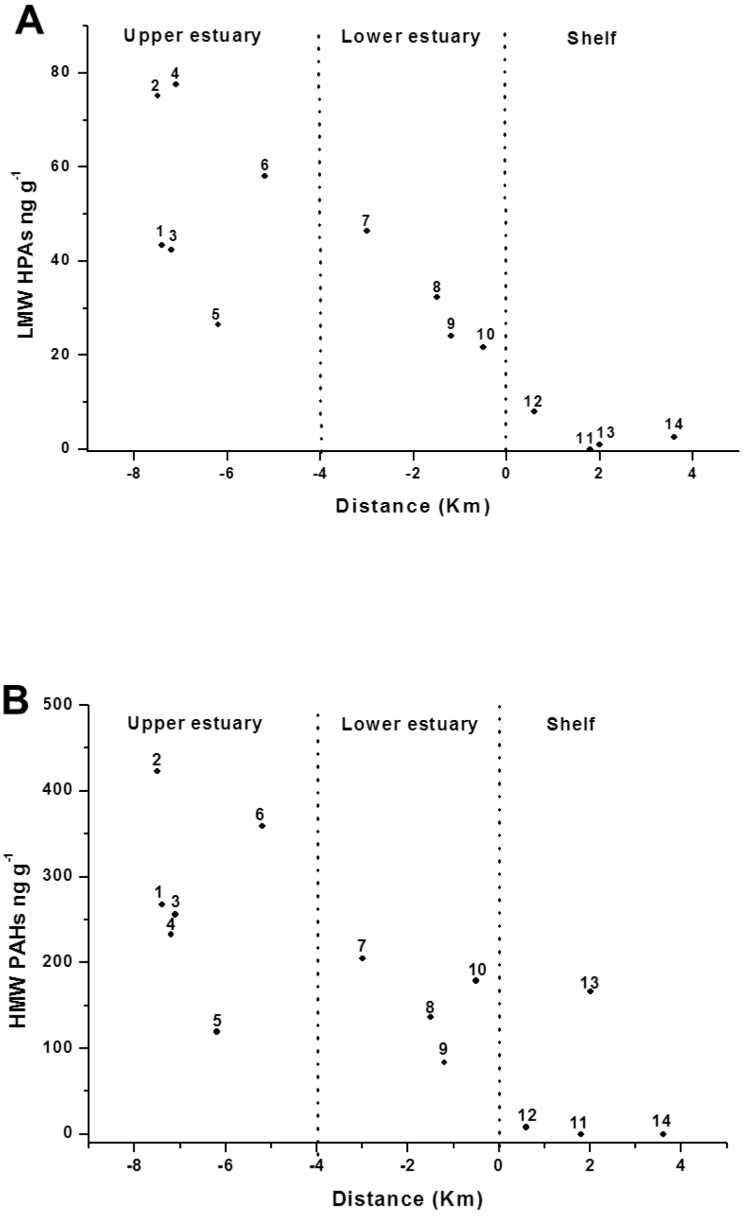

Figure 3. Distribution of Low Molecular Weight (LMW) (A) and High Molecular Weight (HMW) (B) PAHs concentrations (ng g^{-1}) in sediments along the estuary-shelf gradient. The distance reference is the estuarine mouth (0 km).

Conclusion

This study provided information about PAH distribution in sediments from the Capibaribe Estuarine System and adjacent shelf, and revealed how anthropogenic activities may influence the sediment quality. This tropical estuary is receiving PAHs from several sources, but atmospheric input seems to be more relevant. The results suggest that CES is working as contaminants retainer. PAHs are important tools to assess the environmental quality of water bodies and could be used in estuarine management plans.

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5. MANUSCRITO 3: Butilestânicos e redução de imposex em duas áreas portuárias tropicais após o banimento do TBT

Este manuscrito será submetido à Environmental Science and Pollution Research (Anexo III)

Butyltin residues and imposex assessment in two tropical harbor areasof Northeast of Brazil after TBT ban

Abstract

Butyltin (BTs) compounds have been widely used, especially for their biocide properties. Due to its high toxicity, particularly tributyltin (TBT), the use of antifouling paints containing organotin compounds (OTs) was totally prohibited. The present study aimed to investigate the presence of BTs in surface sediments of two tropical harbor areas, the Complex Estuarine System (CES), were is located the Port of Recife and at Suape Estuary, were is located the Port of Suape (Pernambuco State).. Imposex incidence in *Stramonita rustica* populations was evaluated in areas living in of these harbors, and the results were compared to previous studies. The BTs in sediments were analyzed gas chromatograph coupled to a mass spectrometer. Butyltins concentrations ranged from < LOQ to 541.69 ngSn g $^{-1}$dry weight of sediment. TBT levels were relatively high when compared to others areas wordwilde after ban, howerver imposex in *S. rustica* was not observed in the two areas studied. Butyltin Degradation Index indicates recent input of these compounds and it can be attributed to anthropogenic activities, mainly from harbor and maintenance of small boats. The recent TBT input may lead to new imposex events, including in onther gastropod species, and emphasizes the need of more rigorous controls by the government.

Key words: Contamination, sediment, tributyltin

Introduction

Organotin compounds (OTs), particularly tributyltin (TBT), have been widely used as biocides in antifouling paints since the 1950s. Due to their high toxicity to organisms several countries, including the United Kingdom, France, Switzerland, the United States of America and Japan approved regulatory actions for the use of OT-based antifouling paints since the 1980s (Champ 2000). Some years later, the International Maritime Organization (IMO) promoted the International Conference on the Control of Harmful Anti-fouling Systems for Ships, and proposed a document regulating the use of TBT in naval protective systems, and the total ban in September 2008. Since then, some studies have reported a decrease of OT levels in water, sediment and biological tissues from several coastal areas including South Korea, Netherlands, Germany, Belgium and United Kingdom (Choi et al. 2009; Kim et al. 2011;Verhaegen et al. 2012; Law et al. 2012). However, other studies have reported high OT levels in the environment (e.g. Brazil, France, Venezuela), suggesting that TBT-based antifouling paints are still in use in some areas even after the ban (Oliveira et al. 2010; Briant et al. 2013; Paz-Villarraga et al. 2015).

In aquatic environments, OTs have high affinity for particles and are generally accumulated in sediments nearby the input sites (Pinochet et al. 2009). TBT previously accumulated in surface sediments can act as a secondary contamination source to water column and biota (Kim et al. 2011). Studies carried out worldwide have shown the effects of these pollutants on marine organisms (Thain, 1986; Huang and Wang, 1995; McAllister and Kime, 2003;Abidli et al. 2012; Graceli et al. 2013). The most significant effect is associated with endocrine disruption that affects reproduction of marine organisms, especially gastropod mollusks. In these animals, TBT may trigger imposex which is characterized by the appearance of vas deferens and penis in female individuals. At advanced stages, imposex may lead to full obstruction of the palial oviduct, resulting in sterilization (Bettin et al. 1996).

In Brazil, the International Convention on the Control of Harmful Antifouling Systems on Ships (AFS) came into force only in 2010, after approval by the Federal Senate (Decree No. 797/2010). Local authorities are still struggling to control the use of TBT-based antifouling paints but, recent assessments in southern and southeastern Brazil have detected OTs in sites under influence of commercial ports, marinas and shipyards (Santos et al. 2009; Oliveira et al. 2010; Castro et al. 2012a; Buruaem et al. 2013; Sant'Anna et al. 2014). In addition, studies along the Brazilian coast have reported the occurrence of imposex in gastropods, evidencing that the TBT issue is far from being solved (e.g. Fernandez et al. 2002; Castro et al. 2007; Castro et al. 2012a;Borges et al. 2013). In northeastern Brazil, imposex was recorded in *Stramonita rustica* populations in the vicinity of the ports of Recife and Suape (Castro et al. 2007). These ports are very important for national and international trade, receiving ships and cargo from several countries. Despite the occurrence of these biological effects, no studies were carried out for determining BTs concentrations in environmental matrices in these areas.

This is the first study that investigated BTs occurrence in surface sediments from these two tropical estuaries: Suape and Capibaribe Estuarine System, northeastern Brazil. The occurrence of imposex in *Stramonita rustica* populations in the waters surrounding the ports of Suape and Recife, located at CES, was also evaluated. A temporal evaluation is also presented comparing present and previous imposex studies in these areas.

Material and methods

Study area

The State of Pernambuco is located on the northeastern coast of Brazil - a tropical area under direct influence of the intertropical convergence zone (ITCZ). Seasonal ITCZ motions affect rainfall along the coast of Pernambuco. Typically, wet season is observed from March to August while dry season is observed from September to February (Araújo and Pires 1998).

There are two major commercial harbors along the coast of Pernambuco: the ports of Suape (8°23'50.92"S, 34°57'17.60"O) and Recife (8° 3'15.16"S, 34°52'6.48"O) which are located about 40 km apart (Fig.1). Suape Estuary presents a high ecological and socio economic importance, not only for Pernambuco state, but also for the international communities. Suape is the largest port on the northeastern coast and has the biggest shipyard of South America. More than 15 million tons of cargo went through the terminal in 2014 (Porto de Suape 2015). After the implementation of the port, the area underwent several changes, especially in terms of local hydrodynamics and pollution and degradation degree (Souza and Sampaio, 2001). The Port of Recife is on the Capibaribe Estuarine System (CES) - a complex mosaic of channels that drain one of the most populated urban areas on the eastern coast of South America. It is characterized as a dynamic system and the circulation is a balance between the tidal regime and the freshwater outflow, the latter being modulated seasonally according to ITCZ motions. Besides the Port of Recife, several marinas are located on the CES. The Port of Recife is considered one of the most important in the country, receiving long-haul and cabotage ships for import and export of domestic and foreign cargo ships. In

2014, 1.5 million metric tons of various cargo were handled at the Port of Recife (Porto do Recife 2015).

Sediment and organisms sampling

A total of 14 surface sediment samples were collected at Suape estuary: seven in July 2011 (wet season) and seven in February 2012 (dry season) (Fig. 1). Nine samples were collected at CES in December 2012 (dry season) (Fig. 1). Samples were collected with a stainless steel sediment grabber, stored in previously combusted (450 °C for 4 h) aluminum containers, and frozen (-18^{o}C) in the dark until analysis.

Up to 30 adult individuals of the gastropod *Stramonita rustica* were manually caught at each of the 14 sites sampled along the coast: 7 in the vicinity of the Port of Suape and 7 in the vicinity of the Port of Recife, in September 2012 (Fig.1). The gastropod samples were collected from sites where imposex had been previously reported (Castro et al., 2007). After collection, the animals were conducted to the laboratory, narcotized using $MgCl_2$ solution (3.5%) at 1:1 (distilled water: seawater, v/v), and shell lengths were individually measured with a calliper. Shells were then opened and the soft parts were removed. Sex identification was confirmed by the presence of prostate gland in males, and albumen gland, sperm-ingesting and capsule glands in females. The penis length of males and imposex affected females were also measured. Imposex levels were assessed using the following indices: proportion of imposex in females (I%), female penis length index (FPL), relative penis length index (RPLI), and the vas deferens sequence index (VDSI). FPL is the mean penis length of all females in the population including zero values. RPLI is the ratio between mean penis length in females and mean penis length in males, multiplied by 100 (Gibbs and

Bryan 1987). VDSI is based on the development of male sexual characters (particularly the vas deferens) by females. The index was evaluated according to Toste et al. (2013).

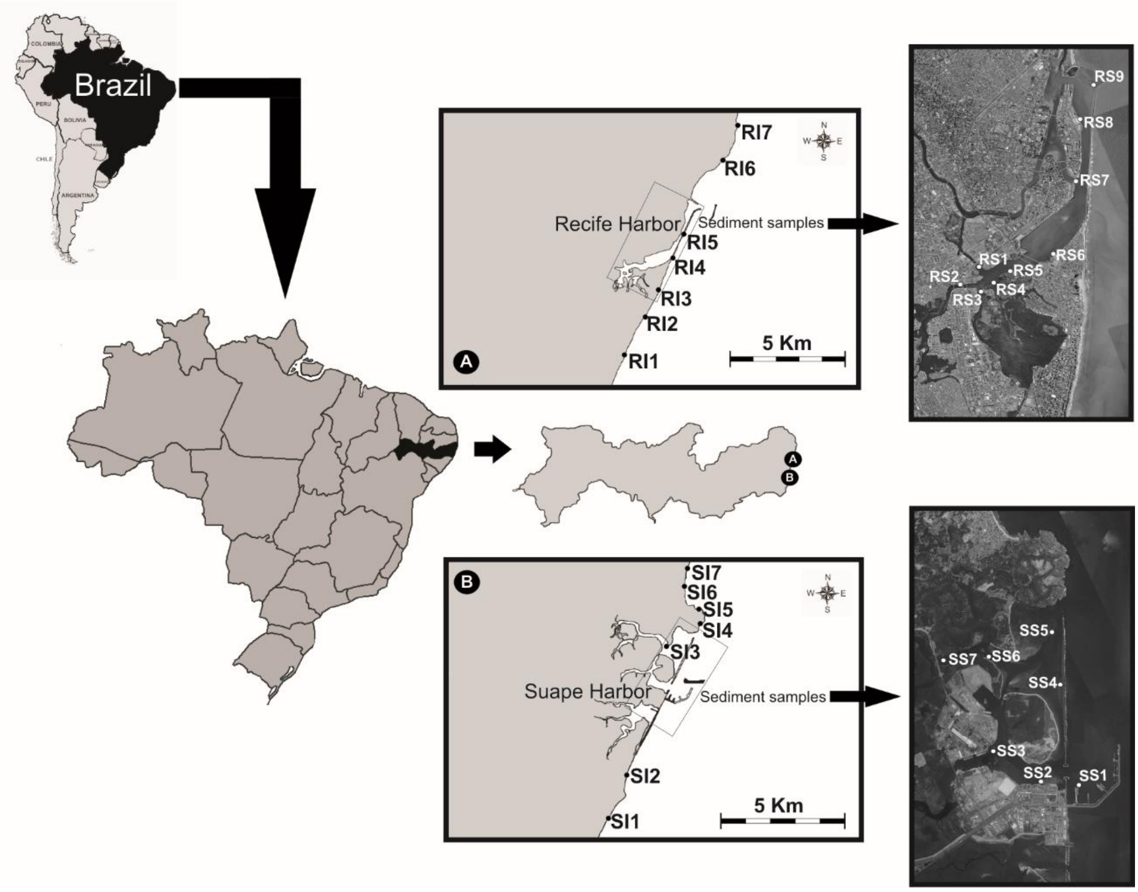

Fig 1. Geographical setting of the study area and location of the sampling sites in the vicinity of the ports of Recife (A) and Suape (B), northeastern Brazil. Sediment samples were collected at sites RS1 to RS9 (Recife), and sites SS1 to SS7 (Suape). Biota samples were collected at sites RI1 to RI7 (Recife), and sites SI1 to SI7 (Suape). In the text samples from dry season are noted as DS and from wet season as WS.

Sediment properties and butyltins determinations

Grain size distribution were gravimetrically determined as sand, silt and clay percentage (Suguio, 1973). Total organic carbon (%TOC) was determined using an elemental analyzer (CHNS Perkin Elmer 2400 Series II). Prior to the analysis, sediment samples were decarbonated in a desiccator containing HCl (37%) vapors.

Butyltins (TBT,DBT and MBT) extraction, derivatization, clean up and analysis followed a procedure described by Castro et al. (2012b). Briefly, 5 g of freeze dried sediment was weighed in 40 mL vials and spiked with 100 ng of tripropyltin (TPrT) as surrogate standard. Organotins were extracted with a mixture of 15 mL of 0.05% tropolone solution in methanol (w/v) and 1 mL of HCl (37%, m/m) in ultrasonic bath for 10 minutes. The extract was concentrated to approximately 2 mL and derivatized with 2 mL of pentylmagnesium bromide in diethylether solution (2 M). The pentylatedbutyltins were recovered by a liquid-liquid three extraction with 5 mL of hexane. The extracts were eluted with 15 mL of n-hexane/toluene (1:1, v/v) in a silica gel chromatography column. Finally, the cleaned up extract was concentrated down to 0.9 mL under a gentle nitrogen flow, and 100 µL of tetrabutyltin solution (1000 ng Sn mL^{-1}) was added as internal standard. Extracts were analyzed in a Perkin Elmer Clarus 500MS gas chromatograph (GC) coupled to a mass spectrometer (MS). The GC was equipped with an Elite-5MS (5% diphenyldimethylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 µm). Quality control was based on regular analyses of blanks, spiked matrices and certified reference material (PACS-2, National Research Council, Ottawa, Canada). Results obtained for the PACS-2 were in good agreement with certified and reported values. The samples recoveries were between 64.18% and 97.12% and RSD (relative standard deviation) below 20% (IUPAC, 2002). All concentrations were reported as ng Sn g^{-1} (dry weight). Quantification Limits (LOQ) were 2.5, 3.0 and 3.0 ng Sn g^{-1} for TBT, DBT and MBT, respectively.

