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CÍNTIA GLASNER RÉGIS

O USO DOS NÁUPLIOS DE *TISBE BIMINIENSIS* (COPEPODA: HARPACTICOIDA) NA AVALIAÇÃO DA TOXICIDADE DOS SEDIMENTOS DO ESTUÁRIO DO RIO CAPIBARIBE E SUA RELAÇÃO COM A GEOQUÍMICA

RECIFE, 2016

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Orientadora: Profa. Dra. Lília Pereira de Souza-Santos

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Cíntia Glasner Régis

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RESUMO

Abordagens que utilizam análises ecotoxicológicas integradas com análises geoquímicas para avaliar a qualidade ambiental de sedimentos tem se tornado cada vez mais importante. Fêmeas ovígeras do copépodo marinho epibentônico *Tisbe biminiensis* tem sido usadas como um organismo-teste autóctone em testes de ecotoxicidade. Entretanto, um protocolo menos laborioso que utiliza náuplios da mesma espécie de copépodos pode também ser apropriado. Há uma carência de trabalhos publicados que abordem a multicontaminação em estuários de regiões tropicais. O presente estudo teve como objetivo avaliar a qualidade dos sedimentos do estuário do rio Capibaribe (localizado no nordeste do Brasil) através da nova aplicação de um teste ecotoxicológico integrado com análises químicas de compostos orgânicos e inorgânicos. Foram analisados 7 pontos ao longo do estuário através de duas metodologias distintas que utilizaram como organismos teste fêmeas de *Tisbe biminiensis* e náuplios do *Tisbe biminiensis*, e tiveram como parâmetros observados sobrevivência das fêmeas, percentual de fecundidade e desenvolvimento dos náuplios do primeiro teste, e sobrevivência dos náuplios e percentual de desenvolvimento dos náuplios no segundo teste Os resultados encontrados indicaram que o teste ecotoxicológico que utiliza náuplios do copépodo é adequado e ainda mais sensível que o teste que usa fêmeas ovígeras. Foram detectados contaminação por hidrocarbonetos policíclicos aromáticos, organoclorados e metais no sedimento e em concentrações superiores aos níveis de segurança em várias estações de coleta, sugerindo que podem ter causado efeito tóxico à biota. Contaminantes orgânicos estiveram concentrados principalmente no alto estuário, provavelmente devido à presença de um ponto de turbidez máxima, porém aparentam não estar sempre biodisponíveis por não ter sido detectada ecotoxicidade nesta estação em uma das campanhas. Análises integradas mostraram um alto grau de comprometimento dos sedimentos das estações localizadas próximo no centro da cidade (S3, S4 e S5) e menor comprometimento das estações localizadas no alto e baixo estuário (S2 e S7).

Palavras-chave: Oceanografia. Contaminação de ecossistema aquático. Teste ecotoxicológico. Estuário do Rio Capibaribe.

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1 INTRODUÇÃO

Regiões costeiras são muito atrativas para ocupação humana (ex. fonte de alimento, recursos financeiros, de transporte-portos, posição estratégica para defesa), porém causam grandes impactos, o que vem sendo agravado com o crescimento desordenado das cidades. Em 2005, estimava-se que 80 por cento da carga de poluição nos oceanos vinha de atividades terrestres, oriundas das próprias áreas costeiras ou carreadas pelos rios de cidades do interior. Uma descarga de imensa variedade de materiais tais como resíduos urbanos, industriais, agrícolas, substâncias químicas oriundas de derrames acidentais e perfuração de petróleo, escoamento de águas pluviais urbanas e agrícolas e a deposição atmosférica afetam as áreas produtivas do ambiente marinho, incluindo os estuários e as águas costeiras. Os estuários são especialmente vulneráveis a esses inputs por serem sistemas aquáticos parcialmente fechados, com troca de água restrita (CINDRIC et al., 2015).

Dentre os principais grupos de contaminantes considerados perigosos para ecossistemas aquáticos estão:

- Óleos e derivados: devido principalmente à sua ampla distribuição global e sua alta toxicidade (MACIEL et al., 2015). Entre estes um dos mais estudados são os hidrocarbonetos monoaromáticos, BTEX (benzeno, tolueno, etilbenzeno e xilenos). Compostos de grande importância, altamente voláteis e solúveis conferindo uma alta toxicidade e mobilidade através do solo e da água subterrânea, sendo os que primeiro atingem o lençol freático. Benzeno e etilbenzeno são classificados como possivelmente carcinogênicos à humanos (grupo 2B) segundo a IARC (Agência internacional de pesquisa sobre o câncer). Os hidrocarbonetos policíclicos aromáticos (HPAs) são compostos formados por dois ou mais anéis benzênicos condensados e têm recebido especial atenção devido a seu alto potencial carcinogênico e mutagênico. De acordo com a Agência de Proteção Ambiental Americana (EPA – US), dentre os mais de 100 HPAs existentes, 16 são considerados prioritários: 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. Sua introdução pode ocorrer através de derrames e acidentes com petróleo e derivados, mas podem também ser oriundos de fontes pirolíticas e ainda de produto de síntese de organismos como plantas, bactérias e fungos, este último em concentrações menores que aquelas geradas pela introdução antrópica (Unep, 2002; Meire et al., 2007; Maciel 2015).

-Organoclorados: Outro grupo de contaminantes são os poluentes orgânicos persistentes (POPs), altamente tóxicos, resistentes ao metabolismo e com alta persistência ambiental e que incluem os pesticidas organoclorados e as bifenilas policloradas-PCBs. São compostos sintéticos produzidos com visão na melhoria da qualidade de vida, mas que com o passar do tempo se mostraram perigosos à saúde ambiental e humana o que culminou em tratados internacionais tais como a Convenção de Estocolmo (2004), atualmente ratificada por 152 países (Stockholm-Convention, 2015), proibindo uso dos POPs. Mas ainda são encontrados ao redor do globo por serem persistentes e serem ilegalmente utilizados (Pazi et al., 2011; Combi et al., 2013; Moura 2016). Os pesticidas organoclorados foram amplamente utilizados durante o século XX contra pragas na agricultura e em ectoparasitas bovinos (OMS 1992). Dentre os pesticidas o DDT foi o pesticida mais utilizado na história do planeta (Miranda Filho et al., 2008). No ambiente, ele pode ser transformado em dois metabólitos, através da via aeróbia se transforma em DDE (diclorodifenildicloroetileno), enquanto que na via anaeróbia o DDT é metabolizado em DDD (diclorodifenildicloroetano) Os PCBs são hidrocarbonetos aromáticos sintéticos, bastante prejudiciais devido a sua apolaridade, lipossolubilidade e resistência à degradação ambiental. Quanto maior o número de átomos de cloro na molécula, mais o PCB se adsorve fortemente aos sedimentos e se tornam portanto mais persistentes, enquanto os menos clorados são mais voláteis. Entre os PCBs mais tóxicos destacam-se os congêneres 77, 126 e 169 enquanto os penta, hexa e hepta-clorobifenilas são os que apresentam maiores níveis de contaminação mundial por terem sido mais comercializados e usados em transformadores (Miranda Filho et al., 2008; Yogui, 2002; Moura, 2016).

-Metais: componentes inorgânicos naturais que entram os ambientes aquáticos via erosão da crosta continental, e podem agir como micronutrientes ou tóxicos para vários organismos aquáticos dependendo de sua concentração e especiação (Donat e Dryden 2001). Dentre os metais considerados como muito tóxicos estão o Cd, Co, Cu, Cr, As, Pb, Ag, Au, Hg e Sb que são altamente reativos e bioacumuláveis nos organismos, o que os torna potencialmente tóxicos para os seres vivos. Já o molibdênio, vanádio, níquel e zinco são exemplos de metais essenciais para vários organismos (Schropp, 1988)

O número crescente de xenobióticos e os efeitos de parâmetros físico-químicos sobre a sua disponibilidade para organismos marinhos dificulta enormemente o monitoramento com base apenas em análises químicas (His et al., 1999). Mesmo se tivéssemos o conhecimento dos tipos de poluentes presentes, a química analítica possui limite de detecção que pode ser superior à concentração que cause efeito biológico adverso, além de determinar o grau e natureza da poluição, mas não fornecer evidências de consequências biológicas. Bioensaios permitem a detecção destes efeitos medindo as repostas biológicas de organismos marinhos (Fathallah et al., 2011) e constituem-se, portanto, ferramentas para compreensão da extensão dos impactos. Em virtude do reconhecimento dos sedimentos atuarem como fonte e sumidouro de contaminantes, este vem se tornando compartimento de estudo fundamental para análise de risco e qualidade ambiental. O uso e incentivo de abordagens que integram as análises químicas e ecotoxicológicas vêm sendo cada vez maior. (Beiras et al., 2011; Borja et al., 2008; Krull et al., 2014; Nipper, 2000)

O Brasil possui uma extensa zona costeira que se estende por mais de 8.500 km voltados para o Oceano Atlântico (MMA, 2015), com grande variedade de características ambientais e que precisa de uma maior opção de testes ecotoxicológicos com espécies autóctones. A busca por novas espécies e metodologias tem sido estimulada também no sentido de aproximar o campo da ciência das empresas e gestores ambientais que buscam resultados mais rápidos (resultante de metodologias menos laboriosas) e menos custosos (Jager et al 2006; Nipper 2000; Faucoult, 2013).