Statistical analysis

Data were tested for normality using the Kolmogorov–Smirnov test. Spearman non-parametric correlation analysis was used to investigate the relationship between the TOC, silt and clay and BTs concentrations. Student's t-test was used to assess the differences between the BTs concentrations during the wet and dry season in Suape estuary. Principal component analysis (PCA) was used in order to evaluate the relationships between the butyltins content and the sediment properties. All statistical analyses were performed by BioEstat5.0 and Statistica 8.0 software using a significance level of 0.05.

Results and discussion

Sediment grain size distribution and TOC content

The grain size distribution at Suape Estuary, in the wet season, was very heterogeneous, and the percentage of sand ranged between 1.18% and 98.59%, silt 1.08% and 55.00%; and clay 0.27% and 59.20% (Table 1). In dry season, except for stations SDS2 and SDS3, there was a predominance of sandy fraction. TOC varied between 0.09% and 2.47% during the wet season and 0.09% and 2.31% in dry season (Table 1). At CES, sediment samples consisted mostly of mud (Silt and Clay), except site #RDS3, with 78.9% of sand. The percentage of TOC in sediments ranged from 2.2% to 3.5%.There was no significant correlation between TOC and the grain size fractions in both sampling seasons at Suape and CES.

Butyltins concentrations in sediments

During the wet season, the concentration of ΣBTs (sum of TBT, DBT and MBT) in sediments from Suape Estuary ranged from < LOQ to 78.39 ng Sn g^{-1} (Table 1). The TBT concentrations ranged from < LOQ to 61.37 ngSn g^{-1}, DBT from <LOQ to 9.29 ng Sn g^{-1} and MBT range from <LOQ to 7.73 ng Sn g^{-1} (Table 1). The highest concentration of BTs was observed at #SWS1 (78.39 ng Sn g^{-1}) that is located in a sheltered spot, next to a dock in the outer port. The low hydrodynamics at this site may

favor sediment deposition and, consequently, contaminants accumulation. In most of the samples, the TBT concentrations were more than 70% of total BTs

In dry season, BTs concentrations ranged from < LOQ to 229.33 ng Sn g^{-1}. TBT, DBT, and MBT concentrations ranged from < LOQ to 130.98 ng Sn g^{-1}, < LOQ to 68.54 ng Sn g^{-1} and < LOQ to 29.81 ng Sn g^{-1}, respectively. The highest concentrations of BTs were recorded at station #SDS4 (229.33 ng Sn g^{-1}), located in a protected area, behind a reef, suggesting that this station also is a deposition area. BTs levels at site #SWS1/#SDS1 were similar in both seasons. The main channel of the port is periodically dredged and, in fact, this practice was occurring at the moment of Feb/2011 sampling (dry season), which can explain the observed decrease in concentrations at #SDS2 and #SDS3. Sediment contamination at sites #SDS5, #SDS6 and #SDS7 was observed during the dry season only.

The BTs showed a strong correlation with silt during the wet season ($r^2 = 0.8154$, $p = 0.0254$). There was no significant correlation between BTs and mud, BTs and their derivatives with clay and TOC ($p>0.05$). Shim et al. (1999) and Oliveira et al. (2010) also reported weak or no correlations for these parameters. Despite the expected correlation with organic carbon (Antizar-Ladislao 2008), sediment complexity such as different organic matter composition, adsorption onto inorganic particles, sediment biological activity, and BT sources may influence this association (Town and Filella 2002).

Except for #SDS2 and #SDS3, samples from dry season showed higher concentrations of BTs than those from wet season (Table 1). The lower precipitation during the dry season may corroborate with a dilution decrease of contaminants that are rapidly adsorbed to the suspended particulate material. In adition, a sediment toxicity study at Suape estuary, comparing the wet and dry season, reported significantly higher mortality of copepods in sediments collected in the dry season (Araújo-Castro et al.

2003). It is important to reinforce that stations #SDS2 and #SDS3 are in an area where dredging is a commum practice. Dredging activities may play an important role in the distribution of BTs in surface sediments in addition to rainfall and might affect remobilization and bioavailability of contaminants since it promotes mixing of anoxic and biologically active surface sediments (Eggleton and Thomas, 2004).

Table 1. Grain size (%), total organic carbon (TOC, %), concentration of organotins (ng Sn g^{-1}dry weight) and butyltin degradation index (BDI) in surface sediments collected in the vicinity of the ports of Suape (Suape Estuary) and Recife (Capibaribe Estuarine System – CES), northeastern Brazil.

Station	Sand	Silt	Clay	TOC	TBT	DBT	MBT	∑BTs	BDI
Suape WS									
SWS1	2.60	55.00	42.40	1.55	61.37	9.29	7.73	78.39	0.78
SWS2	28.57	27.84	43.59	1.10	48.26	< LOQ	< LOQ	48.26	1.00
SWS3	1.18	39.47	59.20	1.28	< LOQ	5.31	< LOQ	5.31	0.00
SWS4	52.28	21.78	25.68	0.09	41.68	7.17	7.39	56.24	0.74
SWS5	98.59	1.08	0.27	0.09	< LOQ	< LOQ	< LOQ	< LOQ	***
SWS6	97.42	1.76	0.44	0.20	< LOQ	< LOQ	< LOQ	< LOQ	***
SWS7	53.75	14.45	31.80	2.47	< LOQ	< LOQ	< LOQ	< LOQ	***
Suape DS									
SDS1	67.48	12.94	18.53	0.59	54.97	8.48	17.57	81.02	0.68
SDS2	1.77	29.82	68.33	1.35	< LOQ	3.68	< LOQ	3.68	0.00
SDS3	45.89	27.96	25.89	1.96	< LOQ	< LOQ	< LOQ	< LOQ	***
SDS4	89.30	8.56	2.14	1.14	130.98	68.54	29.81	229.33	0.57
SDS5	99.49	0.41	0.10	0.12	6.64	< LOQ	3.68	10.33	0.64
SDS6	100.00	0.00	0.00	0.09	5.92	< LOQ	< LOQ	5.92	1.00
SDS7	72.85	9.25	17.23	2.31	13.54	3.26	13.97	30.77	0.44
CES DS									
RDS1	26.64	50.19	23.17	3.48	20.15	21.93	24.81	66.89	0.30
RDS2	20.96	41.99	37.05	2.73	< LOQ	16.70	37.82	54.52	0.00
RDS3	78.99	14.01	7.01	2.21	< LOQ	4.56	9.41	13.97	0.00
RDS4	42.36	30.31	27.33	2.68	338.70	116.21	86.78	541.69	0.63
RDS5	51.79	38.36	9.86	2.62	76.35	69.07	42.18	187.60	0.41
RDS6	20.88	40.50	38.62	2.77	< LOQ	4.93	16.39	21.32	0.00
RDS7	3.62	59.01	37.37	2.56	20.74	6.73	11.39	38.86	0.53
RDS8	21.67	46.98	31.34	2.20	193.21	64.85	23.28	281.35	0.69
RDS9	19.72	79.70	0.58	2.68	37.83	< LOQ	< LOQ	37.83	1.00

*** Not possible to calculate; WS: wet season; DS: dry season; LOQ: Limit of quatification

According TBT levels sediments can be classified as slightly contaminated (10 to 50 ng Sn g^{-1}), moderately contaminated (60 to 200 ng Sn g^{-1}) and highly contaminated (300 to 1000 ng Sn g^{-1}) (Waite et al. 1991). During the wet season, the sediments from #SWS1 and #SWS2 of Suape Estuary could be classified as moderately contaminated, and station #SWS4, as slightly contaminated. In the dry season, the stations #SDS1 and #SDS4 presented moderate contamination and station #SDS7 lower contamination.

At CES, BTs concentrations ranged from 13.97 ng Sn g^{-1} to 541.69 ng Sn g^{-1}. Concentrations of TBT, DBT and MBT ranged from < LOQ to 338.70 ng Sn g^{-1}, < LOQ to 116.21 ng Sn g^{-1} and < LOQ to 86.78 ng Sn g^{-1}, respectively. There was no significant correlation between BTs and either TOC (rs=-0.0669, p=0.8641) or mud (rs=-0.25, p=0.5165). The highest concentrations of BTs were recorded at sites #RDS4 (541.69 ng Sn g^{-1}) and #RDS8 (281.35 ng Sn g^{-1}). The TBT levels, at #RDS4, represents 62% of BTs, suggesting a recent input. This site is located close to Pina river that houses some marinas, docking for boat repairs, beside a fishermen community. The recent inputs of TBT in environment have been associated with antifouling systems used in small boats. High BTs contamination from pleasure and fishing boats, was reported nearby marinas from Venezuela (Paz-Villarraga et al. 2015). BTs in sediments and imposex in marine gastropods from Paraty, Brazil, were attributed to recent inputs of TBT linked to illegal use by small vessels (Borges et al. 2013). The high concentration of TBT reported in front of the Port of Recife (#RDS8) also suggests that there is recent input of this compound, probably coming from port activities. According to the classification proposed by Waite et al. (1991), the stations #RDS2, #RDS3, #RDS6, #RDS7 and #RDS9 were classified as slightly contaminated, stations #RS1,

#RDS5 and #RDS8 as moderately contaminated and stations #RDS4 as highly contaminated.

The highest concentrations observed in sites inside CES are likely due to the intense traffic of small boats. Although the manufacture of TBT-based antifouling paints in Brazil is prohibited since 2007 (NORMAN23/DPC), active ingredients have been reported to be purchased separately from vessel paints and both mixed prior to hull painting (Castro et al. 2012).

TBT concentration in sediments from Suape and Capibaribe estuaries are higher than those found in coastal areas such as Todos os Santos Bay, Brazil (Felizzola et al. 2008); Maizuru Bay, Japan (Eguchi et al. 2010); Zuari estuary, India (Garg et al. 2010); Bolivar harbor, Ecuador (Castro et al. 2012b); Elbe estuary, Germany (Wetzel et al. 2013); Santos, Brazil (Buruaem et al. 2013); Zhuhai, China (An et al. 2013); Kaohsiung Harbor, Taiwan (Dong et al. 2014) and Szczecin Lagoon, Baltic Sea (Filipkowska et al. 2014) (Table 2). Similar concentrations observed in this study were recorded in Jinhae Bay, Korea (Kim et al. 2014). However, compared with some coastal areas, including Itajaí, Brazil (Oliveira et al. 2010); Port of Gdynia, Poland (Radke et al. 2012); Port Camargue, France (Briant et al. 2013) and Caribbean Sea, Venezuela (Paz-Villarraga et al. 2015) concentrations observed in this study were significantly lower (Table 2). Although a reduction in BTs environmental levels is expected in every ocean around the world soon after the implementation of national and international restriction regulations, this will depend on the effectiveness of global TBT ban, the efficacy of local restrictions on production, sale and use of TBT-based antifouling paints and specific characteristics of local sediments (Castro et al. 2012b).

TBT half-life in sediments is variable, may range from one year to more than one decade in anoxic sediments for example (Dowson et al. 1996). In the environment of TBT degradation may occur by various processes, such as ultraviolet radiation, chemical cleavage and biological cleavage, the latter being more common in aquatic environments. (Hock, 2001). To estimate the TBT in the environment is from recent introduction, the butyltin degradation index (BDI) is commonly applied (Oliveira et al. 2010; Castro et al. 2012c; Wetzel et al. 2013; Paz-Villarraga et al. 2015). In this study, the BDI was modified from Díez et al. (2002) and calculated using the equation: $BDI = [TBT]/[MBT + DBT + TBT]$ (Table 1). Values higher than 0.5 are indicative of recent TBT input. In wet season, BDI was higher than 0.5 in stations #SWS1, #SWS2 and #SWS4. In dry season, the stations #SDS1, #SDS4, #SDS5 and #SDS6 showed values higher than 0.5. At CES, BDI was higher than 0.5 at sites #RDS4, #RDS7, #RDS8 and #RDS9. These results pointed out that despite the ban of TBT-based antifouling paints, there still are fresh input of TBT in the two studied areas.

Table 2. Monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT) concentrations (ngSn g^{-1}dry weight) in coastal sediments all over the world.

Location/Country	Sampling year	MBT	DBT	TBT	Reference
Itajaí, Brazil	2008	<dl - 312.2	<dl - 394.4	<dl - 1136.6	Oliveira et al., 2010
Paraty, Brazil	2004-2011	10.1 - 10.2	10.1 - 16.4	16.0 - 205.7	Borges et al., 2013
Santos, Brazil	2007	9.98 - 14.98	14.7 - 15.26	13.72 - 159.2	Buruaemet al., 2013
Port Camargue, France	2009	5 - 710	1 - 5066	1 - 10,738	Briant et al., 2013
Maizuru Bay, Japan	2007	6.5 - 17	1.8 - 8.5	0.9 - 4.7	Eguchi et al., 2010
Zuari estuary, India	2007	3.2 – 68.5	1.1 –29.8	2.1 –64.1	Garg et al., 2010
Port of Gdynia, Poland	2009	134- 968	250 - 2716	1143 - 6408	Radke et al., 2012
Jinhae Bay, Korea	2010	31 - 248	14 - 196	9 - 469	Kim et al., 2014
Sundarban mangrove, India	2009	<dl - 48	<dl - 26.4	<gl - 84.2	Antizar-Ladislao et al., 2011
Bolivar Harbor, Ecuador	2009	44 - 339.9	1.8 - 54.4	12.7 - 99.5	Castro et al., 2012a
Elbe estuary, Germany	2011	<ld - 21	<ld – 10	<ld - 41	Wetzel et al., 2013
Szczecin Lagoon, Baltic Sea	2009	<ld - 33.97	<ld - 38.66	2.23 – 115.2	Filipkowska et al., 2014
Kaohsiung Harbor, Taiwan	2009	0.7 - 5.4	0.5 - 26.1	2.3 - 27.6	Dong et al., 2014
Caribbean Sea, Venezuela	NI	10.4 - 456	14.9 - 990	57.0 - 1929	Paz-Villarragaet al., 2015
Zhuhai, China	2007	9.0 - 136	2.7 - 30.5	<ld - 86.0	Zhang et al., 2013
Suape estuary, Brazil	2011-2012	<ld - 29.81	<ld - 68.54	<ld - 130.985	This study
CES, Brazil	2012	<ld - 86.78	<ld - 116.21	<ld - 338.70	This study

NI=not informed, <dl = detection limit

The PCA results are given in table 3 and fig. 2. The three main components explained together 97.5% of variances. The first component (PC1) explained 44.96% of the total variance, and showed higher and positive correlation with all BTs (Figure 2A). This suggests that the butyltins are from similar sources and undergo by similar processes of accumulation and degradation in both studied areas. Similar results were reported by Filipkowska et al. (2014) in coastal zone of the Southern Baltic Sea. The second component (PC2) (30.00%) showed higher and positive correlation with sand and negative with silt, clay and TOC (Figure 2A). These results indicated that the mud and TOC are not influencing the distribution of compounds, confirming the non-correlation observed between the BTs and their derivatives with the sedimentary parameters, except for the BTs and silt at Suape, during the wet season. The first component showed a positive association between the stations SWS4 during the wet season, and #RDS4, #RDS5 and #RDS8 (Fig. 2B). The RDS4 was the most influenced by PC1 confirming that this component is associated to concentrations of BTs, and showed a negative association between the #SWS5/#SDS5 and #SWS6/#SDS6, which had a lower level of contamination. The influence of the PC1 was not observed on other stations. Based on PCA results, the most critical stations in both study areas are #RDS4, #RDS5 and #RDS8 in the CES and #SWS4/#SWS4 in Suape, in both seasons. The PC2 showed a positive correlation with the stations #SWS4/#SDS4, #SWS5/#SDS5 and #SWS6/#SDS6, #SDS1 in dry season, and #RDS4 in the CES (Fig. 2B). Except the station #RDS4, the others presented higher contents of sand. Stations #RDS1, #RDS2, #RDS6, #RDS7, #RDS9 and #SWS1, #SWS3 in wet season and SDS2 in dry season are negatively influenced by PC2, and these stations presents higher contents of mud. These results reinforce the idea that the component 2 may be associated with sediment properties.

Table 3. Correlation coefficient from principal component analysis (PCA) based on sediment properties and BTs concentrations from Capibaribe Estuarine System and Suape Estuary.