Copépodos marinhos harpacticóides bentônicos vêm sendo frequentemente utilizados em testes letais e sub-letais de toxicidade, em função de sua facilidade de cultivo, facilidade de alimentação, alta fecundidade e resistência a condições laboratoriais. Os seus tamanhos reduzidos facilitam a realização dos testes, barateando os mesmos e aqueles com hábito epibentônico possibilitam testar poluentes tanto na fase aquosa quanto aqueles ligados aos sedimentos (Bengtsson, 1978; Hutchinson et al., 1999; Silva et al., 2000). Especies do gênero Trigriopus, Nitokra, Amphiascus, Acartia e Tisbe estão entre os copépodos mais utilizados em ecotxicologia. Os copépodos marinhos haparticóides bentônicos do gênero *Tisbe* são representantes de um grupo ecologicamente importante da meiofauna e vem sendo frequentemente utilizados em testes de toxicidade (ISO 14669, 1999; Hutchinson et al.. 1999; Miliou et al., 2000; Taylor et al., 2007).

No Brasil, o copépodo *Tisbe biminiensis,* devido a sua sensibilidade, vem sendo usado para avaliação da toxicidade de amostras de sedimento desde 2002 (Araújo-Castro et al., 2006; 2009; 2013 a, b). Neste teste cada recipiente-teste recebe 10 fêmeas ovígeras que são capturadas uma a uma com uma pipeta Pasteur. O bioensaio tem duração de uma semana e a cada dois dias é adicionada 1 ml de suspensão de diatomácea em cada recipiente teste para que não haja falta de alimento durante o experimento. O número de recipientes teste varia de acordo com quantos pontos são coletados na área ambiental do estudo, mas podemos considerar uma média de 100 recipientes teste para 6 estações de coleta, e em cada um devem ser colocado 10 fêmeas, perfazendo 1000 fêmeas sendo retiradas individualmente. Mais recentemente, Lavorante et al.(2013) desenvolveram um protocolo mais simples de avaliação de toxicidade de água com a utilização de náuplios do mesmo copépodo. Neste protocolo uma alíquota contendo cerca de 200 náuplios de copépodos é transferida com pipeta para cada recipiente teste, reduzindo bastante o esforço de capturar os indivíduos separadamente. O experimento dura 72h e não há necessidade de se renovar o alimento durante o teste. Apesar deste protocolo só ter sido testado para avaliar amostras de água não existem motivo *a priori* para ele não poder ser empregue para avaliar amostras de sedimento.

A costa Pernambucana possui um estuário em destaque, o do Rio Capibaribe. Importante sócio e economicamente desde a colonização até os dias atuais para a região metropolitana do Recife, este estuário, encontra-se em situação de extrema poluição, por receber uma grande quantidade de poluentes provenientes dos centros urbanos, esgotos domésticos e industriais sem tratamento adequado e de atividades portuárias e náuticas (Maciel et al 2015; Barros et al., 2009). Estudos anteriores sobre composição do fitoplâncton e produção primária, (Travassos et al., 1991; Santos et al., 2009), indicadores de poluição orgânica (Silva, 2004; Paranaguá et al., 2005; Valença, 2009), coliformes fecais na água (Feitosa, 1988 apud Nóbrega 2011), em bivalves e cracas (Farrapeira et al., 2010) demonstraram que a qualidade da água deste estuário está comprometida e altamente eutrofizada. Também já foi registrado a presença de metais pesados, tais como cromo, ferro, manganês e zinco, com valores acima dos considerados níveis esperados no sedimento, e nos tecidos de animais e vegetais na bacia do Pina (Silva, 2004). Apesar dos níveis de poluição encontrados, os recursos pesqueiros desta região ainda representam o sustento de parte da população que vive em seu entorno.

Este estudo teve como objetivo realizar análises ecotoxicológicas e geoquímicas dos sedimentos do estuário do rio Capibaribe, assim como testar a aplicaçao do protocolo de bioensaio ecotoxicológico para água desenvolvido por Lavorante et al. (2013) para sedimentos utilizando como organismo teste os náuplios do copépodo *Tisbe biminiensis*.

2 OBJETIVOS

2.1 Objetivo Geral

Analisar a qualidade dos sedimentos do estuário do rio Capibaribe através de testes ecotoxicológicos testando duas metodologias associadas a análises geoquímicas

2.2 Objetivos específicos

- Avaliação ecotoxicológica dos sedimentos do estuário do Rio Capibaribe usando as fêmeas ovígeras de *Tisbe biminiensis* em dois períodos: início do período chuvoso e início do período seco;
- Avaliação ecotoxicológica do sedimento do estuário do Rio Capibaribe usando náuplios de *Tisbe biminiensis* em dois períodos: início do período chuvoso e início do período seco;
- Comparação dos dois protocolos de ecotoxicidade quanto à sensibilidade;
- Análise das características do sedimento como o teor de carbono orgânico total, distribuição granulométrica em sedimentos do estuário do Rio Capibaribe;
- Avaliar a qualidade do sedimento do estuário do rio Capibaribe através da integração de dados geoquímicos e ecotoxicológicos através de análises multivariadas.

3 MANUSCRITO:

The use of nauplii of *Tisbe biminiensis* in ecotoxicological test associated with geochemical analyses to assess the sediment quality of a tropical urban estuary in northeast Brazil

Highlights

- A new, little laborious, and easily executable protocol to assess sediment toxicity was positively tested.
- The use of nauplii of *Tisbe biminiensis* increased detection of the toxicity in 35% when compared to adults of the same species.
- Capibaribe river estuary sediment is highly committed as polycyclic hydrocarbons, organochlorine and metals were detected in concentrations above TEL in several stations.
- The biological and geochemical parameters are mainly controlled by hydrodynamics and local contamination of Recife urban area.

Abstract

Approaches that uses both geochemicals and ecotoxicology analyses to assess the environmental quality of sediment has become of great importance. The ovigerous female of the epibenthic marine copepod *Tisbe biminiesis* has been used as an autochthonous organism test to asses ecotoxicity. However, a less laborious protocol using the nauplii from the same species might be suitable. There is a lack of studies addressing the multi contamination of estuaries in tropical areas and therefore the present study aimed to assess the sediment quality of the Capibaribe river estuary (located in the northeast coast of Brazil) through a new ecotoxicity protocol as well as organic and inorganic chemical analyses. Results showed that the nauplii toxicity test was suitable yet more sensitive than the test that uses the ovigerous female. It was also observed that sediments from Cabibaribe river estuary are contaminated by polycyclic hydrocarbons, organochlorine and metals, and in concentrations above TEL in several stations, suggesting they caused toxicity to the biota. Organic contaminants were mostly concentrated in the upper estuary probably due to the presence of an estuarine turbidity maxima, however not always bioavailable. Integrated analyses showed a higher commitment of sediment of stations located closer to the city center (S3, S4 and S5), and slightly commitment of stations located in both head and mouth of estuary (S2 and S7).

Keywords*:* Contamination, copepod, nauplii, Capibaribe river.

Introduction

Estuarine and fluvial systems are among the most impacted environments by anthropic activities, receiving inputs of contaminants from diffuse sources like urban, industrial and agricultural. As partially closed aquatic systems, with limited water exchange, they are especially vulnerable to those inputs (Cindric et al., 2015).

In environmental quality assessments, sediment has been of great importance as a major fraction of many chemicals of concern introduced into aquatic environment associates with bottom sediment after several processes involving absorption, precipitation and biological process (National research council-US, 2003). In the main group of concerning chemicals are the oils, due to its wide distribution and high toxicity (Maciel et al., 2015a), the metals, natural inorganic compounds that may act (depending on both their concentration levels and chemical speciation) as micronutrient or as toxic pollutant for many aquatic organisms (Donat and Dyeden, 2001), and also the highly toxic and metabolic resistance group of the persistent organic pollutants (POPs) (Pazi et al., 2011).

Sediment environmental quality is better assessed with integrated approaches specially chemicals and ecotoxicology analyses (Beiras et al., 2011; Loez et al., 1995; Nipper, 2000). Even though the use of ecotoxicology standardized methodologies is important, the use of autochthonous species from the impacted area is of greater relevance as to not lose or undersize effects of the contaminant on the ecosystem (Nipper et al., 2000). The search for new species and methodologies has been encouraged in order to obtain better models as short tests (to make possible fast decisions on environmental management), little laborious, and with test-organisms of species of ecological relevance with viable laboratory culture (Forbes and Forbes, 1994; Jager et al., 2006; Lavorante et al., 2013).