Variables	PC1	PC2	PC3
Sand	-0.332671	0.915724	-0.157094
Silt	0.410994	-0.766479	0.314061
Clay	0.139028	-0.776339	-0.066982
TOC	0.523846	-0.574131	-0.280719
TBT	0.914258	0.288999	0.107950
DBT	0.925084	0.282225	-0.137453
MBT	0.898909	0.186206	-0.260154
BTs	0.949276	0.281798	-0.007207
BDI	0.269300	0.253076	0.906738

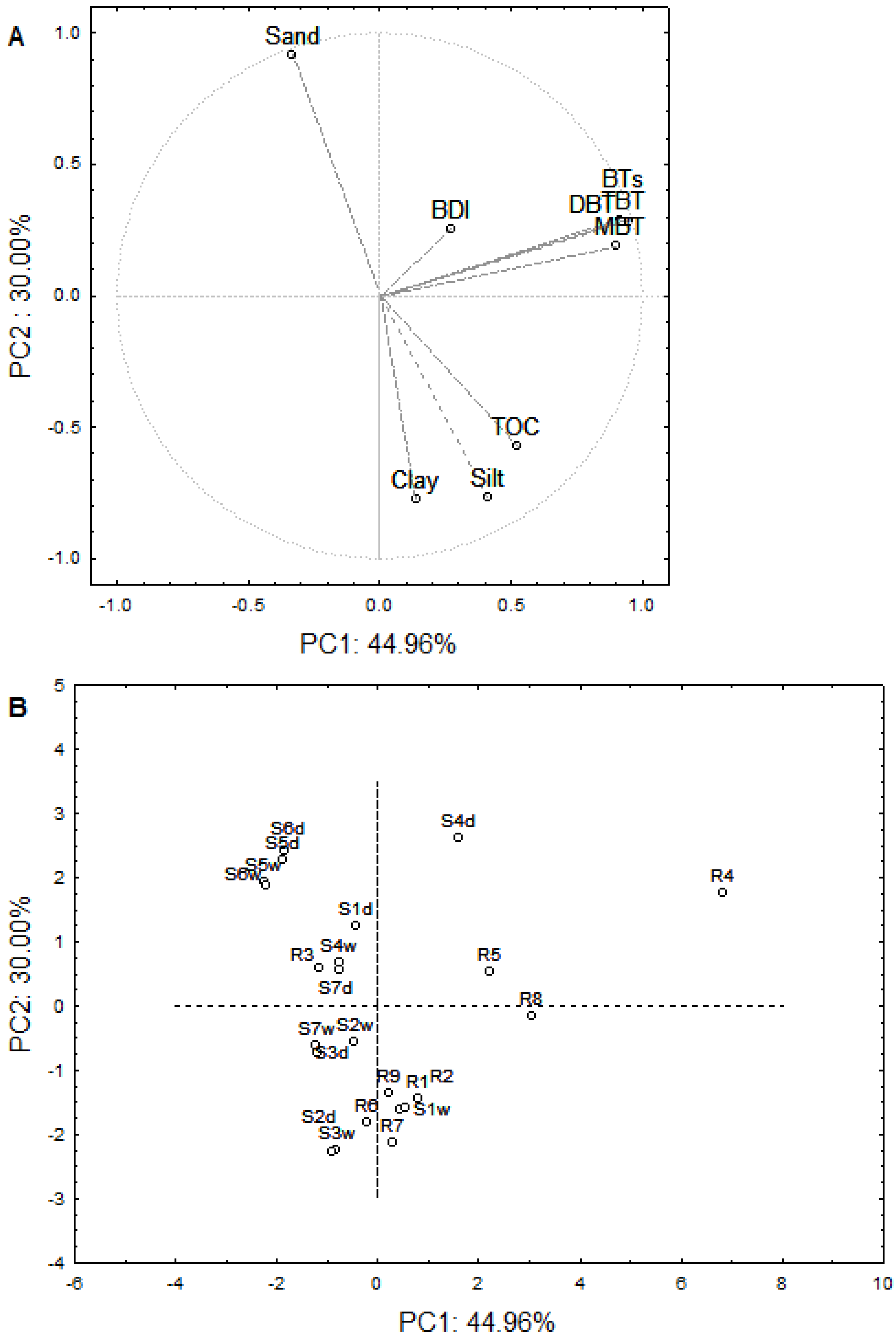

Fig 2. Ordination results of the principal component analysis (PCA) based on sediment properties and BTs concentrations (A) from stations (B) located in the Capibaribe Estuarine System and Suape Estuary.

Imposex levels

Despite high TBT levels observed in surface sediments, imposex was not detected in the organisms collected in the 14 sites (Recife and Suape harbors). These results were different than those observed in 2004, using the same species (*S. rustica*), collected at the same sites in this study. In the previous study the imposex incidence was detected in 3 (RI5, RI4 and RI3) of 7 sampled sites under influence of Port of Recife (Castro et al. 2007). Similarly, in Suape harbor, the same study showed imposex incidence only in SI3. These findings suggest that current TBT concentrations in sediments from Recife and Suape influence areas are lower than were in the past, corroborating with other studies that reported a decrease in the incidence of imposex in Brazilian coastal areas (Castro et al. 2012a). In addition, these results corroborate with lower imposex sensibility of *T. rustica* previously reported by Castro et al. (2012c). On the other hand, Toste et al. (2011) recorded an increase in imposex levels in the gastropod *Stramonita haemastoma* in an area of the coast of Rio de Janeiro. The only site where imposex was registered in stuyd, SI3, is close to the SS6 (sediment sample), that showed evidences of recent input of TBT. Despite the complete ban of organotin compounds in antifouling paints, imposex is still detected around the world, as China (Ho and Leung, 2014); Mexico (Domínguez-Ojeda et al. 2015) and Venezuela (Paz-Villarraga et al. 2015). Therefore, studies investigating the effects of sediment contamination upon other benthonic species are essential and prominent.

Conclusions

Sediments from Suape estuary and CES are freshly contaminated by BTs suggesting that, at these areas, TBT-based antifouling paints are still being used. BTs concentrations varied widely between the two studied areas, but the contribution from port activities could be observed in both sites. The input of TBT from traffic and maintenance of small vessels is very significant, and may explain the higher TBT concentrations identified in sites away from main shipping channels.

Signs of health environmental recovery was observed based on imposex indices, but the observed recent TBT input may lead to new imposex events and emphasizes the need of more stringent controls by the government.

Acknowledgment

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6. **MANUSCRITO 4:** Avaliação da qualidade dos sedimentos de um estuário tropical usando a abordagem da Tríade.

Este manuscrito será submetido à Science of the Total Environment (Anexo IV)

Assessment of sediment quality in a tropical estuary using the Triad approach

Abstract

Many economic development centers, urban areas, harbors and industries are located adjacent to the estuaries, resulting in contamination of these areas by different kind of compounds. Due to their hydrophobic characteristics, several of these contaminants are accumulated in sediments, and the evaluation of this matrix becomes essential. This study investigated the sediment quality in different areas of a tropical estuary, the Capibaribe Estuarine System, Northeast Brazil, through the Sediment Quality Triad (SQT) and multivariate analyses. Sediments were sampled from five stations, and they were chemically analyzed, toxicological bioassays were performed and the structure of the benthic community was evaluated. The results showed that the sediments from CES are contaminated by hydrocarbons, organochlorine and metals, and these compounds are bioavailable causing toxicity to the biota, and they are affecting the benthic community structure. The integrated assessment data indicated that the upper portion of the estuary is the most degraded, and a degradation gradient may be observed from high to lower estuary. The Triad method showed to be effective for assessing the quality and environmental health of the estuarine sediments analyzed.

Key words: contamination, toxicity, benthic community, degradation.

Introduction

Estuaries are among the most affected coastal environments because they are propitious to the establishment of economic centers, ports, industries and urban occupation. As a consequence, this environment receives continuously a range of contaminants from industrial and domestic effluents or indirectly through rivers, soil leaching and precipitation (Blaber 1997; Moraes et al. 2001), resulting in depletion of resources, loss of biodiversity and environmental degradation (Clark, 1995). Many compounds are hydrophobic and tend to associate to particulate matter and their transport and fate depend on the hydrodynamics and physical-chemical properties of the system. The final destination of most contaminants is the deposition in sediments, which become a secondary and important source to the water column and biota (Chapman 1989; Kim et al. 2011). The physical and chemical processes can change the contaminants properties and, in some cases, increase their toxicity (Chapman, 1989). Therefore, the quality of sediments assessment is essential to understanding the environmental health and the potential toxicity.

Several methodologies have been developed to investigate the quality of the sediments, and integrated approaches, such as a simultaneous evaluation of chemical contamination and biological assessments (Chapman et al., 1997). The "Sediment Quality Triad" approach evaluates simultaneously the environmental contamination, toxicity on organisms and the effects of this contamination upon the population structure or communities. This approach has been used in several countries such as United States, Spain, Chile, China, Sweden and Brazil (Besser et al. 1996; Riba et al. 2004; Lee et al. 2006; Shu and Xu 2012; Ribé et al. 2012; Buruaem et al. 2013).

The Capibaribe Estuarine System (CES) (Northeast of Brazil) houses one of the most important harbors in the country, the Port of Recife, and some marinas, resulting in an intense recreational and fishing activities. There is a high density population in the estuary edges and surrounding area. The CES is formed by many rivers that cross some cities and receives a high discharge of untreated domestic and industrial effluents. Despite that, this system functions as nursery for many species, and supplies for feeding and trading to local population, emphasizing its socio-economic and ecological relevance. Regardless of the importance of the CES, few isolated studies has been performed in the area (Sericano et al., 1995; Fernandes et al., 1999; Macedo et al., 2007; Oliveira et al., 2014) but none of them used chemical and biological integrated approach. This paper presents the results of the environmental health investigation of the CES using “Sediment Quality Triad” (SQT) and reinforce the valuable contribution of this approach to understanding the social and ecological risk of this important area.

Materials and methods

Study area

The Capibaribe Estuarine System (CES) is located in Northeast of Brazil (08°04'53.0"S, 34°54'05.8"W) and presents a hot humid tropical climate, with two typical rainfall regime: a dry season, which occurs from September to February, and a wet season, which extends from March to August (Feitosa et al., 1999). The CES is a complex system formed by a group of water bodies, islands and channels, and it is inserted in the midlle of the Recife metropolitan area (RMR), one of the most urbanized areas in Brazil, with a population of about 3.7 million inhabitants (IBGE, 2010). The main water contributor is the Capibaribe River, which crosses much of the Pernambuco State, including several industrial and urban centers. Secondly, there are several small tidal creeks that consist mainly of urban drainage of RMR, turning this area into a real industrial and domestic effluents reservoir.

Sediment sampling

Sediments were sampled in five stations distributed along the CES in May, 2014 (Figure 1). The sampling sites were chosen based on previous results of hydrocarbons and organotins sediment contamination from CES (Maciel et al., 2014; Zanardi-Lamardo et al., 2014). Sediments were sampled using a stainless steel "van Veen" grab sampler. The superficial sediment layer (~2 cm) was collected and immediately stored in aluminum containers previously calcined (450° C for 4h) for organic chemicals analyses, in acid washed plastic bags for analysis of metals, and in glass containers for toxicity bioassays. The samples were transferred to the laboratory in a refrigerated case, and the toxicity bioassays were performed immediately. Samples for chemical analysis were kept at -18° C until the laboratory analysis. In parallel, three replicates samples were collected at each station also using a van Veen grab sampler, and the whole material (4.41×10^{-6} m^3) was transferred to plastic bags for benthic community evaluation.

Sediment properties

Grain size distribution was determined according to Suguio (1973), by dry sieving (grains > 63µm) and pipetting methods (grains < 63 µm) and the results were interpreted using Sysgran 3.0 software. An aliquot of 4g of sediment dried and homogenized was calcinated for 6 hours at 450 °C to determine the percentage of organic matter (OM) (Heiri et al., 2001). Total organic carbon (TOC) and nitrogenous (N) percentages were determined by an Elemental Analyzer (EuroVector, EA 3000).

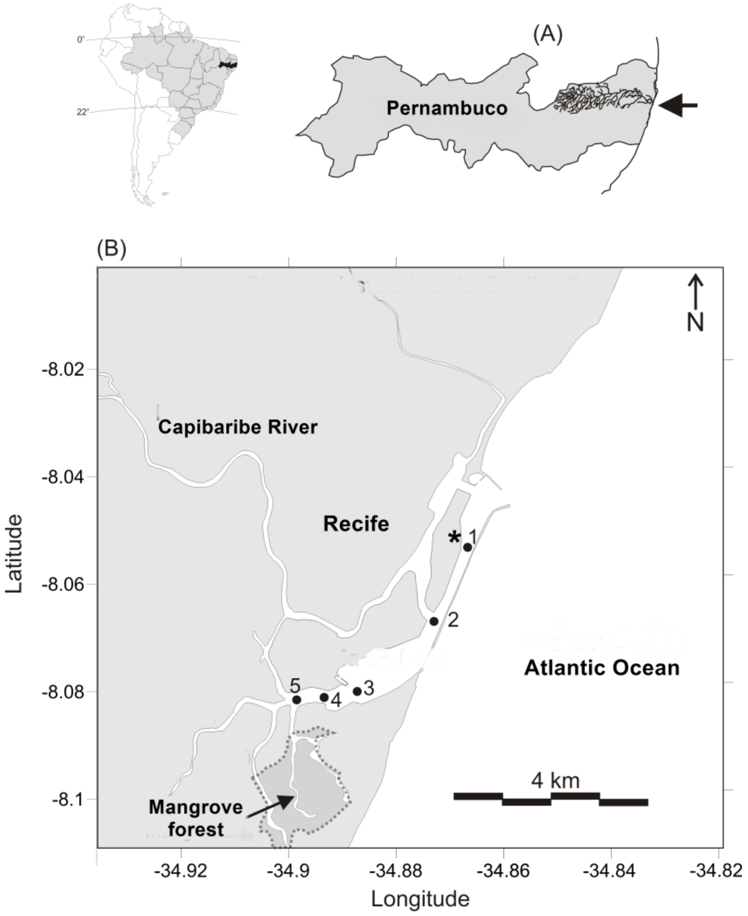


Figure 1. State of Pernambuco in South America (A) and Capibaribe Estuarine System (B) with sampling stations (1 to 5). (*) indicates the Port of Recife.

Chemical analyses

Hydorcarbons

An aliquot of 5g of sediment freeze-dried was extracted in a Soxhlet system with a mixture of n-hexane/dichloromethane (1:1, v/v) to investigate aliphatic (AHs) and polycyclic aromatic hydrocarbons concentrations (PAHs) (UNEP, 1992). The extracts were purified and fractionated by silica/alumina gel chromatography (5% deactivated) and eluted with n-hexane to separate the AHs, followed by a mixture of hexane/dichloromethane (7:3, v/v) to get the PAHs. The AHs were analyzed in a gas chromatograph (Agilent Technologies 6890) equipped with a flame ionization detector (GC-FID), and the PAHs in a gas chromatograph (Agilent Technologies 6890) equipped with mass spectrometer (GC-MS).

Quality control was based on the analysis of procedural blanks, matrix spikes and Standard reference material (National Institute of Standards & Technology-USA - SRM 1944). The recovery of the hydrocarbons surrogate standards, eicosene and hexadecene for AHs and naphthalene-D8, acenaphthene-D10 and phenanthrene-D10 for PAHs, varied between 63% and 99% and 48% and 112%, respectively. The recovery of AHs in a matrix spiked varied between 47% and 110%. The standard reference material was analyzed in duplicate and the results fell within the standard deviation for 80% of the compounds analyzed, and the relative standard deviation (RSD) varied 2% to 17%.

Organochlorine

The Organochlorine compounds were extracted in the same way that the HCs (UNEP, 1992), and the extracts were purified in alumina (5% deactivated) chromatographic column, and eluted with a mixture of hexane/dichloromethane (7:3, v/v). The compounds were analyzed in a gas chromatograph (Agilent Technologies 6890) equipped with mass spectrometer (GC-MS).

Quality control was based on regular analyses of blanks, duplicates and certified reference material (National Institute of Standards & Technology-USA - SRM 1944). The recovery of surrogate standard ranged from 62% to 122% for Polychlorinated Biphenyls (PCBs) and 64% to 113% for Organochlorine Pesticides (POC). The results of standard reference material were within the standard deviation for 80% of the compounds analyzed.

Metals

Metals analyses (Al, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Zn) were performed in sediment samples through acid digestion, and the identification was by inductively coupled plasma mass spectrometry (ICP-MS). The analyses were conducted at the SGS GEOSOL LABORATÓRIOS LTDA (http://www.sgsgeosol.com.br). The standard reference material (Canadian Certified Reference Materials TILL-3) fell within the standard deviation for all compounds analyzed. The coefficient of variation (CV) of duplicate varied from 4% to 12%.

Sediment toxicity

To evaluate the toxicity of sediments from CES, benthic copepods of the species *Tisbe biminiensis* (Volkmann-Rocco, 1973) were exposed to the muddy fraction of sediments (<63µm) and to elutriate. The endpoints were the mortality, the inhibition of the total fecundity (nauplii + copepodites) and the development inhibition (copepodites percentage). The sensitivity of the ovigerous females set was tested through bioassays performed with the reference substance Dichromate Potassium ($K_2Cr_2O_7$) (Araújo-Castro et al., 2009). The sediment control was obtained from Maracaípe Estuary, located far away from urban areas, with no ports or marinas surrounding.

Exposition to the muddy fraction

Three sub-samples of each three replicate were sieved in mesh <63 µm for associated fauna removal, and avoid predation of copepods. Two (2) g of muddy fraction of each sample were added in glass containers, added 20 mL of suspension of diatom *Chaetoceros calcitrans* (0.2 µg chl-a mL^{-1}), and incubated at 28°C under 12/12 h light/dark photoperiod (Araujo-Castro et al., 2009). After 24 h, 10 ovigerous female of

T. biminiensis with 10 to 12 days age were added to each container. Bioassays lasted seven days, keeping the same photoperiod and temperature and adding food every 2 days. At the end of the bioassay the organisms were stained with Rose Bengal and fixed with formaldehyde (4%).

Elutriate

In order to obtain the elutriate, the sediments were homogenized and mixed with 1:4 (v:v) filtered seawater in a mechanical shaker for 30 minutes (Lazorchak and Josephs, 2003). After sedimentation, 25 mL of the supernatant was immediately transferred to glass recipients. Four replicates for each treatment were used. 1 mL of the suspension of nauplii of *T. biminiensis,* with approximately 500 (±30) individuals, was added for each recipient (Lavorante et al., 2013).

Benthic community

The sediment samples were sieved in mesh size of 500 microns for separation of macrofauna. The major taxonomic groups were separated with the support of a stereoscopic microscope. Analyses and ecological indices were expressed in relation of average value for three replicates by sampling site. Density of major taxonomic groups, Polychaeta, Gastropoda, Bivalvia and others, was determined and expressed in percentage. Simpsons Diversity Index was calculated to assess and quantify the biodiversity.

Data analyses and integrated approach

The data normality was verified through the Komogorov-Smirnov test. The one-way ANOVA (α=0.05) was used to assess significant differences between the endpoints evaluated in bioassays comparing the samples of the CES and control, applying the subsequent Dunnett’s test. The lethal concentration for 50% of the organisms (LC_{50}) was calculated using the Trimmed Spearman-Karber method.

An integrated approach was made through the Sediment Quality Triad (SQT) based on Chapman et al. (1987), that compares the alteration degree at each station to a reference site, using the ratio-to-reference (RTR). Another similar approach has being used alternatively (Cesar et al., 2009; Buruaem et al., 2013) when a decontaminated site is not available and, in this case, the index RTM (Ratio-to-mean) is used. This ratio is calculated dividing the level or concentration of each variable by the arithmetic mean of all stations. This approach results are represented by triangles, and the degradation degree of sediment is related to the area of the triangles calculate by Heron's Formula: $A = \sqrt{p(p-a)} * (p-b) * (p-c)$, in which the letters a, b and c are the measurements of the sides of the triangle and p is semiperimeter. The larger the area the higher the degradation degree. A schematic representation of the components used in this study to assess the sediment quality of CES can be visualized in Figure 2. Multivariate analysis was conducted using a principal component analysis (PCA) in the software Statistica 8.0.

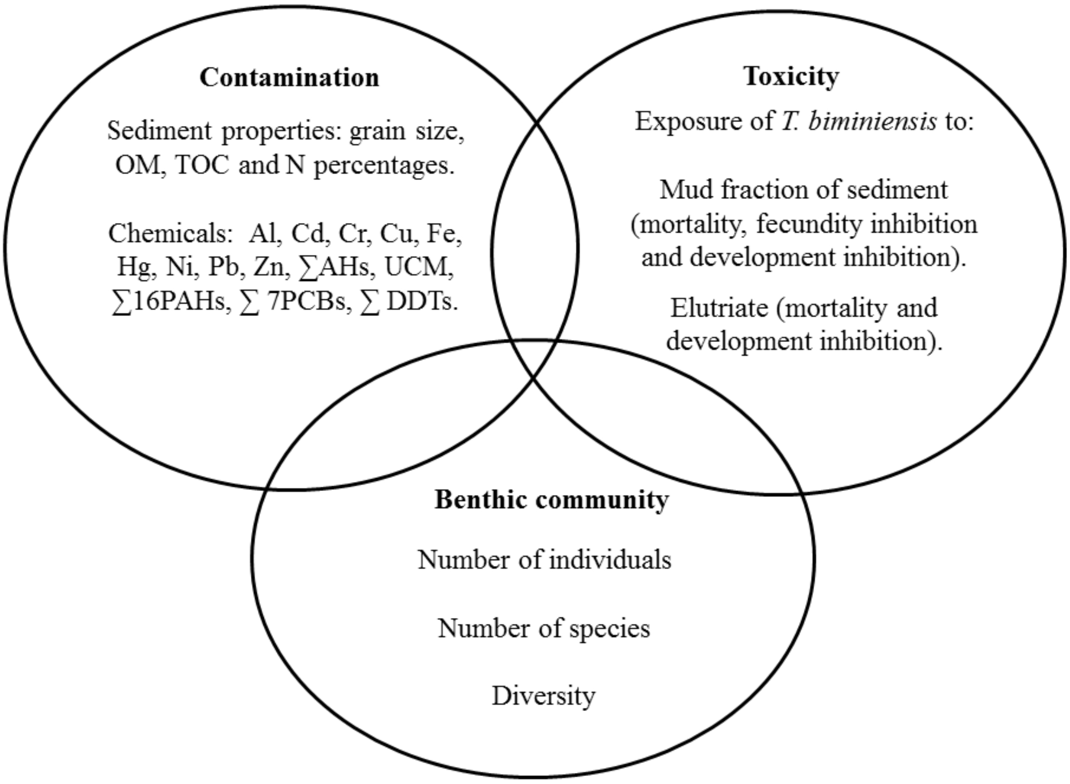


Figure 2. Schematic representation of the components used to evaluate the sediment quality of Capibaribe Estuarine System. Adapted from Chapman et al., 1997.

Results

Sediment properties and chemical analysis

The sediment properties and chemical analysis include depth sampling site, salinity, grain size, OM, TOC, Nitrogen and chemicals (Table 1). The highest percentage of mud was recorded at the mouth of the estuary (#S1, 92.3%) and at samples located at upper portion of the estuary (#S4 and #S5) with 73.1 and 66.2%, respectively. The samples from the upper estuary also showed the highest levels of organic matter (OM), total organic carbon (TOC) and nitrogen (N) (Table 1). A strong and significant correlation was observed between the TOC and OM (r=0.9095, p=0.0322) and between TOC and N (r=0.9865, p=0.0019) for all samples. The δ13C ranged between -23.5‰ and -25.4‰. Sediments from #S1, #S4 and #S5 presented the highest concentrations for most of the analyzed metals.

Table 1. Physical and chemical properties of sediments from Capibaribe Estuarine System (CES), May 2014.

			Samples				
Variables	**S1**	**S2**	**S3**	**S4**	**S5**	**ERL**	**ERM**
Depth (m)	12.5	6.6	2.1	2.2	1.4		
Salinity	24	20	25	15	12		
Sand (%)	5.6	54.1	58.1	24.0	33.5		
Mud (%)	92.3	40.8	37.1	73.1	66.2		
OM (%)	12.4	12.9	7.8	19.8	20.6		
TOC (%)	3.0	2.0	2.3	4.5	4.8		
N (%)	0.3	0.2	0.2	0.5	0.5		
δ13C (‰)	-23.5	-25.4	-25.2	-24.6	-25.3		
Al (μg g^{-1})	2.0	1.3	1.2	2.3	1.9		
Cd (μg g^{-1})	0.2	0.1	0.2	0.4	0.3	1.2	9.6
Cr (μg g^{-1})	46.0	33.0	31.0	58.0	47.0	81	370
Cu (μg g^{-1})	31.1	24.5	34.5	57.8	49.6	34	270
Fe (μg g^{-1})	2.3	1.7	1.5	2.6	2.3		
Hg (μg g^{-1})	0.2	0.1	0.1	0.3	0.2	0.15	0.71
Ni (μg g^{-1})	13.4	10.6	11.1	17.2	14.1	20.9	51.6
Pb (μg g^{-1})	24.2	17.7	25.4	35.8	31.1	46.7	218
Zn (μg g^{-1})	130.0	96.0	148.0	275.0	208.0	150	410
Total AHs (μg g-^{1})	21.9	29.2	156.3	439.7	474.2		
UCM (μg g-^{1})	<DL	<DL	121.6	386.4	404.2		
PAHs (ng g^{-1})	287.5	15999.4	469.6	561.5	538.7	4022	44792
p,p'-DDD	<DL	5.8	<DL	<DL	3.4		
o,p'-DDE	<DL	<DL	<DL	1.1	<DL		
p,p'-DDE	3.2	5.6	4.8	6.6	5.1	2.2	27
Σ DDTs (ng g^{-1})	3.2	11.4	4.8	7.7	8.5		
Σ PCB	9.4	5.1	7.4	13.1	13.3		

ERL= effects range low; ERM= effects range median; OM= Organic Matter; TOC= Total Organic Carbon; N= Nitrogen; AHs= Aliphatic hydrocarbons; UCM= Unresolved complex mixture; PAHs= ∑ 16 Polycyclic aromatic hydrocarbons; Σ DDTs = o,p'-DDT + p,p'-DDT + o,p'-DDD + p,p'-DDD + o,p'-DDE + p,p'-DDE; Σ PCB= PCB-28 + PCB-52 + PCB-101 + PCB-118 + PCB-138 + PCB-153 + PCB-180.

Total aliphatic hydrocarbons (AHs) and unresolved complex mixture (UCM) concentrations ranged from 21.9 µg g^{-1} (#S1) to 474.2µg g^{-1} (#S5) and <DL (#S1, #S2) to 404.2 µg g^{-1} (#S5), respectively (Table 1). PAHs concentrations ranged from 538.7 ng g^{-1} (#S5) to 16217.0 ng g^{-1} (#S2). The Σ DDTs ranged from 3.15 ng g^{-1} (#S1) to 11.39 ng g^{-1} (#S2), with concentrations of p,p'-DDD between 3.4 ng g^{-1} (#S5) and 5.8 ng g^{-1} (#S2), and p,p'-DDE between 3.2 ng g^{-1} (#S1) and 6.6 ng g^{-1} (#S4). The o,p'-DDE was found only at station #S4, 1.1 ng g^{-1}. Concentrations of o,p'-DDT, p,p'-DDT, and o,p'-DDE were <DL. The lowest Σ PCBs (7 PCBs) was registered at #S2 (5.08 ng g^{-1}) and the highest was at #S5 (13.30 ng g^{-1}). Except the PAHs, and DDTs, the chemicals analyzed showed a strong and significant correlation whit TOC (%) (Table 2).

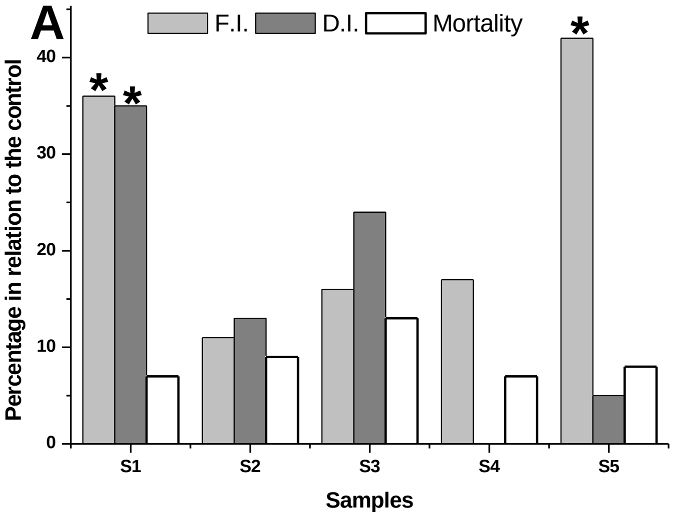
Table 2. Pearson correlation results between TOC (%) and the contaminants analyzed. Significant results are in bold ($\alpha<0.05$)

Contaminants	*R*	gl	p
∑Metals	**0.901**	3	**0.0366**
AHs	**0.910**	3	**0.0321**
UCM	**0.911**	3	**0.0313**
PAHs	-0.566	3	0.3197
PCBs	**0.983**	3	**0.0027**
DDTs	0.053	3	0.933

AHs= Aliphatic hydrocarbons; UCM= Unresolved complex mixture; PAHs= ∑16 Polycyclic aromatic hydrocarbons; Σ DDTs = o,p'-DDT + p,p'-DDT + o,p'-DDD + p,p'-DDD o,p'-DDE + p,p'-DDE; Σ PCB= PCB-28 + PCB-52 + PCB-101 + PCB-118 + PCB-138 + PCB-153 + PCB-180.

Sediment toxicity

The sediments bioassays results (mud fraction and elutriate) were normalized to the control data. A fecundity inhibition was observed in females exposed to the muddy fraction of the samples #S1 and #S5 ($F_{(5,11)}$=6.485, p=0.0052) and a significant inhibition of organisms development was observed at sample #S1 ($F_{(5,11)}$=3.4056, p=0.0419). None of the samples presented significant differences on females mortality ($F_{(5,11)}$= 1.2852, p=0.3371) (Figure 3A). For elutriate, the inhibition of the development was higher and significantly different of the control in samples #S1 and #S5 (F(5,13)= 12.3665, p=0.0003) and only at #S5 the mortality rate was statistically different from the control (F(5,13)= 164.3675, p=0.0001) (Figure 3B).



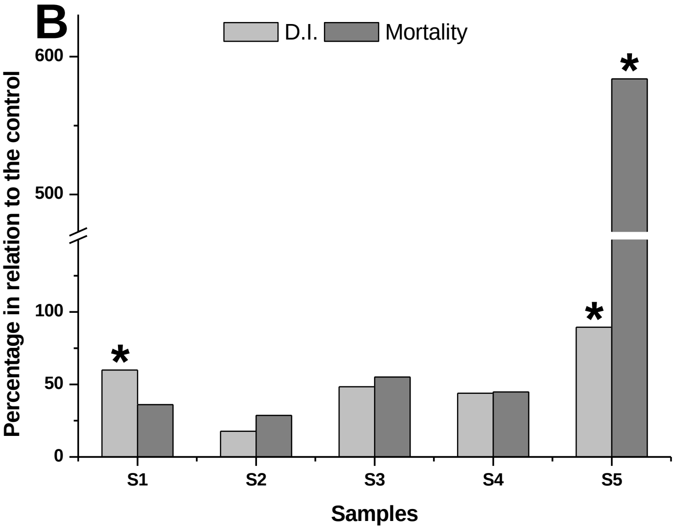


Figure 3. Fecundity inhibition (F.I.), development inhibition (D.I.) and mortality percentage related to the control in *Tisbe biminiensis* after 7 days of exposure to muddy fraction (A) and elutriate (B) of sediments from the Capibaribe Estuarine System. (*) significantly different from control.

Benthic community

Some benthic community descriptors were analyzed to investigate the environmental health (Table 3). The station #S2 showed the higher average of the total individuals, while at the station #S5 just one individual was found. Polychaeta was the most abundant group in the samples, except at #S3, where the Bivalvia was the dominant group. The most abundant species was *Streblospio benedicti*, followed by *Mytlella charruana* and *Sigambra grubii*.

Table 3. Benthic community descriptors of stations located in the Capibaribe Estuarine System, May 2014.

	Stations				
	S1	**S2**	**S3**	**S4**	**S5**
Number of species	6	5	6	2	1
Number of Individuals	38	163	49	71	1
Diversity	0.8	0.4	0.8	0.7	0.1
Polychaeta (%)	87	93	29	81	100
Bivalvia (%)	3	0	68	6	0
Gastropoda (%)	3	6	3	12	0
Others (%)	7	1	0	1	0

Integrated analysis of data

Results from contamination, toxicity and benthic descriptors were converted in indices using RTM (Table 4) and they are presented in a three-dimensional graphic (Figure 4). The triangles area ranged from 0.51 (#S2) to 1.33 (#S4) but, at station #S5, an elevated value (26.66) was observed, indicating a high degradation at this site (Figure 4). The station #S5 had the largest RTM values for toxicity and benthos and the second largest for chemical (Table 4). The RTM indices integrated (triangles area) showed the following trend for sediment degradation: #S5> #S4> #S1>#S3>S#2.

Table 4. RTM values for chemical, toxicity and benthos descriptors.