In this matter, the use of the epibenthic marine copepod *Tisbe biminiesis* as an ecotoxicology organism-test has been studied over the last decade (Araújo-Castro et al.,2009; Costa et al., 2014; Lavorante et al., 2013; Souza-Santos et al., 2015) and is considered of having a high potential as it has an easy laboratory culture that achieves high density and has a short life cycle (Ribeiro et al., 2011). The use of the ovigerous female for sediment ecotoxicology tests is well developed and presented good outcomes (Oliveira, D. et al., 2014; Maciel et al. 2015b). Although this methodology requires certain ability and training as high numbers of ovigerous females must be added to test-recipients manually. That can imply in a little amount of people being able to apply this methodology as well as a limited number of site samples, as higher the numbers of sites higher will be the number of ovigerous female to be manually collected. Based on the water toxicity assessment test developed by Lavorante et al. (2013), this study intends to analyze whether sediment ecotoxicology assessment could be made in a less laborious test using nauplii of *Tisbe biminiesis* as organism-test inoculated in test-recipients in groups.

Furthermore, there is a lack of studies addressing the multi contamination of sediment, mostly in estuarine areas, of tropical regions where developing cities are located. The tropical estuary of the Capibaribe river is located in the northeast coast of Brazil and most part of it cuts the urban center of Recife (1.5 million inhabitants) (IBGE, 2010). It has been characterized as a flat, shallow and partially mixed estuary (Monteiro et al., 2011) that although receives a high amount of discharges from the urban center, is still important economically and ecologically (Maciel et al., 2015b).

Besides helping the improvement of knowledge of pollutants dispersion and its dynamic correlation to ecotoxicology data in tropical estuaries, this study is also of major local relevance as few studies are available for the Capibaribe river estuarine area and mostly restricted to Pina sound area (Fernandes et al., 1999; Macedo et al., 2007; Maciel et al., 2015a; Maciel et al., 2015b; Oliveira, D. et al., 2014). The present study aims to assess the sediment quality of the Capibaribe river estuary through the comparison of a new ecotoxicity protocol to the older one as well as organic and inorganic chemical analyses.

Materials and methods

Study Area

There are 42 municipalities in the watershed of the Capibaribe river which is 240 km long. Recife is located in its estuarine portion which has narrows channels 3 to 12 m deep. Closer to the mouth it´s the deepest part, due to frequently dredged (where Recife´s port is located) followed by the shallowest part of the channel (Monteiro et al., 2011), fact that hampers the entrance of water from the ocean during high tides but, at the same time, helps the elimination of pollutants, as the narrowing of the main channel and sudden change of depth generates a jet effect that contributes to the flushing (Araújo and Pires, 1998).

The Capibarive river estuary is a part of the Capibaribe estuarine system (CES) which is formed by the its confluence with the Pina sound formed by several creeks (Tejipió, Jiquiá, Jordão and Pina rivers) and carries considerable amount of untreated industrial and domestic effluents (Fig.1) (Santos et al., 2009).

Water circulation in the estuary is controlled by semi-diurnal mesotides with a range up to 3 m during spring tides (Maciel et al., 2015a). Average discharge is 11 m^3s^{-1}, yet 50% of the time it is $2m^3s^{-1}$. (Oliveira, T. et al 2014; Schettini et al., 2016)).

Sediment Sampling

Surface sediment samples (top 2 cm) were collected from river banks at 7 stations in October 2014 (dry season) and May 2015 (wet season): S1 and S2 were located at the upper estuary, S3, S4 and S5 located at the densest part of the city, and stations S6 and S7 were located at lower estuary close to the port of Recife (Fig. 1).

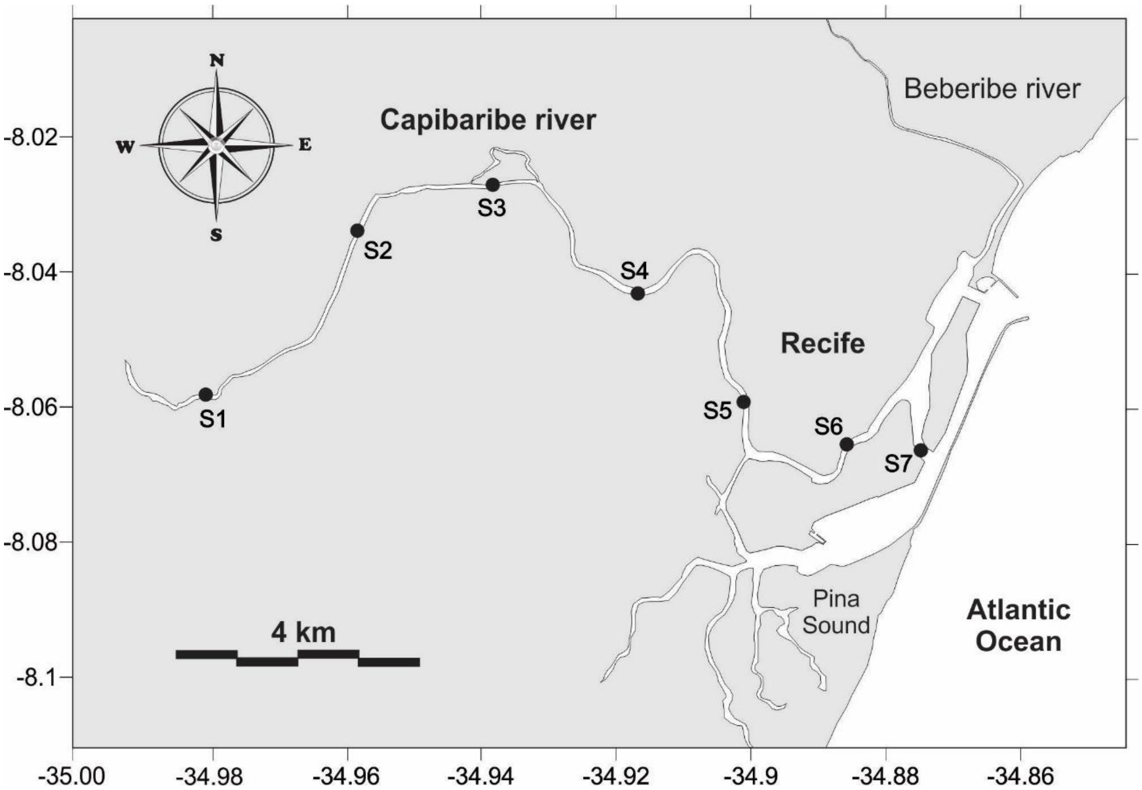


Fig.1: Sampling stations in Capibaribe river estuary.

Sediment samples for toxicity tests were collected in triplicate with a stainless steel spoon from river banks during ebb tide. The samples were homogenized and stored in plastic bags for metal analysis and in aluminum containers (previously calcined at 450ºC for 4 hours) for the geochemical analysis. All samples were stored following regimentation standards (ABNT, 2006). Two sediment samples were also collected from the Maracaípe estuary, which has been considered a clean environment (Araújo-Castro et al., 2009), and used as negative control for toxicity tests.

Toxicity tests

Prior to the tests, sediment samples were sieved through 63 µm for removing sand, plant debris and benthic fauna. Toxicity tests were simultaneously carried out following two different protocols.

The first one were conducted with *T. biminiensis* ovigerous females following the methodology proposed by Araújo-Castro et al. (2009), which basically consists in the addition of ten *T. biminiensis* ovigerous females, manually collected, in each replicate of test. The endpoints evaluated after seven days of incubation at 28 ºC and a 12-h light/dark photoperiod were: female survivorship (evaluated by rose the bengal stained animals), fecundity rate (total stained offspring produced by 10 females during 7 days) and offspring development (calculated as the percentage of copepodites in the total number of individuals from the offspring).

While the second used newly hatched nauplii of *T. biminiensis* as test organisms following the methodology proposed by Lavorante et al. (2013) for water samples, applied for sediment samples in this work. The methodology uses a suspension of 200 newly hatched nauplii in each replicate of the test and evaluates, as endpoints, the copepod survivorship (stained animals), calculated based on the difference between initial mean number of nauplii inoculated at test-recipients and final stained animals at the end of the test, and the nauplii development, calculated as the percentage of copepodites (stained copepodites) out of the total number of survivors after three days of incubation at controlled temperature of 28ºC and a 12-h light/dark photoperiod. The coefficient of variation (CV) of the initial mean number of nauplii inoculated at the test-recipients must be less than 20% so the test can be considered valid.