	Stations				
	S1	**S2**	**S3**	**S4**	**S5**
Chemical	0.8	0.9	0.7	1.3	1.2
Toxicity	1.2	0.6	1.0	0.5	1.7
Benthos	1.0	0.9	0.9	1.8	24.6

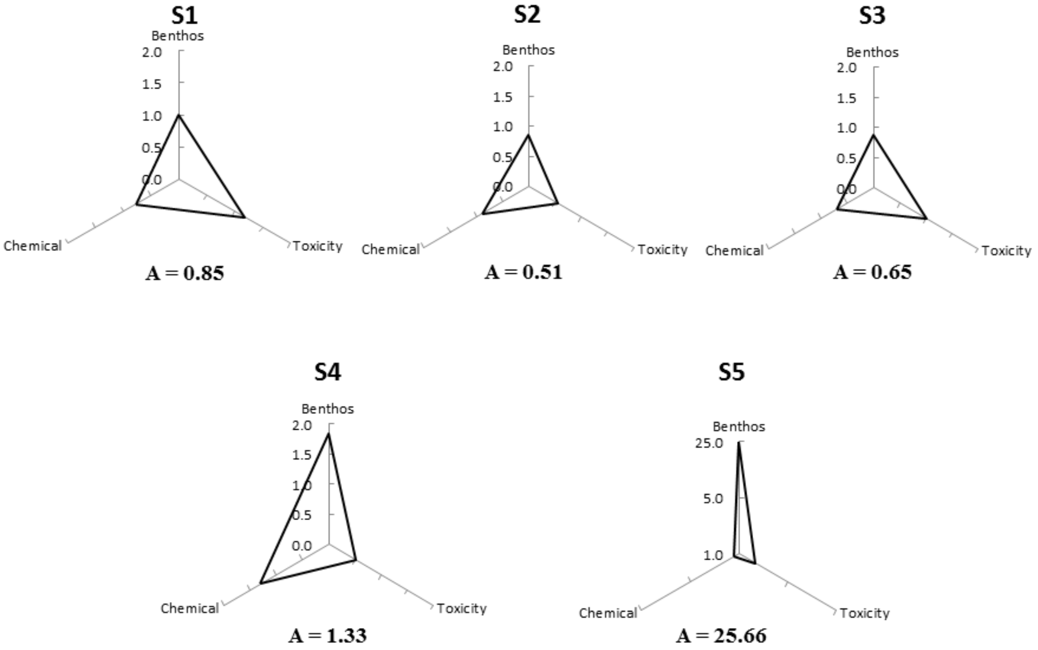


Figure 4. Ratio-to-mean (RTM) in a three-dimensional plot and respective triangle areas (A) for sediments from Capibaribe Estuarine System (CES).

The results of the PCA are given in table 5 and figure 5. The three axes (PC1, PC2 and PC3) explained together 90.9% of the variances. The first axis (PC1) showed higher correlation with mud, TOC, OM, N, AHs, UCM, PCBs, and all metals (Table 5). The PC1 represents a negative association with the number of species and individuals (Table 4). The second axis (PC2) showed higher and positive correlation with sand and ∑DDTs and negative with diversity and development inhibition (DI) in the mud fraction bioassay (Table 4). The PC3 represents a negative association between development inhibition and mortality of elutriate (Table 5). Considering the spatial distribution, the PC1 correlates positively with stations #S5 and #S4 and negatively with #S3 and #S2. This component did not explain the S1. The PC2 represents a positive association between the stations #S2 and #S5, and negative for the #S1. The findings from PCA may direct to that the component 1 reflects a pollution gradient, and there are two distinct groups: the first located in the inner part of the estuary (#S4 and #S5) and the second located at the main channel (#S3 and #S2). These data corroborate the results from the classical approach of the triangles, which showed #S5 and #S4 as the most degraded sites.

Table 5. Correlation coefficient from principal component analysis (PCA) based on chemicals, toxicity and benthos descriptors from Capibaribe Estuarine System.

VARIABLES	PC1	PC2	PC3
Sand (%)	-0.586	0.705	-0.194
Mud (%)	0.620	-0.680	0.146
OM (%)	0.871	0.345	0.118
TOC (%)	0.987	0.118	-0.094
N (%)	0.998	-0.033	-0.045
Al (μg g^{-1})	0.864	-0.343	0.343
Cd (μg g^{-1})	0.930	0.037	0.185
Cr (μg g^{-1})	0.922	-0.175	0.339
Cu (μg g^{-1})	0.933	0.196	0.038
Fe (μg g^{-1})	0.870	-0.254	0.340
Hg (μg g^{-1})	0.954	-0.018	0.258
Ni (μg g^{-1})	0.933	-0.113	0.323
Pb (μg g^{-1})	0.926	0.026	-0.002
Zn (μg g^{-1})	0.907	0.161	0.144
Total AHs (μg g-^{1})	0.869	0.419	-0.170
UCM (μg g-^{1})	0.874	0.405	-0.153
PAHs (ng g^{-1})	-0.625	0.511	0.397
Σ PCBs	0.990	-0.032	-0.130
Σ DDTs (ng g^{-1})	-0.050	0.896	0.270
FI Mud	0.517	-0.381	-0.596
Mortality Mud	-0.579	0.241	-0.496
DI Mud	-0.559	-0.796	-0.230
DI Elutriate	0.634	-0.197	-0.737
Mortality Elutriate	0.518	0.360	-0.701
Number of species	-0.841	-0.519	0.067
Number of individuals	-0.606	0.382	0.682
Diversity	-0.245	-0.680	0.373

OM= Organic Matter; TOC= Total Organic Carbon; N= Nitrogen; AHs= Aliphatic hydrocarbons; UCM= Unresolved complex mixture; PAHs= ∑ 16 Polycyclic aromatic hydrocarbons; Σ DDTs = o,p'-DDT + p,p'-DDT + o,p'-DDD + p,p'-DDD o,p'-DDE + p,p'-DDE; Σ PCB= PCB-28 + PCB-52 + PCB-101 + PCB-118 + PCB-138 + PCB-153 + PCB-180; FI= Fecundity inibition and DI= Development inibition.

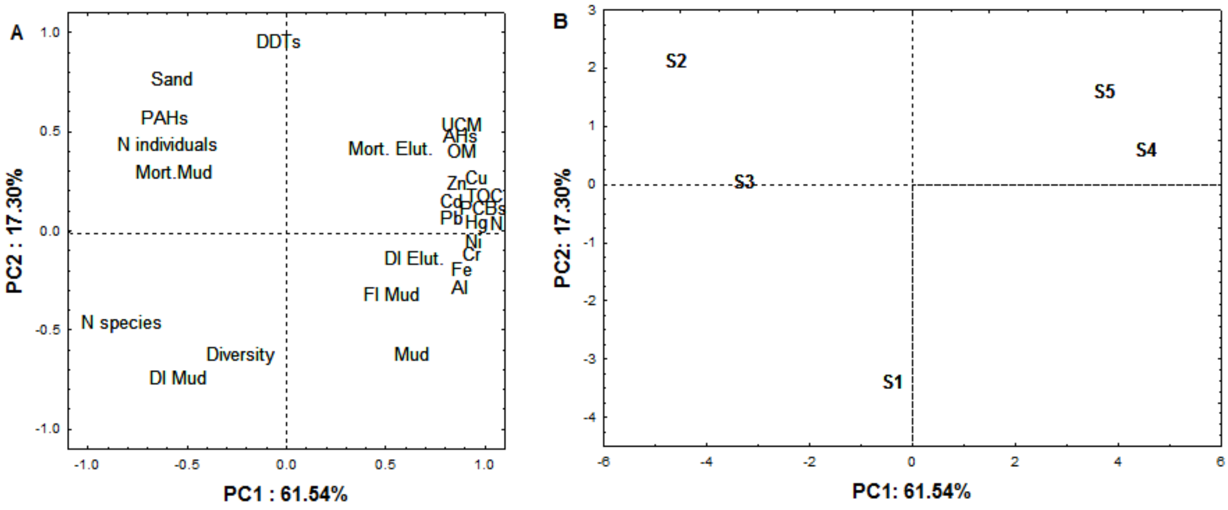


Figure 5. Ordination results of the principal component analysis (PCA) based on chemicals, toxicity and benthos variables (A) from stations (B) S1, S2, S3, S4 and S5 of the Capibaribe Estuarine System.

Discussion

Chemical contamination, toxicity and benthic community descriptors

The sediments of CES are mostly mud (silt and clay) and might be a result of continental contribution, considering the substantial river and runoff input to this estuary. The high average content of organic matter (OM, 14.7 % ± 5.4) recorded in the CES, is common on tropical ecosystems with very high productivity and allochthonous contributions (de Assis Esteves; 1998). The results indicate a decrease in contents of mud, OM, TOC and nitrogen towards to the lower estuary. The higher concentrations at #S1, compared to #S2 and #S3, are probably due to the influence of the activities at Port of Recife. A strong and negative correlation between the TOC and salinity was observed (r=-0.8224, gl=4, p= 0.0445), corroborating with the decrease pattern observed. The values of δ13C observed in this study suggest that the C3 plants (e.g. mangroves) are the main source of organic matter to the area (–24‰ to –30‰), as well as estuarine phytoplankton (–20‰ to –35‰) (Bouillon et al., 2011).The strong correlation observed between TOC and N ($r=0.9865$, $p=0.0019$) suggests that even though there are distinct organic matter sources in the estuary such as sewage, terrestrial plants and marine phytoplankton, they are introduced in nearly constant proportion (Ruttenberg and Goñi, 1997).

Most of the contaminants showed a similar distribution, with highest concentrations in the upper portion of the estuary, except for PAHs and DDTs that were higher at #S2. Besides the input sewage, the higher concentration of PAHs may be from pirolitic sources, as the fossil fuels burning. The same pattern distribution was observed in this area previously, using AHs, UCM and PAHs concentration as indicators: a decreasing pattern from inner estuary towards to the sea and adjacent platform (Maciel et al., 2014, Zanardi-Lamardo et al., 2014). These results highlight the important input from the CES contributors rivers to the sedimentary organic matter formation and contamination of sediments.

Despite the AHs could be introduced naturally in the environment, high concentration (e.g. 100 µg $g-^{1}$) of these compounds are associated to anthropic sources, as oil and derivatives (Volkman et al., 1992). The concentrations of AHs and UCM recorded at stations #S3, #S4 and #S5 suggest oil contamination at these areas. Based on the PAHs concentrations, sediments may be classified as slightly contaminated (0 – 100 ng g^{-1} de Σ 16HPAs), moderately contaminated (100 – 1000 ng g^{-1} de Σ 16HPAs), highly contaminated (1000 – 5000 ng g^{-1} de Σ 16HPAs) and very highly contaminated (>5000 ng g^{-1} de Σ 16HPAs) (Baumard et al., 1998). Considering that, the estuarine system can be classified as moderately contaminated, except station #S2, which showed a very high concentration characteristic of extremely contaminated environments.

The concepts "effects range low" (ERL) and "effects range median" (ERM) have been used to assess the impact of contaminated sediments upon aquatic biota (Long et al., 1995). Concentrations equal or higher than ERL, but lower than ERM, represent a possible-effects range, but higher than ERM represents a probable-effects range (Long et al., 1995). None of the compounds showed concentrations higher than the ERM (Table 1). Only at station #S2 the PAHs concentration was higher than ERL (4022 ng g^{-1}). Among metals, Cu was higher than ERL (34 µg g-1) at stations #S3, #S4 and #S5, and Hg were higher than ERL, (0.15 ng g^{-1}) at stations #S1, #S4 and #S5. For Zn, stations #S4 and #S5 showed concentrations higher than ERL (150 ng g^{-1}). Concentrations of p,p'-DDE at all stations were higher than the ERL established (2.2 ng g^{-1}). All stations had PCBs concentrations lower than the ERL (Table 1). Concentrations higher than the ERL, even though for few contaminants, may become a matter of great concern, especially considering the potential synergistic effect that could lead to an increased risk of toxicity (Costa et al., 2008). Unfortunately, such approach are barely studied.

The significant reduction in total fecundity of organisms exposed to the mud fraction, observed at stations #S1 and #S5, may be related to a high load of many contaminants reaching these areas. The #S1 is in front of the Port and receives oil and other contaminants, mainly from port activities. The #S5 is located in a confluence area of the rivers that flow to the estuary, carrying a huge amount of sewage mostly untreated. The total fecundity of *T. biminiensis* females showed a significant and inverse correlation with TOC ($r = -0.9429$, $p= 0.0048$), suggesting that the stressor agents are mainly associated to sediments with higher carbon content. This is confirmed by the significant correlation between the contaminants and TOC (Table 2). Despite the toxicity of sediments from #S1 and #S5, a reduction in percentage of copepodites was observed only at #S1 and, consequently, an inhibition in development. The #S5 showed high levels of OM and TOC, and due to the detritivorous feeding habits of *T. biminiensis,* this may have stimulated the growth of microbial communities, used as food, and ensured the development of nauplii in the bioassays. Oliveira et al. (2014) reported the toxicity of sediment in the mangrove forest located near the upper portion of the CES (Figure 1), and they attributed the effects to the presence of Zn and Cr in sediments. In the present study, the sediments from the inner portion of the CES also presented concentrations of Zn, Cu, Hg and p,p'-DDE higher than the proposed safety limits, making them potential ecotoxicological stressors.

The results from the elutriate test also pointed out toxicity to the samples from stations #S1 and #S5. However, in opposition to that observed in the bioassay using mud, organisms exposed to the elutriate showed inhibition in development and a high mortality rate at #S5. The elutriate test is performed to simulate the resuspension of contaminated sediments, that may occur during dredging activities or a dramatic event like a storm. These situations may turn contaminants available to the water column (Lazorchak and Josephs, 2003) and the oxigenation may cause changes in the physico-chemical properties of the compounds, often resulting in increased toxicity (Carvalho et al., 1998). Dredging activities frequently occurs in the area of the Port of Recife, where is located the Station S1 (Porto do Recife, 2015). A strong correlation was observed between the mortality at elutriate bioassay and the percentage of nitrogen ($r=0.9992$, $gl=3$, $p < 0.0001$). It is possible that the ammonia, present in the pore water, has contributed to the toxicity increase, once it has been identified as the agent for toxicity observed in pore water tests (Chapman et al., 2002). Ammonia has been known to be very toxic to aquatic organisms including copepods (Buttino 1994; Di Marzio et al. 2009; de Sousa et al. 2012).

The evaluation of benthic community pointed stations #S2 and #S5 as the poorest in terms of diversity, but #S2 showed the highest number of individuals, with the predominance of the bivalve *M. charruana*. This specie has shown resistance to disturbance and successfully established in unconsolidated sediment in the area (Maciel, 2011). In addition, as mentioned above the stations #S2 and #S5 had highest concentrations of some contaminants, with concentrations above established safety levels, which may be influencing negatively the structure of the benthic community.

The polychaete *S. benedicti* was the most abundant specie in the other stations, in accordance to a local previous study (Lima, 2006). This specie is considered a good pollution indicator because colonize disturbed and eutrophic environments (Levin, 1986). This is a clear indication that, even thoug this estuarine system could be classified as moderately contaminated, the benthic estructure reflects the impact of this contamination. Although the environmental characteristics such as salinity, temperature, grain size, population dynamics, and biological interactions are determining factors in the benthic community structure (Rosenberg, 1995), the influence of pollution on the benthic community structure is clearly noted, especially at station #S5.

Sediment quality and integrated analyses

Both RTM and PCA approaches indicated the upper portion of the estuary as the most critical area and shows the influence of contaminants on the structure of the benthic community. This gradient is in accordance to that pattern observed for contaminants only, considering the proximity to the sources. The toxicity does not seem to be a decisive agent for determining this sequence.

The PCA results showed the strong afinity of organic material, TOC and N in complexing the hydrophobic contaminants, such metals, PCBs and hydrocarbons (Figure 5 and Table 5). In addition, by the negative correlation, PC1 also explain how these components interfere on diversity and number os species, favouring a dominant specie instead of a great diversity. The association between the mortality and inhibition of development in elutriate with the most contaminants indicate that the remobilization of these compounds into the aqueous phase may have occured, resulting in adverse effects on the organisms (Carvalho et al., 2008).

The integrated results as well as the sediment contamination indicated a degradation decrease towards the sea. This patterns has already been recorded previously in this region (Maciel et al., 2014; Zanardi-Lamardo et al., 2014) and in other estuarine areas, as Pearl River Estuary, China (Wang et al., 2002), Phraya River, Thailand (Boonyatumanond et al., 2006); Santos-São Vicente Estuarine System, Brazil (Buruaem et al., 2013) and Elbe Estuary, Germany (Wetzel et al., 2013).

The impact of sewage in environment has been focus of several studies, since it brings several contaminants. These compounds may cause oxygen depletion due to the nutrient enrichment of the waters and can be very toxic to organisms (Costanzo et al. 2001; Oberrecht 2005; Berbel et al. 2015). This estuary was already classified as eutrophic with high concentrations of ammonia and phosphate, mainly in the upper portion, and human activities (e.g. sewage) was considered the main input of nutrients and contaminants (Nascimento et al., 2003). Although the most critical stations are located inside the estuary, the pollution recorded surrounding the harbor area cannot be neglected. In spite of having less chemical contamination compared to the other stations, the #S1 showed toxicity in both bioassays. This area (#S1) is located in the mouth of the estuary, where the hydrodynamic favors the oxygenation of the sediments and consequently changes the physical-chemical properties of the same, increasing contaminants mobilization and toxicity (Carvalho et al., 1998). In addition, this area is constantly dredged (Porto do Recife, 2015) that also causes sediment remobilization and the effects on bioavailability of the contaminants are the same (Eggleton and Thomas, 2004).

This approach, in order to integrate physical-chemical variables with biological data proved to be extremely important (Riba et al., 2004; Lee et al., 2006; Ribé et al., 2012), providing a more realistic representation of health and environmental quality of the estuarine sediments. If only contaminants were considered, the #S2 should be the worst in opposition to the toxicity results that would classify the #S4 as non-critical. The SQT provided an objective identification of all stations and showed where contamination is causing real damage. Results from chemical, toxicity and ecological analysis impart strong evidence presently available for determining pollution-induced degradation (Chapman, 1990).