Five concentrations of potassium dichromate (ranging from 0 to 25mg $K_2Cr_2O_7$) and zinc sulfate heptahydrate (ranging from 0 to 9 mg $ZnSO_4$ $.7H^2O$) were used as reference substances in the ovigerous female and nauplii protocols, respectively, for assessing sensibility of the test organism (positive control). The estimated LC_{50} 96h for ovigerous females of *T. biminiensis* and estimated LC_{50} 72h for newly hatched nauplii of *T. biminiensis* was calculated using the Trimmed Spearman-Karber method (Hamilton et al., 1977) and were within the standard deviation published by Araujo-Castro et al. (2009) and Lavorante et al. (2013), respectively.

Sediment properties

The analysis of grain size of the sediments was done using the procedures described by Suguio et al., (1973) and the sediment was classified in relation to its percentage of sand and clay. The content of carbonate was evaluated according to the methodology of Costa (20116) with minor adaptation, which is based on the removal of carbonate by hydrochloric acid digestion followed by samples weight loss (gravimetric determination). The total organic carbon (TOC) and nitrogenous (N) percentage were determine through an elemental analyzer coupled to an isotope ratio mass spectrometry (EA-IRMS), after the carbonate of the sediment was acid digested as already described.

Metals

Prior to chemical analyses, sediment samples were oven dried (105 ºC), ground and sieved through a 63 µm sieve. A total of 23 metals (Ag, Al, As, Ba, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sr, Ti, V, W, Zn, Zr) and the non-metals P and S were extracted from sediment through partial extraction and analyzed by inductively coupled plasma mass spectrometry (ICP-MS). The standard reference material tested (Canadian Certified Reference Materials - TILL 3 Geochemical soil and TILL reference material) was within the expected values and the recovery ranged between 86,84% and 105,8%. The coefficient of variation (CV) of the duplicate was less than 20 %. Only 9 (Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, Zn) of 25 elements analyzed has been used to assess the impact of contaminated sediments upon aquatic biota (Long et al., 1995, Macdonald et al., 1996, Buchman, 2008).

BTEX

Benzene, toluene, ethylbenzene and xylenes (BTEX) were analyzed according to EPA (2014). The recovery of surrogate was higher than 75% for all elements tested.

Organochlorines and Polycyclic Aromatic Hydrocarbons (PAHs)

Polychlorinated biphenyls (182 congeners), chlorinated pesticides (cyclodienes, hexaclorociclohexane- HCHs, Dichlorodiphenyltrichloroethane -DDT and metabolites, Mirex) and polycyclic aromatic hydrocarbons (16 priority PAHs according to EPA) were analyzed based on procedures described by Bicego et al. (2006). Briefly, sediment samples were extracted in Soxhlet apparatus and cleaned up using column adsorption chromatography. Extracts were injected in a gas chromatograph coupled to a mass spectrometer (GC-MS). The average recovery of the surrogate standard and standard reference material was 81,1% and 104,5% respectively, with the first one varying from 77,8% to 84,4% and the last one varying from 97,2% to111,9%

Statistical analysis

The ecotoxicological data normality was verified using the Kolmogorov-Smirnov test, and *Student* t-test was performed to evaluate differences between stations and control data. The significance level used for all tests was set at 0.05. The Principal Component Analysis (PCA) was also used for evaluating potential interrelationships between all chemicals analyzed and toxicological data. For interpreting PCA data, the loadings were considered as having good association with respective PC when higher or equal to 0.55, according the criteria proposed by Tabachnick and Fidell (2007)

Results

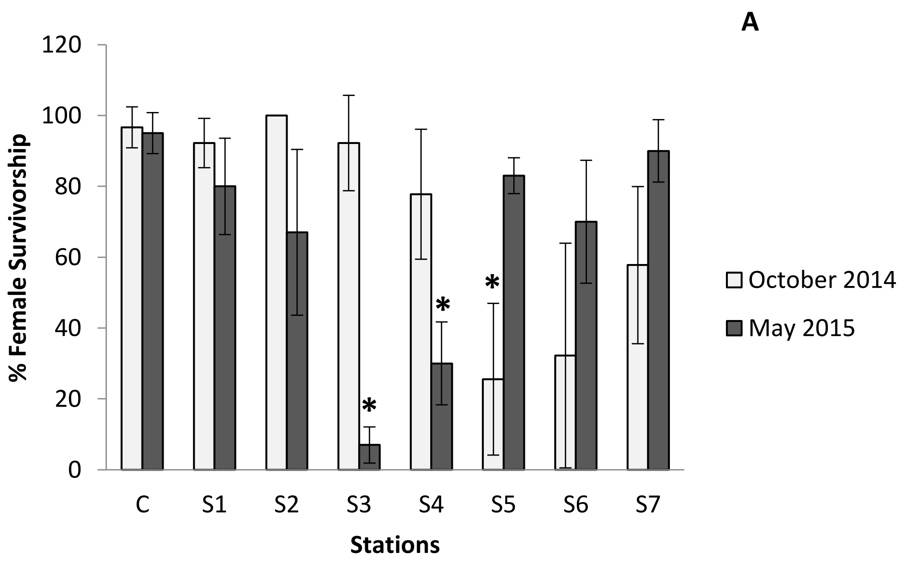
Toxicity tests

Based on Souza-Santos et al. (2006), Araujo-Castro et al. (2009) and Lavorante et al. (2013), seawater parameters were successfully maintained within acceptable ranges during toxicity tests: 33 to 36 for salinity, 7.2 to 8.1 for pH, and 3.9 to 5.6 mg L^{-1} for dissolved oxygen. Survival of organisms exposed to the control sediment was higher than 80%.

Coefficient of variation (CV) of nauplii inoculated, calculated for the experiments that used nauplii as test-organism, were 6,77 ± 9 and 9.99 ± 2.9 (mean ± standard deviation) respectively in October of 2014 and in May of 2015 what validates the sampling method so the test was considered valid.

In October 2014, survivorship of ovigerous females of *T. biminiensis* in the toxicity tests was only significantly lower than the control at station S5 (Fig. 2a). The offspring development rate was significantly lower than the control at station S1 (Fig. 3a) while fecundity rate was not lower than the control at any stations (Fig. 4). Survivorship of *T. biminiensis* in the nauplii toxicity tests was significantly lower than the control at stations S1, S3, S4 and S5 (Fig. 2b), whereas the nauplii development rate was significantly lower than the control at stations S1, S5 and S6 (Fig. 3b). All endpoints (except offspring development of ovigerous female test) had lowest values in station S5.

In May 2015, survivorship of ovigerous females of *T. biminiensis* in the toxicity test was significantly lower than the control at stations S3 and S4 (Fig. 2A). The offspring development was significantly lower than control at stations S3, S4 and S5 (Fig. 3B) while fecundity rate was significantly lower than the control at stations S2, S3 and S4 (Fig. 4). Survivorship of *T. biminiensis* in the nauplii toxicity tests was significantly lower from the control at stations S1, S2, S3, S4 and S5 (Fig. 2B), whereas the nauplii development rate was significantly lower than the control at stations S3, S4 and S6 (Fig. 3B). All endpoints had lowest values in station S3.



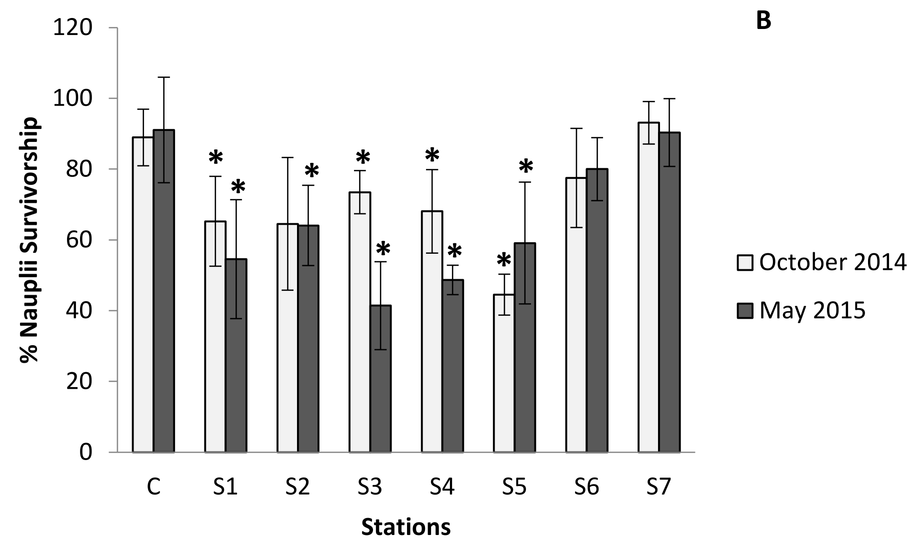
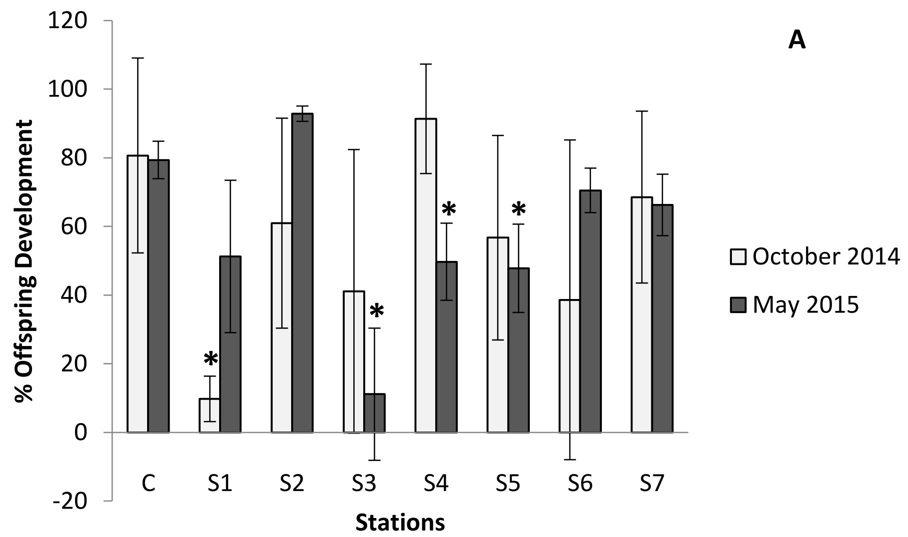