Conclusion

The CES is contaminated by hydrocarbons, metals and organochlorines and, in some stations, the contaminants might be a risk to biota. The inner part of the estuary receives most of the contaminants probably brought by its tributaries that carry industrial and domestic sewage discharges, causing toxicity and changes on benthic community structure. The integrated analyses indicated the presence of a degradation gradient, towards the mouth of the estuary, but also showed that the input from port activities is significant. The Triad method showed to be effective for assessing the quality and environmental health of the estuarine sediments analyzed.

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CONCLUSÃO GERAL

Os sedimentos do Sistema Estuarino do Rio Capibaribe estão contaminados por hidrocarbonetos alifáticos (HAs) e policíclicos aromáticos (HPAs), organoestânicos (OTs), organoclorados (OCs) e metais pesados. Cerca de 10% dos AHs foram introduzidos pela degradação das folhas do mangue remanescente na região. Entretanto, o aporte antrópico é mais significativo e os resultados indicam a contaminação dos sedimentos por petróleo e seus derivados. A principal rota de entrada dos HPAs é através de queima de combustíveis fósseis ou matéria orgânica, mas a introdução direta do petróleo também foi significativa, principalmente nas regiões onde desembocam os rios formadores do SERC e na área portuária. Este estudo mostra que existe um potencial risco para a biota e, consequentemente, para a saúde humana, considerando-se que alguns HPAs estão acima dos limites de segurança estabelecidos por agências reguladoras internacionais.

A contaminação dos sedimentos por organoestânicos ocorre principalmente nas proximidades do Porto de Recife, das marinas e locais onde ficam atracados barcos de pesca, evidenciando que, apesar do banimento do TBT na fabricação de tintas anti-incrustantes, esta prática ainda existe. Isto sugere que a fiscalização e monitoramento do seu uso pelos órgãos competentes não está sendo realizado de forma eficaz. As concentrações dos pesticidas organoclorados apresentaram-se relativamente baixas, e a ausência de DDT com a presença de seus metabólitos, especialmente DDE, indicam aporte antigo. Os metais estão distribuídos de forma mais homogênea ao longo do estuário, entretanto, alguns apresentam concentrações que podem resultar em danos a biota local.

Alguns pontos do estuário, especialmente situados na porção superior e na área portuária, apresentaram toxicidade para a biota, sugerindo que os contaminantes presentes nos sedimentos estão biodisponíveis. Isto reflete na organização da estrutura da comunidade bentônica, que apresentou dominância de espécies indicadoras de poluição.

A avaliação dos dados químicos integrados aos dados ecotoxicológicos e ecológicos, indicam a presença de um gradiente de degradação no sentido do estuário para o mar, evidenciando o papel do SERC como um filtro para a plataforma adjacente. Vale ressaltar que o estuário também pode atuar como uma fonte dos contaminantes aprisionados, em determinadas condições, tais como tempestades e inudações.

A aplicação da Tríade da Qualidade do Sedimento, demonstrou ser uma ferramenta eficaz e importante para avaliar a qualidade e saúde ambiental dos sedimentos estuarinos. O SERC pode ser considerado como um ambiente moderadamente contaminado, e alguns pontos com elevado grau de contaminação. Neste sentido, é fundamental que se faça um monitoramento contínuo, utilizando variáveis químicas e biológicas, para avaliar a longo prazo o grau de degradação deste sistema estuarino.

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APÊNDICE 1

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AVALIAÇÃO DA TOXICIDADE DOS SEDIMENTOS DO SISTEMA ESTUARINO DO RIO CAPIBARIBE (PERNAMBUCO, BRASIL) UTILIZANDO O COPÉPODO BENTÔNICO *Tisbe biminiensis* VOLKMANN ROCCO (1973)

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RESUMO

Os sedimentos concentram uma fração considerável dos contaminantes que chegam aos estuários. Estes compostos podem comprometer a sobrevivência, fecundidade e desenvolvimento dos organismos bentônicos. O objetivo deste estudo foi avaliar a toxicidade dos sedimentos superficiais do Sistema Estuarino do Rio Capibaribe (SERC) sobre o copépodo bentônico *Tisbe biminiensis*. Amostragens de sedimento foram realizadas em cinco estações do SERC. Hidrocarbonetos de petróleo dissolvidos e/ou dispersos na água (HAPDDs) foram analisados em amostras de água de fundo para avaliação do aporte recente destes compostos. Efeitos subletais sobre *T. biminiensis* foram observados para as amostras coletadas em frente ao Porto do Recife, na desembocadura norte do Rio Capibaribe e na porção superior do estuário. As concentrações de HAPDDs registradas no SERC sugerem que a contaminação ocorre de forma crônica e é proveniente principalmente da descarga de efluentes domésticos e industriais, como também das atividades portuárias.

Palavras chave: Fecundidade total, percentual de copepoditos, contaminação, hidrocarbonetos de petróleo.

ABSTRACT

Part of the contaminants that reach the estuaries are deposited in sediments. These compounds may affect survival, fecundity and development of benthic organisms. This study aimed to evaluate the toxicity of the surface sediments from the Capibaribe Estuarine System (CES) on benthic copepod *Tisbe biminiensis*. Sediment samples were collected in five stations of the CES. Dissolved/dispersed petroleum hydrocarbons (DDPHs) was measured in bottom water samples from the same sites, to estimate the recent input of such compounds. Sub-lethal effects on *T. biminiensis* were observed for samples collected in front of the Harbor of Recife, in the northern mouth of the Capibaribe River and in the upper portion of the estuary. The DDPHs concentrations recorded in the CES suggest chronic contamination and pointed out the domestic and industrial effluents, and harbor activities, as the main source to this system.

Key Words: Total fecundity, copepodites percentage, contamination and petroleum hydrocarbons.

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INTRODUÇÃO

Estuários são ecossistemas costeiros que funcionam como barreiras biogeoquímicas, retendo as partículas em suspensão e os poluentes adsorvidos à estas (SHUBEL AND CARTER, 1984; TOLOSA et al., 2004). Nestes ecossistemas, o aporte de contaminantes se dá através do lançamento de efluentes industriais e domésticos; da lixiviação do solo na área da bacia de drenagem; de fontes pontuais localizadas no estuário (portos, marinas e estaleiros) e também através da deposição atmosférica de partículas (e.g., fuligem resultante da combustão de combustível fóssil) (MORAES et al., 2001).

O material particulado em suspensão e o sedimento podem atuar como vias de exposição a poluentes para organismos filtradores e detritívoros, bem como fontes secundárias de liberação de contaminantes para a coluna d'água (CHAPMAN, 1989; KIM et al., 2011). Estes poluentes podem ocasionar efeitos relevantes sobre a biota, tais como redução na fecundidade dos organismos e atrasos no desenvolvimento (CHAPMAN, 1990). Além disso, propriedades físico-químicas dos poluentes (ex. elevada capacidade de se particionar para matrizes orgânicas) e características ambientais (ex. anoxia na coluna d'água) podem favorecer a retenção destes poluentes no estuário, prolongando a toxicidade.

Testes de toxicidade são comumente utilizados para avaliar a qualidade de sedimentos estuarinos (ABESSA et al., 2012; ARAÚJO-CASTRO et al., 2013; OLIVEIRA et al., 2014). Dentre os organismos utilizados nos bioensaios destacam-se os copépodos bentônicos. Estes microcrustáceos são fáceis de cultivar em laboratório, possuem ciclo de vida curto, e são sensíveis a poluentes (ARAUJO et al., 2009). Além disso, copépodos com hábito epibentônico possibilitam avaliar diferentes vias de exposição a poluentes, tais como sedimento e água (ARAÚJO-CASTRO et al., 2009; ARAÚJO-CASTRO et al., 2013). O copépodo epibentônico *Tisbe biminiensis* é uma espécie promissora para ser utilizada em bioensaios, visto que se mostra sensível a poluentes inorgânicos (ARAUJO-CASTRO et al., 2009; LAVORANTE et al., 2013) e orgânicos (COSTA et al., 2014), sendo cultivado em laboratório de forma padronizada (RIBEIRO e SOUZA-SANTOS, 2011). O Sistema Estuarino do Rio Capibaribe (SERC) está localizado na zona urbana da cidade do Recife, e recebe um elevado aporte de poluentes através de fontes difusas (e.g., inúmeras galerias de esgotos ou águas pluviais) e pontuais (e.g., Porto do Recife e marinas) (NÓBREGA, 2011). Apesar da intensa degradação, trata-se de um ambiente de relevante importância ecológica e socioeconômica. Entretanto, ainda são poucos os estudos visando avaliar o grau de contaminação e os efeitos da poluição neste estuário sobre a biota (SERICANO et al., 1995; FERNANDES et al., 1999; LIMA et al., 2002; MACEDO et al., 2007; OLIVEIRA et al., 2014). Dentre os diversos contaminantes presentes, destacam-se os hidrocarbonetos policíclicos aromáticos, uma vez que que alcançam os estuários através de diversas fontes, tais como derrames de óleo, queima de combustíveis fósseis e de efluentes domésticos (FAVROD, 2012; CABRAL, 2014; ZANARDI-LAMARDO et al., 2014). Estes autores registraram elevadas concentrações de HPAs na água (ex. >7,00 μg L-1) e em sedimentos (>400 ng g-1) do SERC.

Alguns estudos têm investigado a presença de óleo na coluna d'água através da determinação de hidrocarbonetos de petróleo dissolvidos e/ou dispersos na água (HAPDDs) (ZARNADI et al., 1999; BÍCEGO et al., 2002; LEMOS et al., 2014). Esta técnica, apesar de não discriminar os componentes aromáticos individuais, é bastante simples, apresenta baixo custo e gera respostas em curto prazo. A quantificação destes compostos na água é importante, pois permite avaliar a contaminação recente de ambientes aquáticos.

O objetivo deste estudo foi investigar a toxicidade de sedimentos superficiais do SERC através de bioensaios com o copépodo bentônico *T. biminiensis*, bem como analisar as concentrações de HAPDDs em amostras de água de fundo do estuário, uma vez que, devido ao hábito epibentônico destes organismos, a água representa uma importante via de exposição.

ÁREA ESTUDADA

O SERC é formado pela confluência dos rios Capibaribe, Tejipió, Jiquiá, Jordão e Pina, sendo separado do oceano através de um dique natural de arenito (SANTOS et al, 2009). Estes rios atravessam zonas urbanas e industriais, recebendo uma elevada carga de efluentes, a maior parte sem tratamento. O SERC é caracterizado como um ambiente estuarino bastante dinâmico, com a circulação determinada principalmente pelo regime de marés semidiurnas e o influxo de água doce modulado sazonalmente. O clima é do tipo As', tropical quente-úmido, com dois períodos pluviométricos distintos, uma estação seca (setembro a fevereiro) e uma estação chuvosa (março a agosto) (FEITOSA et al, 1999). Apesar da intensa degradação, apresenta fundamental importância ecológica e socio-econômica para a população ribeirinha e todo o estado de Pernambuco, especialmente devido a sua atuação pesqueira. Seus bancos intermareais apresentam grande produtividade e são intensivamente explorados pela população local, especialmente para a captura de moluscos (LIMA, 2003).

MATERIAL E METÓDOS

Amostras de sedimento foram coletadas em duas campanhas, realizadas em março e maio de 2014. A primeira campanha teve um caráter exploratório, sendo coletados sedimentos em cinco estações do SERC (Fig. 1), os quais foram utilizados apenas para os bioensaios. Infelizmente, a amostra do ponto cinco foi perdida devido à um problema operacional. A partir dos resultados obtidos, uma nova coleta foi realizada (maio). Em ambas as campanhas, foram coletadas três réplicas por estação. Os sedimentos foram coletados com auxílio de um amostrador de fundo tipo "van Veen" de aço inoxidável, separando-se a camada superficial (2 cm) com auxílio de uma colher de aço inoxidável. Alíquotas de cada amostra foram armazenadas em recipientes de vidro para condução dos bioensaios. Na segunda campanha, uma alíquota de cada amostra também foi separada e acondicionada em recipientes de alumínio previamente descontaminados e, no laboratório, armazenadas em freezer a -20°C, até a realização das análises granulométricas e determinação dos teores de carbono orgânico total (COT). Amostras de água de fundo (4 L) foram coletadas em cada estação, com auxílio de garrafas âmbar presas à um suporte de aço inox, para a determinação dos HAPDDs. Em seguida, 20 mL de n-hexano (grau pesticida) foram adicionados em cada amostra para posterior extração em laboratório. Brancos de campo foram realizados adicionando-se 20 mL de n-hexano em frascos âmbar de forma a determinar possíveis contaminações durante a coleta. A localização geográfica dos pontos de coleta pode ser visualizada na Tabela 1.

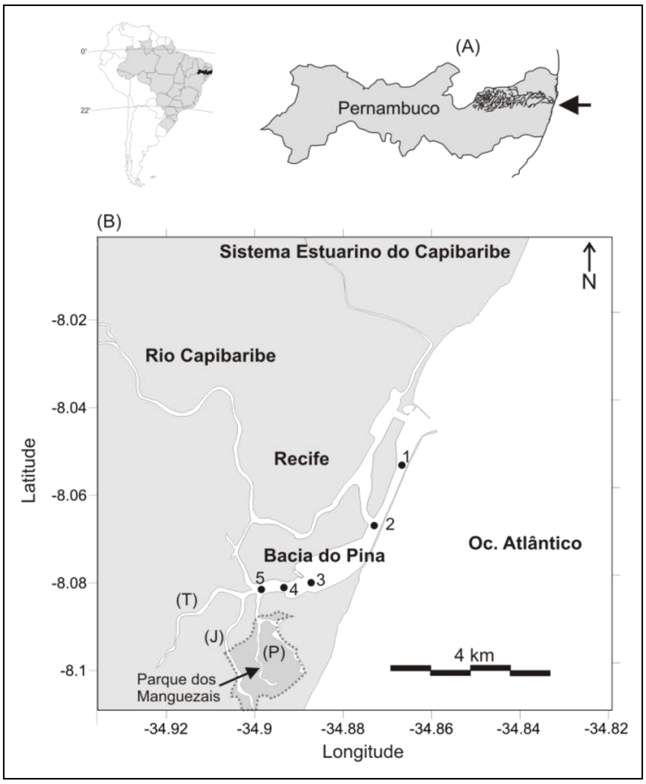


Figura 1. (A) Localização do Sistema Estuarino do Capibaribe (SERC), Pernambuco, Brasil. (B) Estações de coleta de sedimento em março e maio de 2014. (T) = Rio Tejipió, (J) = Rio Jordão e (P) = Rio Pina.

Testes de toxicidade com sedimento

Os bioensaios foram conduzidos segundo metodologia estabelecida por Araújo-Castro et al. (2009). Inicialmente, as amostras foram peneiradas em malha de 63 µm para remoção da fauna associada. Cerca de 2 g da fração lamosa de cada amostra foram pesados em recipientes de vidro. Após pesagem, 20 mL de suspensão da diatomácea *Chaetoceros calcitrans* (0,2 µg chl-a mL^{-1}), foram adicionados em cada recipiente. Os recipientes foram acondicionados em estufa incubadora à 28ºC. Após 24 h, 10 fêmeas ovígeras de *T. biminiensis* com idade controlada (entre 10 e 12 dias) foram adicionadas em cada recipiente. Os bioensaios tiveram duração de 7 dias, mantendo-se os recipientes na estufa incubadora com fotoperíodo de 12/12 h (claro/escuro) e adicionando-se alimento a cada 2 dias. Para controle dos bioensaios, uma amostra de sedimento lamoso foi coletada no Estuário do Rio Maracaípe, no qual não há instalações portuárias nem marinas, e processada da mesma forma que as amostras do SERC. Para cada amostra foram feitas 3 réplicas. Ao fim do teste os organismos foram corados com Rosa de Bengala e fixados com formol a 4%. O parâmetro biológico utilizado para avaliar a toxicidade foi a fecundidade total (náuplios + copepoditos) e o percentual de copepoditos na prole das fêmeas. Para avaliar a sensibilidade do lote de fêmeas ovígeras, foram realizados bioensaios com a substância de referência Dicromato de Potássio ($K_2Cr_2O_7$) (ARAUJO-CASTRO et al., 2009).

Análise granulométrica e do teor de COT

A análise granulométrica das amostras foi realizada conforme metodologia estabelecida por Suguio (1973). A fração arenosa (> 63 µm) foi separada com auxílio de um agitador de peneiras e a fração lamosa (< 63 µm) foi separada através do método da pipetagem. A determinação dos teores de COT foi feita na amostra total do sedimento em um Analisador Elementar acoplado a Espectrômetro de Massas de Razão Isotópica (EA-IRMS), após acidificação das amostras com HCl (1 M) por 24 h.