Fig. 2. Survivorship rate of *T. biminiensis* exposed to sediment of the Capibaribe estuary: (A) ovigerous females test and (B) nauplii test. Results are presented as mean ± standard deviation. Asterisks indicate significant difference between the control (C) and sampling sites (S1-S7; $p < 0.05$).



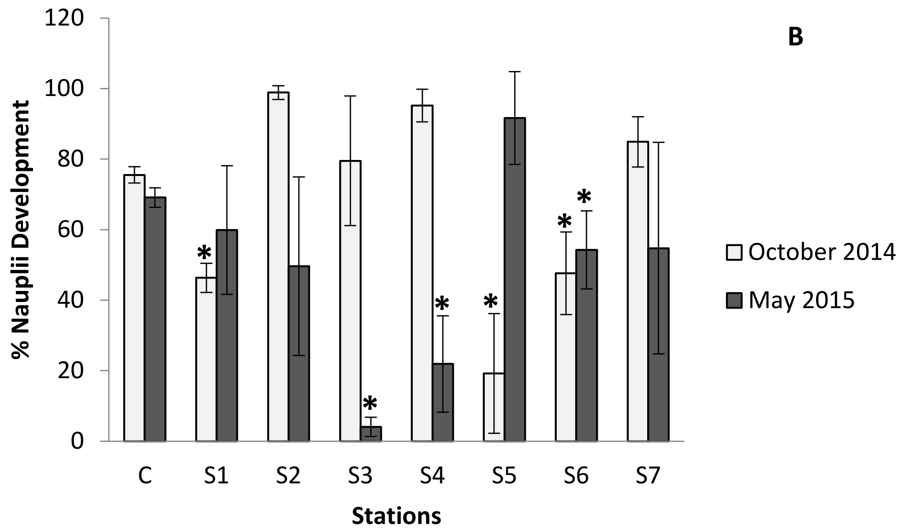


Fig. 3. Offspring development rate of *T. biminiensis* exposed to sediment of the Capibaribe estuary: (A) ovigerous females test and (B) nauplii test. Results are presented as mean ± standard deviation. Asterisks indicate significant difference between the control (C) and sampling sites (S1-S7; $p < 0.05$).

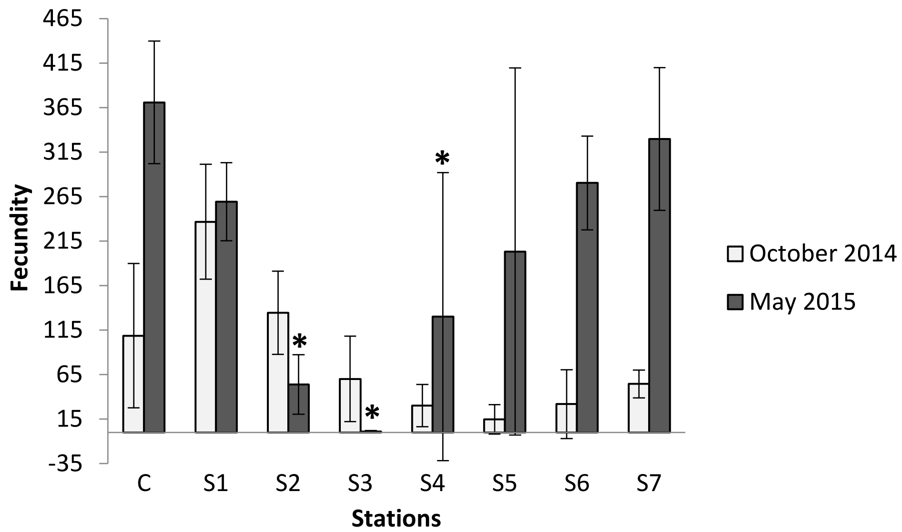


Fig. 4. Fecundity rate (expressed as total offspring produced by 10 females during 7 days) of *T. biminiensis* ovigerous females exposed to sediment of the Capibaribe estuary. Results are presented as mean ± standard deviation. Asterisks indicate significant difference between the control (C) and sampling sites (S1-S7; $p < 0.05$).

Sediment properties

Grain size distribution, carbonate, TOC and total nitrogen are shown in Table 1. The grain distribution of the sediment, both in dry and wet season, followed a pattern in which sediments closer to the estuary mouth had lower muddy fraction and higher sand predominance, which tends to invert towards the estuary head. In May of 2015 (beginning of rainy season) the station S7 had lower muddy fraction than in October of 2014 (beginning of dry season). In stations S5 and S6 the opposite was observed, higher muddy fraction in May of 2015 than in October of 2014. TOC and nitrogen concentration increased from stations S1 to station S2 (October of 2014), and to station S3 (May of 2015) and decreased towards stations S7. Carbonate values in October of 2014 increased from the upper to lower estuary with exception of S1 and S5, which had higher values (19.6% and 5.6% respectively). In May of 2015, it showed an opposite trend with values decreasing from the upper to lower estuary.

Table 1. Properties of the Capibaribe estuary sediment collected in October 2014 and May 2015.

		Sand (%)	**Mud (%)**	**CO_3^{-2}(%)**	**TOC (%)**	**TN (%)**
October 2014	S1	4.09	95.91	19.62	3.16	0.29
	S2	11.1	88.9	0.68	5.83	0.55
	S3	19.4	80.6	5.62	3.84	0.36
	S4	18.98	81.02	1.51	5.18	0.51
	S5	30.91	69.09	3.07	3.55	0.33
	S6	50.98	40.02	3.15	3.22	0.29
	S7	40.0	60.0	3.74	1.64	0.14

May 2015	S1	3.94	96.06	24.04	4.00	0.48
	S2	18.83	81.17	13.11	5.35	0.57
	S3	7.53	92.47	13.80	7.40	0.91
	S4	11.65	88.35	7.17	5.44	0.57
	S5	10.05	89.95	7.97	5.25	0.58
	S6	29.88	70.12	5.41	4.02	0.29
	S7	48.27	51.73	4.85	1.19	0.14

CO_3^{-2}= carbonate; TOC= total organic carbon; TN= total nitrogen

Chemical analysis

Concentrations of chemical contaminants as well as their threshold effect level (TEL) and probable effect level (PEL) are shown in Table 2. In general, it was observed a peak in the concentration of metals at station S5 with decreasing trends both upstream and downstream. On the other hand, organic contaminants usually peaked at stations S2 and S3.

Table 2. Chemical contamination of the Capibaribe estuary sediments collected in October 2014 and May 2015.