Extração e análise de HAPDDs

Para extração dos HAPDDs, as garrafas âmbar foram agitadas vigorosamente por 2 minutos e a fase orgânica separada em um funil de separação. Os extratos foram secos com Na_2SO_4 (previamente calcinado a 450 °C), concentrados a 10 mL em um rotaevaporador e armazenados em freezer a -22°C até as análises. Os HAPDDs foram quantificados em um espectrofluorímetro, usando um comprimento de onda de 310 nm para excitação e 360 nm para a medida de emissão (Zanardi et al, 1999; LEMOS et al., 2014). Uma série de diluições do óleo Carmópolis intemperizado (0 a 5,0 µg L^{-1}) foi preparada e utilizada na preparação da curva analítica e quantificação dos HAPDDs (Zanardi et al, 1999; Bícego et al., 2002; Lemos et al., 2014). A equação de reta obtida foi (sinal de fluorescência) = 5,3871[HAPDDs] + 0,0344, r^2= 0,9998. A média dos valores de fluorescência dos brancos de campo foi subtraída de cada amostra. O limite de detecção do método (LD) foi determinado como sendo três vezes o desvio padrão das concentrações do branco, resultando em 0,003 µg L^{-1} equivalente em óleo Carmópolis.

Análise de dados

A Análise de Variância (ANOVA, α = 0,05) foi utilizada para avaliar diferenças significativas em relação aos parâmetros toxicológicos entre as estações do SERC e o tratamento controle, aplicando-se o teste a posteriori de Dunnet. A normalidade dos dados foi verificada através do teste de Komolgorov–Smirnov. Análises de Correlação de Pearson foram realizadas entre os parâmetros ecotóxicológicos e os dados abióticos. Para dados não-paramétricos utilizou-se a Correlação de Spearman. A concentração letal a 50% dos organismos (CL50) foi calculada através do método Trimmed Spearman-Karber.

RESULTADOS

As CL50-96 h do Dicromato de Potássio obtidas durante os bioensaios das duas campanhas de amostragem estiveram dentro da faixa estabelecida por Araújo-Castro et al. (2009) (9,45 ± 1,68) mg L^{-1}.

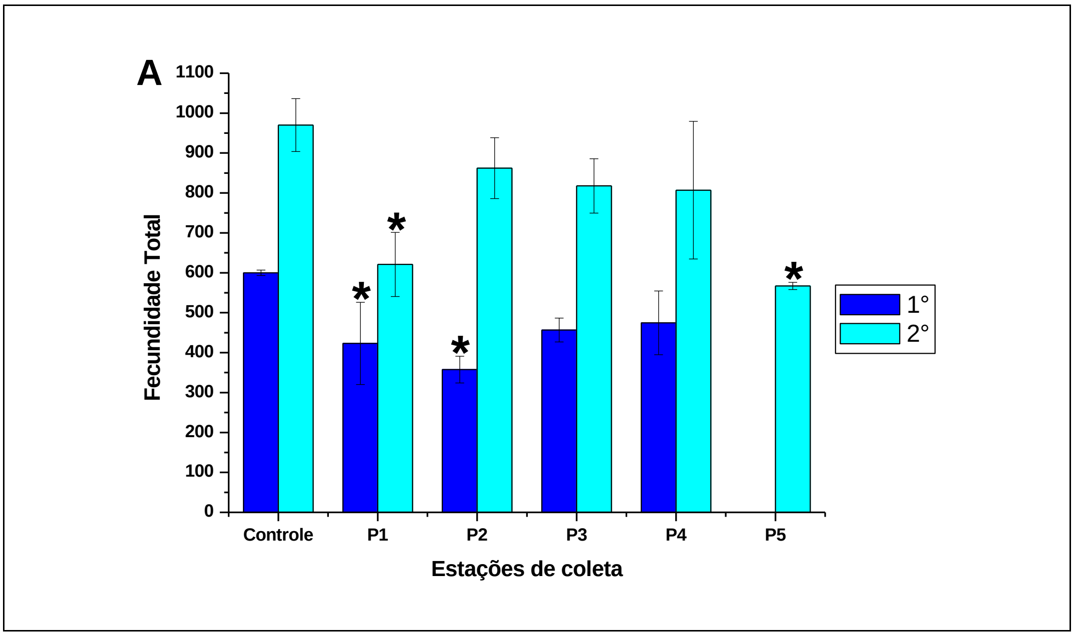
Na primeira campanha houve uma redução significativa na fecundidade total das fêmeas expostas ao sedimento das estações 1 e 2 em relação ao controle ($F_{(5,11)}$ = 4,0952, p = 0,0254) (Fig. 2 (A)). Em todas as estações o percentual de copepoditos manteve-se acima de 80%, não havendo diferenças significativas relativas ao controle ($F_{(5,11)}$ = 0,7444, p = 0,5817) (Fig. 2 (B)). Na segunda campanha foi observada uma redução significativa na fecundidade total das fêmeas expostas ao sedimento das estações 1 e 5 quando comparadas ao controle ($F_{(5,11)}$=6,485, p=0,0052) (Fig. 2 (A)). Em relação ao percentual de copepoditos, a estação 1 (32 ± 3.34%) diferiu significativamente em relação ao controle (50 ± 1.13%) (F(5,11)=3,4056, p=0,0419) (Fig. 2 (B)).

A análise granulométrica das estações indicou que o percentual de lama variou de 24% (controle) a 92% (estação 1) (Tabela 1). O teor de COT variou entre 1,43% (controle) e 4,76% (estação 4) (Tabela 1). As concentrações médias de HAPDDs variaram de 0,47 ± 0,08 (estação 1) a 5,63 ± 0,25 µg L^{-1} (estação 5) eq. de óleo Carmópolis (Tabela 1). A fecundidade total das fêmeas de *T. biminiensis* apresentou correlação inversa e significativa com o percentual de lama (r=-0,8218, p=0,0448) e o teor de COT (r = -0,9429, p= 0,0048) das estações (Fig. 3). Não houve correlações significativas entre o percentual de copepoditos e os parâmetros acima descritos.

Tabela 1 - Localização das estações de coleta e resultados da análise granulométrica (% de lama), teores de carbono orgânico total (% COT) e concentração média ($\mu g\ L^{-1}$ equivalente em óleo Carmópolis) e desvio padrão amostral (n=3)de HAPDDs de água de fundo. Amostras coletadas no Estuário de Maracaípe (controle) e no Sistema Estuarino do Rio Capibaribe, em maio de 2014.

	Latitude	Longitude	COT	Areia	Lama	HAPDDs
Controle	08º32’21.8”	35º00’14.5”	1,43	75,36	23,68	---
P1	08°03'21,2''	034°52'0,36''	2,98	5,64	92,27	0,47 (±0,08)
P2	08°04'02,8''	034°52'24,9''	1,99	54,08	40,76	4,60 (±1,93)
P3	08°04'43,9''	034°52'42,2''	2,26	58,06	37,05	3,78 (±1,18)
P4	08°05'02,4''	034°53'28,3''	4,54	23,97	73,09	1,74(±0,19)
P5	08°04'59.3''	034°53'48,4''	4,76	33,53	66,2	5,63 (±0,25)

(---) Não analisado.



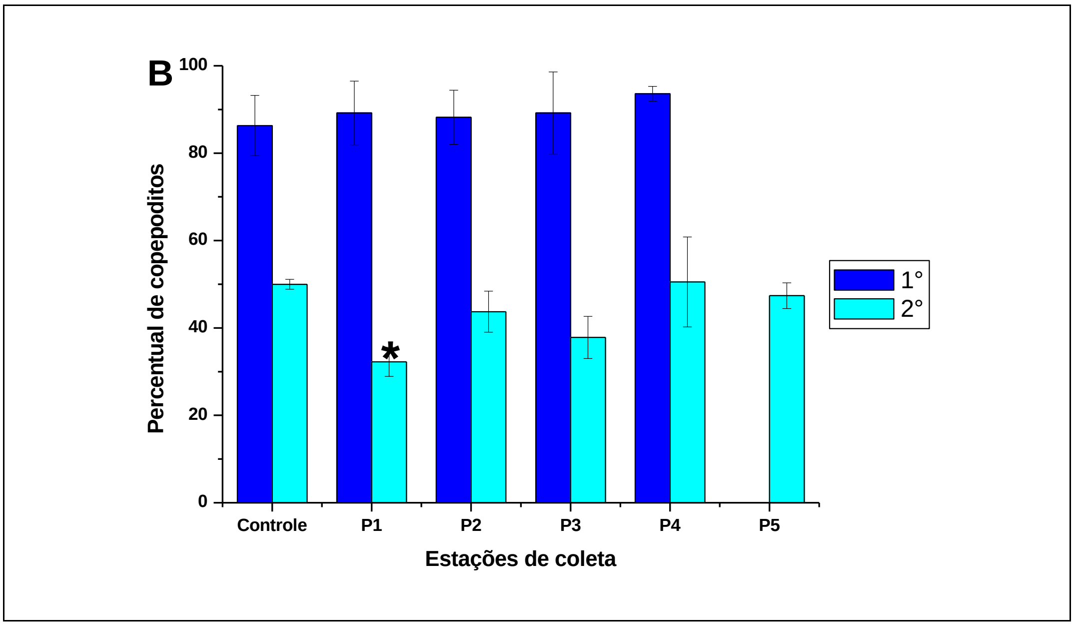


Figura 2. (A) Fecundidade total média (±D.P.) de náuplios + copepoditos e (B) percentual médio de copepoditos de *Tisbe biminiensis* após 7 dias de exposição a sedimentos coletados no Sistema Estuarino do Rio Capibaribe e Rio Maracaípe durante a 1° e 2° campanhas. (*) Amostras significativamente diferentes do controle.

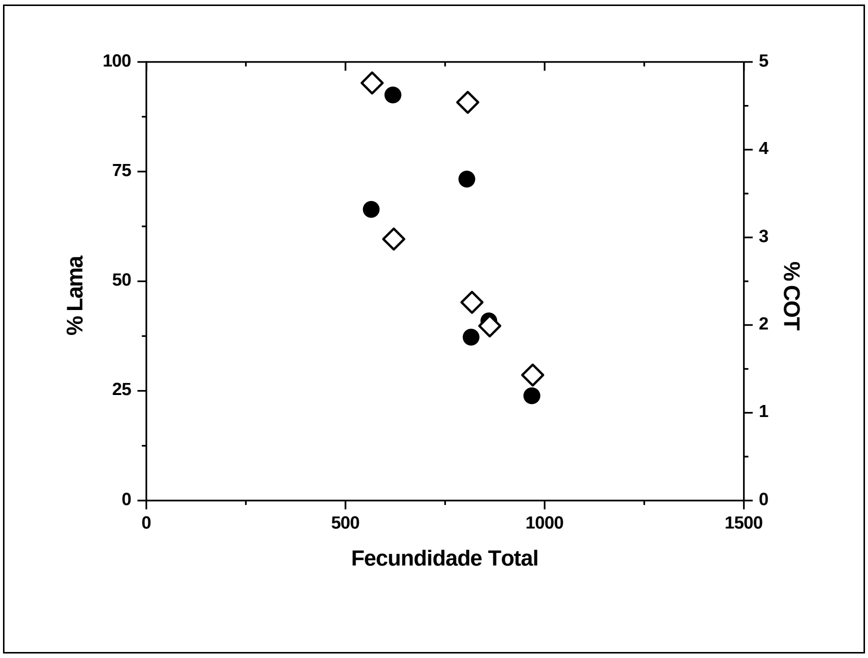


Figura 3 – Gráfico de dispersão da fecundidade total (náuplios + copepoditos) das fêmeas de *Tisbe biminiensis* em função do percentual de lama (•) e do percentual de carbono orgânico total (COT) (◇) nas amostras de sedimento do controle e do Sistema Estuarino do rio Capibaribe (SERC).

DISCUSSÃO

Os resultados dos bioensaios indicam toxicidade nas amostras de sedimento coletadas nas estações 1 (localizada em frente ao Porto do Recife), 2 (na desembocadura norte do rio Capibaribe) e 5 (localizada na confluência dos rios que formam o SERC). O ponto 1 recebe, de forma contínua, o aporte de óleo e outros contaminantes, derivados das embarcações e atividades portuárias. Os pontos 2 e 5 estão situados próximos às desembocaduras dos rios formadores do SERC, que atravessam a região metropolitana do Recife. O Rio Capibaribe ainda recebe uma elevada carga de efluentes de vários pólos industriais do estado de Pernambuco, localizados no seu entorno, em sua maior parte sem tratamento. Além disto, no SERC há o aporte atmosférico de contaminantes oriundos principalmente da queima de combustíveis fósseis (Zanardi-Lamardo et al., 2014). Estes resultados demonstram que em alguns pontos do SERC os sedimentos estão contaminados, e que estes contaminantes estão biodisponíveis e causam efeitos sobre a biota.

A presença de contaminantes em sedimentos do SERC foi reportada em estudos prévios. Elevados níveis de contaminação por metais pesados já foram registrados (MACEDO et al., 2007). OLIVEIRA et al. (2014) indicaram o efeito dos metais Zn e Cr sobre a toxicidade dos sedimentos no Parque dos Manguezais situado próximo a porção superior do SERC. Uma grande preocupação em relação à introdução de metais pesados no ambiente deve-se ao fato de que os mesmos podem bioacumular e causar efeitos tóxicos para diferentes espécies da biota e para o homem (TAVARES E CARVALHO, 1992; VIRGA et al., 2007). A exposição a metais pesados pode causar efeitos adversos, afetando principalmente o sistema neurológico (efeitos neurotóxicos), sistema reprodutivo (efeitos na reprodução e teratogênicos), sistema imunológico e hematológico, entre outros, além dos efeitos carcinogênicos (JÄRUP, 2003). A contaminação de sedimentos do SERC por hidrocarbonetos de petróleo foi reportada recentemente (Maciel et al., 2014a). Estes mesmos pesquisadores também observaram elevadas concentrações de hidrocarbonetos policíclicos aromáticos (HPAs), com concentrações do dibenzo[a,h]antraceno superiores aos limites de segurança estabelecidos pela "Canadian Environmental Agency" (CCME) (ZANARDI-LAMARDO et al., 2014). O dibenzo[a,h]antraceno está entre os 16 HPAs considerados como poluentes prioritários pela USEPA (United States Environmental Protection Agency) devido ao seu elevado potencial mutagênico e carcinogênico, o que o torna uma ameaça a biota (IARC, 1983).

Além de metais pesados e hidrocarbonetos, a ocorrência de compostos orgânicos de estanho (OTs) também já foi reportada no SERC, com concentrações elevadas de Tributilestanho (TBT) em diversos pontos (MACIEL et al., 2014b). As maiores concentrações (> 100 ng Sn g^{-1}) foram registradas em frente ao Porto do Recife e na desembocadura do Rio Pina, onde estão localizadas as estações 1 e 5, respectivamente, do presente estudo. O TBT é considerado o mais perigoso de todos os compostos de estanho e um dos compostos mais tóxicos lançados no ambiente aquático (PLETSCH et al., 2010). Vários estudos em diversas partes do mundo têm mostrado os efeitos deletérios do TBT sobre os organismos (RUIZ et al., 1995; McALLISTER e KIME, 2003; ROACH e WILSON, 2009; GRACELI et al., 2013). Dentre estes, os mais significativos estão relacionados à ação de desregulador endócrino que o TBT apresenta, afetando principalmente a reprodução de organismos aquáticos. A presença destes contaminantes (hidrocarbonetos, metais, organoestânicos) pode estar relacionada com a toxicidade dos sedimentos amostrados. Vale ressaltar que o efeito sinérgico entre estes contaminantes pode ocorrer, ocasionando um aumento significativo da toxicidade (Costa et al., 2008).

Aliado a estes dados pretéritos, a presença de HAPDDs nas amostras de água de fundo, indica um aporte recente de óleo na região. O gradiente observado no sentido porção superior - baixo estuário confirma que o aporte ocorre principalmente pelos rios que formam o SERC. As concentrações de HAPDDs foram, em média, 10 vezes superiores à linha de base proposta para as águas do Atlântico Sul (0,31 µg L^{-1} eq. óleo Carmópolis), indicando que as amostras estão potencialmente contaminadas por HPAs (BÍCEGO et al., 2002), e classificam o estuário como cronicamente contaminado (Lemos et al., 2014). Estas concentrações também foram maiores do que as registradas para outras áreas estuarinas do estado de Pernambuco, como o Canal de Santa Cruz (0,23 a 0,37 µg L^{-1} eq. óleo Carmópolis) (SANTOS, 2015) e Suape (0,05–4,59 µg L^{-1} eq. óleo Carmópolis) (LEMOS et al.; 2014). A contaminação por HAPDDs no SERC já havia sido reportada para águas superficiais por Favrod (2012), que registrou concentrações entre 0,27 e 7,86 µg L^{-1} eq. óleo Carmópolis, com as menores concentrações em frente ao Porto do Recife, e as maiores na porção superior do estuário, onde desembocam os rios formadores do SERC. Estes resultados confirmam o observado neste estudo e corroboram com a hipótesede que estes rios são a principal via de aporte de poluentes para o estuário, e que a entrada de hidrocarbonetos políciclicos aromáticos neste sistema estuarino ocorre de forma contínua. A baixa concentração observada na estação em frente ao Porto do Recife é provavelmente resultante da influência da entrada das águas oceânicas, uma vez que a hidrodinâmica local é um fator preponderante na dispersão e diluição destes contaminantes (FAVROD, 2012; CABRAL et al., 2014).