	October 2014							**May 2015**								
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**TEL[1]**	**PEL[1]**
Ag (µg.g^{-1})	0.49	<0.01	0.47	0.60	**1.99**	<0.01	<0.01	<0.01	<0.01	0.63	**1.05**	**1.64**	**2.96***	0.73	0.73	1.70
As (µg.g^{-1})	3	1	**9**	2	5	<1	<1	<1	<1	2	3	4	5	**10**	7.24	41.60
Cd (µg.g^{-1})	0.35	0.36	0.31	0.46	**0.69**	0.19	0.13	0.07	0.18	0.42	0.50	0.57	0.54	0.30	0.68	4.20
Cr (µg.g^{-1})	51	50	48	**54**	**66**	32	41	41	41	51	**54**	**56**	**59**	51	52.30	160
Cu (µg.g^{-1})	**52.40**	**40.80**	**39.60**	**61.50**	**75.60**	**19.70**	**20.30**	17.10	**21.50**	**56.20**	**70.90**	**80.50**	**73.40**	**43.60**	18.70	108
Hg (µg.g^{-1})	0.07	0.03	0.04	0.04	0.08	0.02	0.02	0.06	0.07	**0.23**	**0.33**	**0.41**	**0.61**	**0,22**	0.13	0.70
Ni (µg.g^{-1})	**18.80**	**18.50**	15.60	**20.30**	**23.30**	13.80	**16.80**	**16.10**	**16.80**	**17.20**	**17.50**	**16.80**	**16.80**	11.60	15.90	42.80
Pb (µg.g^{-1})	**35.50**	**33.20**	28.40	**35.40**	**41.70**	24	20.80	16.30	19.70	**35.70**	**37.80**	**38.50**	**40.90**	27.80	30.24	112
Zn (µg.g^{-1})	**164**	**140**	**155**	**209**	**274***	66	62	53	91	**212**	**259**	**306***	**257**	**181**	124	271
Benz(a)anthracene (ng.g^{-1})	54.43	**81.91**	21.12	23.67	11.53	5.17	7.67	27.21	**138.83**	37.99	34.39	19.37	4.65	3.09	74.80	693
Benzo(a)pyrene (ng.g^{-1})	72.47	**104.69**	25.39	32.96	14.01	9.81	10.15	36.81	**121.59**	35.80	33.14	20.49	nd	5.05	88.80	763
Chrysene (ng.g^{-1})	57.87	106.36	33	40.40	21.81	8.32	9.92	33.03	**178.91**	58.02	53.74	34.59	8.55	4.65	108	846
Dibenz(a,h) anthracene (ng.g^{-1})	**15.12**	**20.09**	**7.21**	**9.40**	4.47	2.58	2.78	**6.96**	**25.21**	**12.75**	**8.97**	**6.27**	2.42	1.95	6.22	135
Acenaphthene (ng.g^{-1})	1.53	2.53	1.04	1.08	< 0.72	< 0.7	< 0.73	0.93	**7.06**	1.57	1.52	1.03	< 1.02	< 0.83	6.71	88.90

Acenaphthylene ($ng.g^{-1}$)	**16.33**	**2.,12**	4.92	3.27	1.76	< 0.7	0.93	**6.07**	**17.80**	5.80	3.39	3.08	< 1.02	0.91	5.87	128
Anthracene ($ng.g^{-1}$)	10.89	21.36	6.06	5.85	2.95	2,24	1.70	7.45	32.12	7.76	7.64	5.40	1.93	1.57	46.90	245
Phenanthrene ($ng.g^{-1}$)	34.94	75.45	42.56	36.99	23.00	17.72	9.99	19.15	**135.44**	46.11	49.39	44.55	7.40	4.01	86.70	544
Fluoranthene ($ng.g^{-1}$)	77.06	**150.32**	47.69	48.42	22.16	16.22	12.90	46.23	**286.81**	77.17	62.41	49.89	18.46	6.67	113	1494
Fluorene ($ng.g^{-1}$)	5.81	17.08	7.19	4.79	1.98	0.75	0.90	2.93	13.79	7.30	6.80	4.49	1.31	< 0.83	21.20	144
Naphthalene ($ng.g^{-1}$)	3.59	1.46	1.46	11.28	5.18	nd	1.15	11.68	20.49	4.17	5.08	11	1.12	< 0.83	34.60	391
Pyrene ($ng.g^{-1}$)	100	**172.12**	63.54	65.26	33.59	25.48	10.99	50.83	**262.29**	81.70	73.92	76.72	11.98	6.07	153	1398
∑PAHs ($ng.g^{-1}$)	732.34	1205.60	390.44	454.79	222.83	130.48	121.06	398.85	**1745.57**	583.59	489.53	406.71	93.46	57.72	1684	16770
p,p'-DDD ($ng.g^{-1}$)	nd	nd	nd	nd	nd	nd	nd	nd	**4.48**	nd	nd	nd	nd	< 0.83	1.22	7.81
p,p'-DDE ($ng.g^{-1}$)	**4.18**	**6.51**	**6.79**	**6.42**	**2.68**	1.98	1.04	**4.86**	**7.51**	**7.65**	**6.42**	**5.96**	1.52	**3.31**	2.07	3740
p,p'-DDT ($ng.g^{-1}$)	nd	nd	nd	nd	nd	nd	**6.23***	nd	**11.83***	nd	nd	**5.41***	**4.49***	**2.28**	1.19	4,77
DDT + DDE + DDD ($ng.g^{-1}$)	**4.18**	**6.51**	**6.79**	**6.42**	2.68	1.98	**7.26**	**4.86**	**32.72**	**7.65**	**6.42**	**11.38**	**6.01**	**5.58**	3.89	51.70
∑ PCB ($ng.g^{-1}$)	10.23	12.24	10.04	10.75	8.53	5.35	3.36	3.42	15.35	8.64	7.99	12.09	2.87	3.62	21.60	189

[1] TEL, threshold effect level; PEL, probable effect level (Macdonald et al. 1996); Values higher than TEL are in bold; Asterisks indicate values higher than PEL; ∑ PCB= PCB-28 + PCB-52 + PCB-101 + PCB-118 + PCB-138 + PCB-153 + PCB-180; ∑PAHs= 12 listed on this table plus benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene and indeno(1,2,3 cd)pyrene; nd= not detected

Multivariate analysis

In October 2014, the first principal component (PC1) explained 48% of the data variance. It had negative correlation with %Sand, mortality of females (MF), inhibition of fecundity (IF), Mn and DDT; this axis showed positive correlation with %Mud, TOC, nauplii mortality (MN), all PCBs except PCB90/101 and PCB175, all PAHs except naphthalene and 2- methylnaphthalene, metals Al, Fe, Pb and V. The second principal component (PC2) explained 24% of the data variance and had positive correlation with MN, inhibition of nauplii development (IND), mostly metals (including highly toxic Cd, Cr, Cu, Hg, Pb), PCB 90/101, PHAs naphthalene and 2- methylnaphthalene (Fig. 5a, 5b and table 3). This axis had negative correlation with PCBs 175 and 183 only.

Table 3. Correlation coefficient from PCA integrating sediment properties, chemicals concentration and toxicities from sediment samples of Capibaribe river estuary collected in October 2014.

Variable	PC1	PC2	Variable	PC1	PC2	Variable	PC1	PC2
%SAND	**-0.89**	-0.05	Ni	0.43	**0.69**	Benzene	**0.81**	0.39
%MUD	**0.87**	0.10	P	0.46	**0.75**	Toluene	0.18	**0.87**
TOC	**0.83**	0.00	Pb	**0.66**	**0.67**	Ethylbenzene	**0.81**	0.39
%N	**0.82**	0.03	S	0.35	**0.89**	Naphthalene	0.34	**0.63**
MF	**-0.65**	0.46	V	**0.67**	0.47	2-Methylnaphthalene	0.47	**0.61**
IF	**-0.55**	0.43	Zn	0.50	**0.85**	Acenaphthylene	**0.83**	-0.46
IOD	0.15	0.10	Zr	0.32	**0.67**	Acenaphthene	**0.90**	-0.44
MN	**0.55**	**0.64**	DDT	**-0.59**	-0.30	Fluorene	**0.86**	-0.42
IND	-0.29	**0.52**	DDE	**0.82**	0.04	Fenantrene	**0.90**	-0.30
Al	**0.92**	0.23	PCB-90/101	0.24	**0.70**	Anthracene	**0.86**	-0.47
As	0.17	**0.55**	PCB-95	**0.82**	-0.15	Fluoranthene	**0.88**	-0.45
Ba	-0.36	**0.64**	PCB-110	**0.93**	0.14	Pyrene	**0.91**	-0.39
Cd	0.47	**0.80**	PCB-118	**0.93**	0.09	Benzo[a]anthracene	**0.87**	-0.43
Cr	0.54	**0.75**	PCB-138/160	**0.96**	0.06	Chrysene	**0.92**	-0.37
Cu	0.53	**0.82**	PCB-139/149	**0.83**	-0.10	Benzo[b]fluoranthene	**0.84**	-0.48
Fe	**0.74**	**0.54**	PCB-153	**0.95**	-0.04	Benzo[k]fluoranthene	**0.87**	-0.43
Hg	0.36	**0.74**	PCB-175	0.30	**-0.64**	Benzo[a] pyrene	**0.86**	-0.44
K	0.39	**0.81**	PCB-180	**0.74**	-0.51	Indene [1,2,3-	**0.90**	-0.40

						cd]pyrene		
Mg	0.25	**0.60**	PCB-182/187	**0.72**	-0.48	Dibenzo[a,h]anthracene	**0.92**	-0.35
Mn	**-0.68**	0.05	PCB-183	**0.65**	**-0.55**	Benzo[ghi]perylene	**0.95**	-0.31
Mo	0.27	**0.87**	∑ PCB	**0.97**	0.15	∑HPAs	**0.91**	-0.40
Na	0.09	**0.65**						

Values in bold indicates significant correlation (≥0.55) with the respective axis.

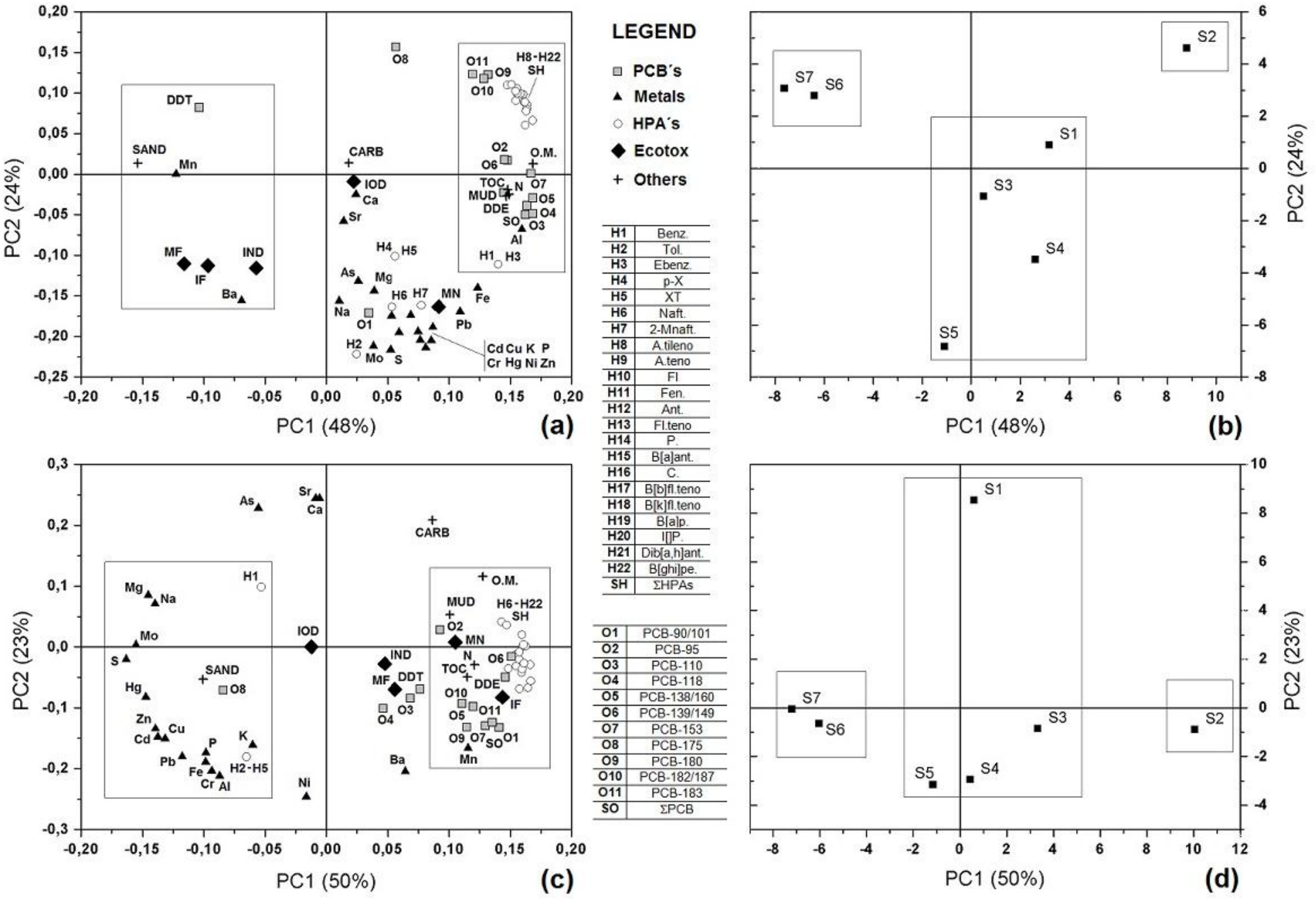


Figure 5. Ordination results of the principal component analysis (PCA). (a) and (b): respectively loading plot and score from October of 2014. (c) and (d): respectively loading plot and score plot from May of 2015. MF- mortality of female´s test; IF- inhibition of fecundity- female´s test; IOD- inhibition of offspring development- female´s test; MN- mortality of nauplii´s test; IND- inhibition nauplii development- nauplii´s test.

In May 2015, PC1 explained 50% of the data variance and had positive correlation with %Sand and 11 metals including Cd, Cu, Pb and Hg. This axis had negative correlation with % Mud, TOC, inhibition of fecundity (IF), MN, with all PAHs, 6 PCBs, DDE, and metals Mn and Ti. PC2 explained 23% of the data variance and showed positive correlation with carbonate, As, Ca, Sr, and negative correlation with 13 metals, ethylbenzene, p-xylene and total xylene (Fig. 5c, 5d, and table 4).

Table 4. Correlation coefficient from PCA integrating sediment properties, chemicals concentration and toxicities from sediment samples of Capibaribe river estuary collected in May of 2015.

Variables	PC1	PC2	Variables	PC1	PC2	Variables	PC1	PC2
%SAND	**0.58**	-0.27	Mn	**-0.67**	**-0.67**	Toluene	0.38	**-0.76**
%MUD	**-0.58**	0.27	Mo	**0.90**	-0.01	Ethylbenzene	0.38	**-0.76**
TOC	**-0.66**	-0.22	Na	**0.81**	0.24	p-Xylene	0.38	**-0.76**
Carbonate	-0.51	**0.84**	Ni	0.10	**-0.98**	Total Xylene	0.38	**-0.76**
%N	**-0.69**	-0.11	P	**0.58**	**-0.65**	Naphthalene	**-0.83**	0.15
MF	-0.32	-0.24	Pb	**0.69**	**-0.71**	2-Methylnaphthalene	**-0.85**	0.14
IF	**-0.83**	-0.31	S	**0.95**	-0.09	Acenaphthylene	**-0.93**	0.05
IOD	0.07	0.06	Sr	0.04	**0.98**	Acenaphthene	**-0.86**	-0.18
MN	**-0.61**	0.09	Ti	**-0.61**	**-0.67**	Fluorene	**-0.95**	-0.28
IND	-0.28	-0.10	V	0.32	**-0.91**	Fenantrene	**-0.91**	-0.29
Al	0.51	**-0.84**	W	-0.15	**0.93**	Anthracene	**-0.90**	-0.09
As	0.32	**0.91**	Zn	**0.82**	-0.52	Fluoranthene	**-0.90**	-0.16
Ba	-0.37	**-0.78**	DDE	**-0.85**	-0.18	Pyrene	**-0.92**	-0.19
Ca	0.03	**0.98**	PCB-90/101	**-0.81**	-0.51	Benzo[a]anthracene	**-0.91**	-0.11
Cd	**0.80**	**-0.58**	PCB-138/160	**-0.64**	-0.36	Chrysene	**-0.93**	-0.17
Cr	0.55	**-0.82**	PCB-139/149	**-0.88**	-0.05	Benzo[b]fluoranthene	**-0.93**	-0.02
Cu	**0.77**	**-0.59**	PCB-153	**-0.75**	-0.50	Benzo[k]fluoranthene	**-0.95**	-0.02
Fe	**0.58**	**-0.75**	PCB-180	**-0.66**	-0.51	Benzo[a] pyrene	**-0.93**	-0.02
Hg	**0.86**	-0.35	PCB-182/187	**-0.69**	-0.38	Indene [1,2,3-cd]pyrene	**-0.92**	-0.07
K	0.35	**-0.69**	PCB-183	**-0.69**	-0.38	Dibenzo[a,h]anthracene	**-0.97**	-0.14
Mg	**0.84**	0.31	∑ PCB	**-0.78**	-0.49	Benzo[ghi]perylene	**-0.97**	-0.23
						∑HPAs	**-0.94**	-0.13

Values in bold indicates significant correlation (≥0.55) with the respective axis.