A correlação negativa observada entre a fecundidade total e o percentual de lama, bem como com o %COT, é indicativo da dinâmica de concentração de poluentes orgânicos nos sedimentos mais finos e com maiores teores de carbono, e do potencial de toxicidade da amostra. Nos bioensaios com *T. biminiensis*, a maior toxicidade (menor fecundidade) foi observada na estação com maior %COT (estação 5). Quanto ao hábito alimentar, o *T. biminiensis* pode ser classificado como um detritívoro epibentônico e, portanto, os detritos no sedimento são uma importante via de exposição a poluentes para esta espécie. A ausência de correlação significativa entre o percentual de copepoditos e os parâmetros acima mencionados pode ser um efeito secundário da poluição orgânica no SERC (predominantemente oriunda de esgotos domésticos). Este aporte pode ter estimulado o crescimento das comunidades microbianas, utilizadas por *T. biminensis* como alimento, e garantido o desenvolvimento dos náuplios nos bioensaios. Esta hipótese, entretanto, deve ser testada em futuros bioensaios. Esta correlação negativa entre teor de finos e COT com a fecundidade pode ser explicada pelo fato de que a maioria dos contaminantes, devido a sua baixa solubilidade, são rapidamente adsorvidos ao material particulado em suspensão, e posteriormente tendem a se depositar no sedimento, ligando-se especialmente às partículas finas (WANG et al, 2001; TOLOSA et al., 2004; YANG et al., 2012). Além disto, os sedimentos que possuem elevados teores de silte e argila apresentam maior capacidade de retenção de matéria orgânica e, consequentemente, dos contaminantes (KOWALSKA et al., 1994). Vários estudos têm reportado fortes correlações entre o COT e diversos poluentes, corroborando com esta teoria (NAN et al., 2008; HE et al., 2009; PINOCHET et al, 2009; MARTÍNEZ-SANTOS et al., 2015).

CONCLUSÕES

Os sedimentos de alguns pontos do SERC apresentaram toxicidade sub-letal para *Tisbe biminiensis*, sugerindo que o mesmo possa estar ocorrendo para outros organismos bentônicos. Alguns pontos críticos foram identificados e associados à proximidade das fontes de poluição, tais como a desembocadura do SERC, onde está instalado o Porto do Recife, e a desembocadura dos rios que formam o estuário, que recebem o aporte de esgotos domésticos e industriais na bacia de drenagem do SERC.

As concentrações de HAPDDs observadas nas amostras de água de fundo reforçam a idéia de que a contaminação do SERC ocorre de forma crônica e que os contaminantes são, principalmente, trazidos pelos rios formadores do SERC. Este estudo mostra que é fundamental a realização de pesquisas mais direcionadas/específicas que avaliem os níveis de contaminação do SERC, investigando as principais fontes de contaminantes para área. O monitoramento contínuo de parâmetros biológicos torna-se imprescindível para avaliar os possíveis efeitos desta contaminação sobre a biota.

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ANEXO 1

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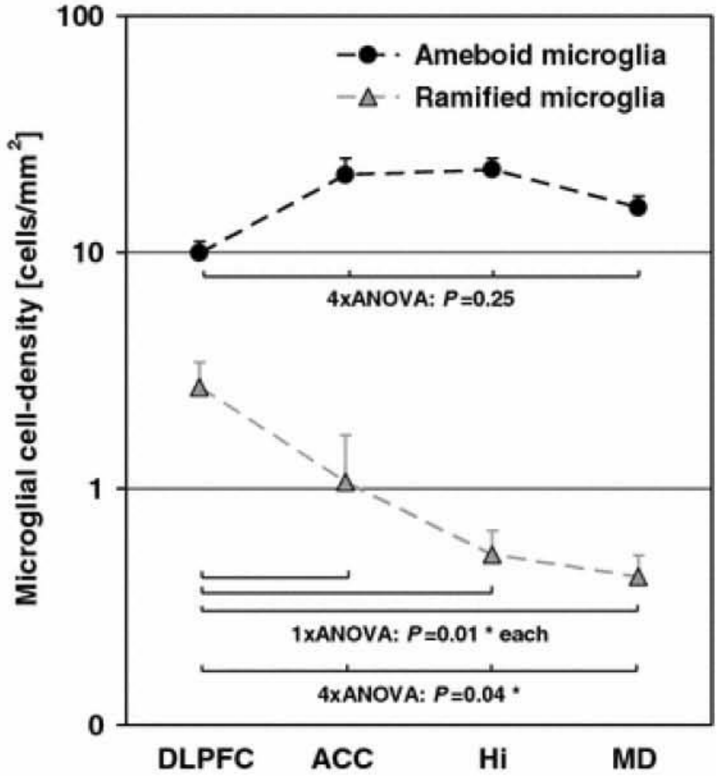

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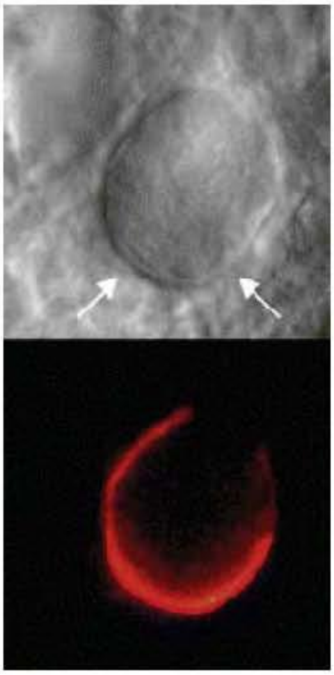

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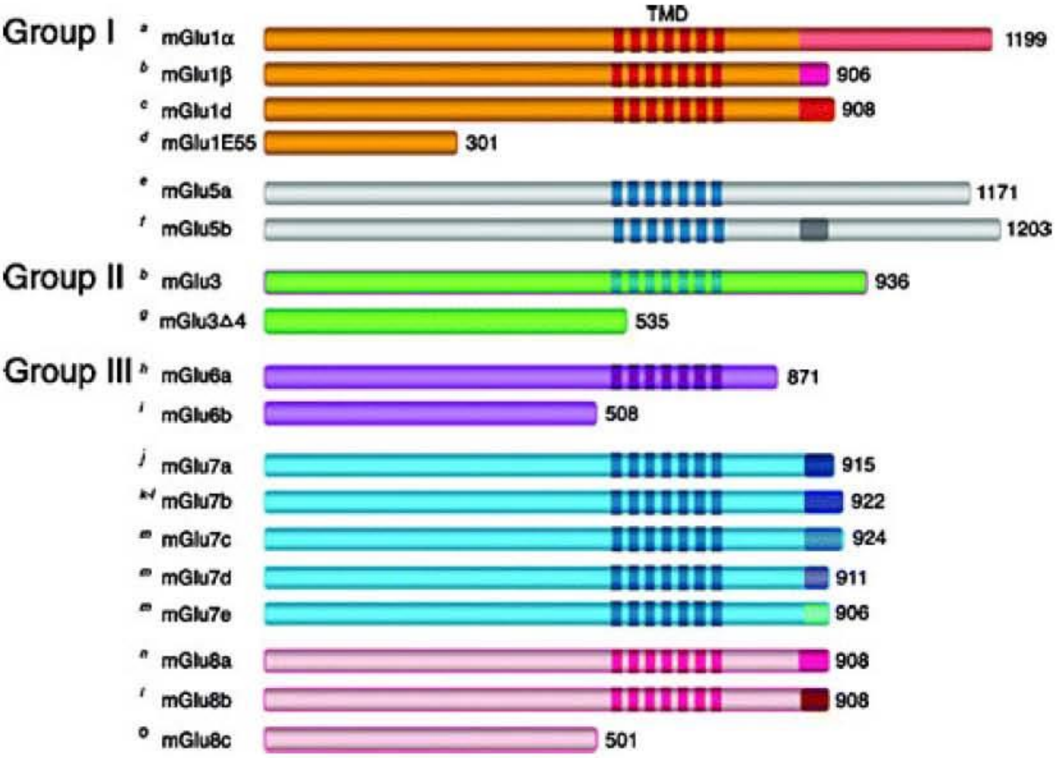

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This result was later contradicted by Becker and Seligman (1996).
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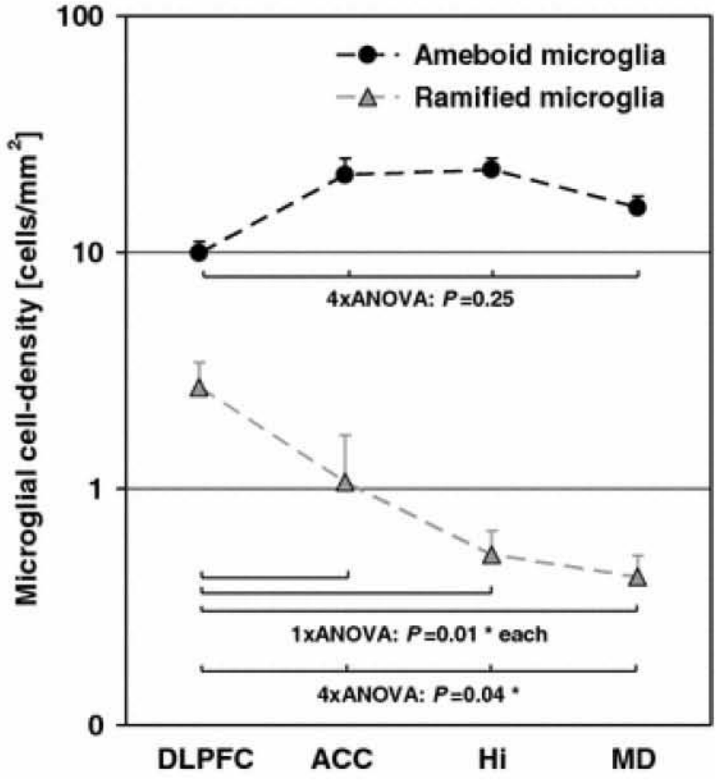

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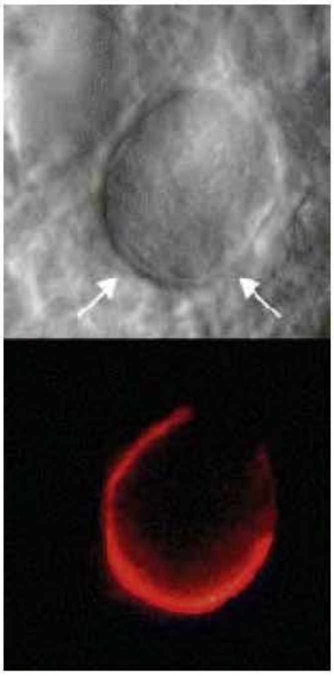

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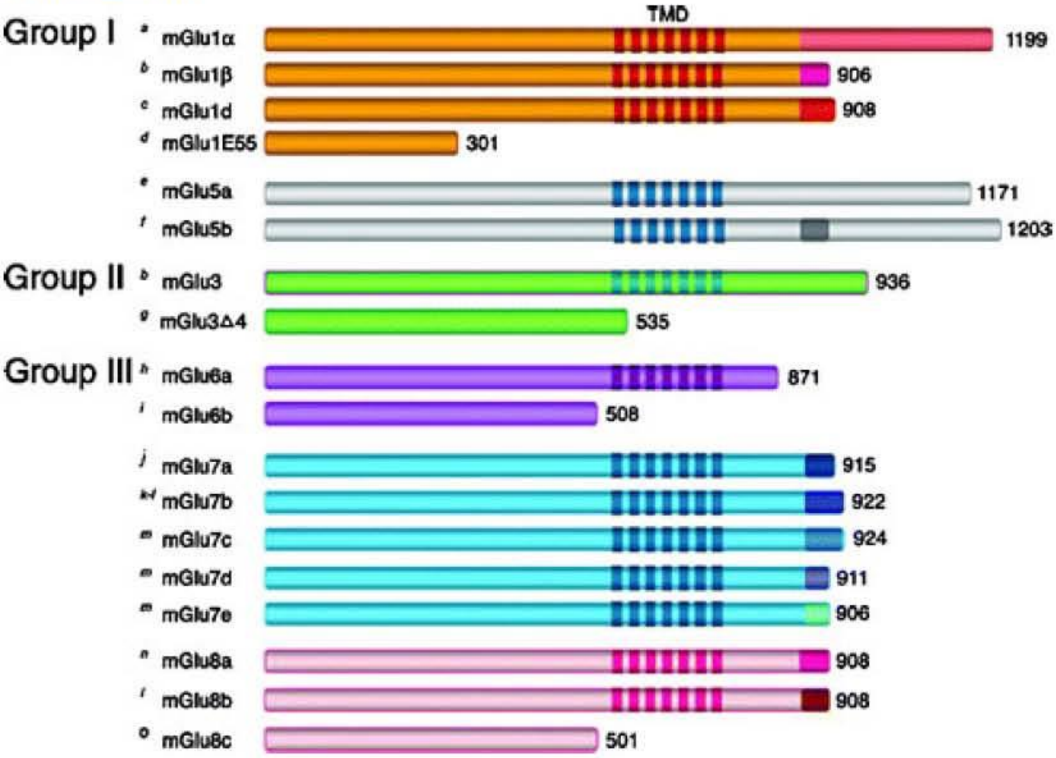

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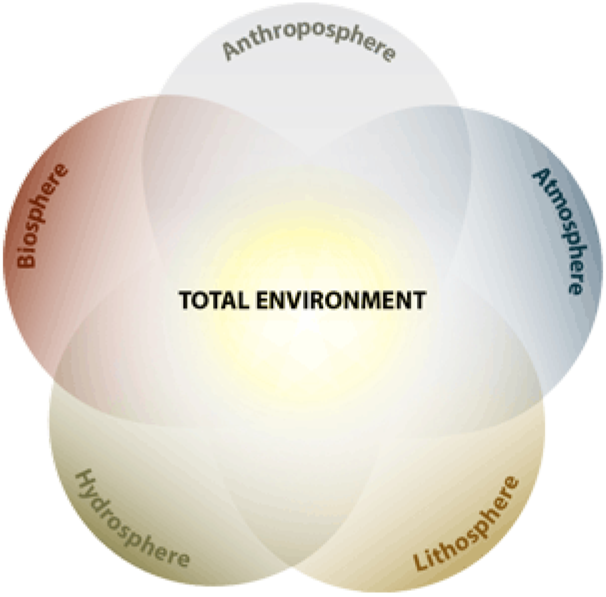

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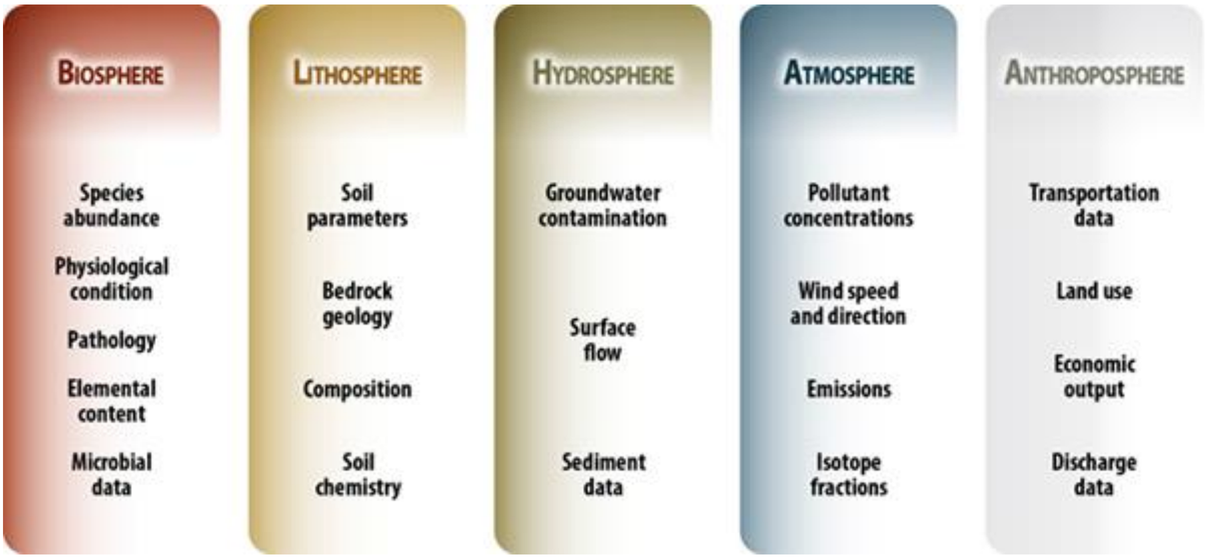

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