Discussion

The ecotoxicological test that uses *T. biminiensis* nauplii as test-organisms proved to be suitable for assessing quality of the sediment as all stations that were toxic in the previous methodology were also in this. In fact, it can be considered more sensitive, as some stations that were not toxic in the ovigerous female test, were considered toxic on the nauplii test (stations S3, S4 and S6 in October of 2014; S1 and S6 in May of 2015). Also stations that were sensitive to sublethal endpoints were now sensitive to lethal endpoints (stations S1 in October of 2014; S2 and S5 in May of 2014). This is a great outcome, a less laborious methodology that can be used as an alternative to assess the sediment toxicity, and that also requires a shorter amount of time to be executed and therefore is less onerous (Zagatto and Bertoletti, 2008). Also this results reinforces the knowledge, that earlier developmental stages of copepods are more sensitive than older stages in life cycle, shown in some studies under laboratory conditions for specific contaminants. Araújo-Castro et al. (2009) stated that *T. biminiensis* nauplii were more sensitive to potassium dichromate than ovigerous female of the same species. Bao et al. (2014) showed higher sensitivity of the nauplii of marine copepod *Tigriopus japonicas* to zinc pyrithione alone and in combination with copper than of adult copepods. Huang et al. (2006) found that nauplii were more sensitive to bis(tributyltin) oxide than copepodites of calanoid copepod *Pseudodiaptomus marinus*. Saiz et al. (2009) stated that naupliar stages of the marine cyclopoid copepod *Oithona davisae* had lower tolerance to PAH (naphthalene and 1,2-dimethylnaphthalene) than adults.

Grain size variation between stations appears to occur due to hydrodynamic with stations closer to estuary mouth (S7) having higher % Sand as a consequence of higher energy of tidal currents, and stations closer to estuary head having higher Mud %. The lower muddy fraction in the beginning of rainy season (May of 2015) in station S7 in relation to the beginning of dry season (October of 2014) is probably an effect of the decrease of river influx during the dry season and consequent decrease of water stream velocity. As turbulence water may not reach the lower estuary, the fines particles will no longer stay suspended and will settle to the bed before reaching station S7, which may accumulate in the middle estuary (stations 5 and 6).

The highest concentration of TOC and %N in station S2 in October of 2014, and in stations S3, in May of 2015 is probably associated to the presence of an estuarine turbidity maxima (ETM) in the Capibaribe river estuary that was reported by Schettini et al. (2015) of being located around 15 km from the estuary mouth, in between stations S2 and S3. One of the implications of the presence of the ETM is the accumulation of organic matter and also the presence of pollutants adsorbed to fine clay sediments (Morgan et al.,1997; Brunk et al., 1996). The lowest TOC and %N concentrations in stations S7 is probably associated with the influence of hydrodynamics.

In this study we compared the concentrations from chemicals of the sediment of the Capibaribe river estuary to the threshold effect level (TEL) and to the probably effect level (PEL) published by Macdonald et al. (1996). In this matter, the commitment of sediment of the Capibaribe river estuary appear to be severe as in each station we detected at least one metal above TEL. From stations S1 to S4 at least one PAH were above TEL, DDE were above TEL from stations S1 to S5, and DDT was above PEL at stations S2, S5 and S6 (May of 2015).

Metals (Zn, Cr) above TEL were detected by Oliveira, D. et al (2014) in values mostly higher than the ones found in this study in the mangrove and Pina sound area (Fig. 1). The station S5 had the highest values of metals, in both collections dates, and 7 (Ag, Cd, Cr, Cu, Ni, Pb, Zn) out of 9 metals above their TEL values, in which Zn was even above PEL. This station is closer to the connection between Capibaribe river main channel to the Pina sound and is probably influenced by the contamination found in that area. Stations S5 also had the lowest values in toxicity endpoints of October of 2014 which were probably associated with these metal concentrations as they were above the concentration limit of not causing toxic effect to organisms (Macdonald et al.,1996).

The metals concentrations found in this study is in the average of others tropical estuaries: it was higher than what Krull et al. (2014) reported for the Subaé estuary (Brazil), smaller than what Cordeiro et al. (2015) report for Guanabará bay (Brazil), similar to what Swarnalatha et al (2015) reported for Akkulam-Veli (India) and to what Abrahim et al. (2008) reported for Tamaki estuary (New Zealand).

PAHs concentrations had a similar pattern in both collections dates. They were detected at higher levels in stations S2 wherein 7 were above TEL (table2). Dibenz(a,h)antharecene was above TEL in stations S1, S2, S3, and S4. Maciel et al (2015a) studying the distribution of the 16 PAHs considered as priority by the US Environmental protection Agency (US-EPA) in the Capibaribe estuarine system found only Dibenz(a,h) antharecene above TEL in stations located in the Pina sound, which is connected to the Capibaribe river main channel closer to stations S5. The author also highlighted the harmful potential of causing effects to local biota as it´s a compound considered by IARC (International Agency for research on Cancer, 1983) as highly carcinogenic and teratogenic. According to the sum of 16 PAHs (∑16PAHs), in both collection dates, sediments from stations S1, S3, S4, S5, S6 and S7 were classified as moderately contaminated (100-1000ng.g^{-1} of ∑16PAHs) while stations S2 were classified as highly contaminated (1000-5000ng.g^{-1} of ∑16PAHs) (Baumard et al., 1998). Smaller concentrations were reported by Oliva et al. (2015) in sediments of Bahía Blanca estuary (Argentina), and similar results were reported by Araghi et al. (2014) in Gorgan Bay (Iran).

Station S3 had higher As values, and stations S4 had higher fluoranthene and 2metyl-naphthalene values than all other stations. Those stations were toxic for nauplii but not for ovigerous female. The difference of sensibility from organisms might be related to those, although it´s hard to affirm that relaying in only these observations, what would require further studies.

In October of 2014, DDE and DDT were detected. The first one was above TEL in stations from S1 to S5, while DDT was detected only at stations S7 and was above PEL. In May of 2015, DDT was detected at alarming levels above PEL in stations S2 (highest value) S5 and S6. DDE was also detected and above TEL in all stations but station S6. The high DDT and metabolites (DDE/DDD) levels found in this study, in May of 2015 where they were not detected in October of 2014 shows that they are being illegally used, what is of concern as the use of DDT is forbidden since 2004 (D´amato et al., 2002) for its highly toxic characteristics.

The higher concentrations of organic chemicals in stations S2 and S3 can also be explained by the presence of ETM, as stated before (Morgan et al.,1997; Brunk et al., 1996). However, even being the station with higher organic chemicals concentration, station S2 were not considered toxic by any endpoint analyzed in October of 2014, what can be due to the organic compounds not being bioavailable or that *Tisbe biminiensis* is not much sensitive to those compounds. In May of 2015 stations S3 was the stations with highest organic concentrations and also lowest toxicity endpoints. What can be related to a deleterious effect of the organic compounds that would be now bioavailable.

Addressing the multivariate analyses, in October of 2014, estuarine hydrodynamics seems to be associated with PC1 as percentage of sand and mud had strong and opposite correlations with this axis and stations S6 and S7 (lower estuary) are negatively loaded on PC1 along with sand (Fig. 5). Conversely, stations S2 (upper estuary) are positively loaded on

PC1 along with mud, metals and organic contaminants (Fig. 5). PC2 is probably associated with toxicity as mortality of nauplius (MN) and inhibition of naupliar development (IND) are positively correlated with this axis along with mostly metals, and S2 (the least toxic) and S5 (the most toxic) are strongly loaded on opposite sides of PC2 while other stations are gradually loaded in between accordingly to its toxicity (Fig. 5b). From the score plot (Fig 5a) it looks like there is a strong correlation between MN and heavy metals as Pb, Cd, Cu Cr, Hg as they are situated close together, meaning that higher the concentrations of those metals higher were the nauplii mortality (MN). A high correlation of Mo and S with stations S5 indicate that it's a reductive environment and probably with high influence of sewage.

PCA of data from May of 2015 seems to have the PC1 axis associated with estuarine hydrodynamics similarly to results observed in October 2014. From PC2 the clearly correlation was with station S1 with Ca and Sr, however this was not expected as the stations closer to estuary mouth usually have higher contents of carbonate source (Oliveira, T. et al. 2014). From the score plot (Fig. 5d) is possible to observe a correlation from the mortality of nauplii (MN) with organic chemicals, meaning that these compounds were most likely influencing the adverse effect on the organisms in this collection date, which was not found in October of 2014. Also there was lack of correlation between metals and toxicity endpoints in May of 2015. Thus, even though high concentrations of chemicals are present in sediment it does not mean it is bioavailable and it will not necessarily cause prejudice effect to the biota (Poleto et al., 2008). Baird and Cann (2011) reported different samples having the same concentration of ions of a metal but varying by a factor of at least ten, in terms of toxicity for organisms associated with the metal.

Conclusions

A little laborious methodology to assess sediment ecotoxicology using nauplii of *Tisbe biminiensis* was proved to be suitable and more sensitive than a methodology that uses the ovigerous female from the same species. This study provided information about contamination by polycyclic hydrocarbons, organochlorine and metals in the tropical estuary of Capibaribe river, that were mostly bioavailable and causing harmful effect on the biota, with higher commitment of sediment located in the city center and slightly commitment of stations located in both head and mouth of estuary. It also reinforced the probable occurrence of an estuarine turbidity maxima, where the concentration of organic chemicals and total organic carbon was present.

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