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Sediment–water interface and water toxicity tests with mysids (Crustacea, Mysidacea) to assess the quality of tropical estuaries

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Abstract. The current research aimed to evaluate the environmental quality of Poxim river estuary (Sergipe, Brazil) using the acute toxicity test with superficial water, and sediment-water interface (SWI) as an innovative approach with the microcrustacean *Mysidopsis juniae*. Superficial water and sediments were sampled in three sites along the extension the Poxim estuary, from the inner regions to the mouth of the estuary. The mysids were exposed to water and to the SWI system to test for acute toxicity (96 h). Water samples were less toxic to mysids than SWI samples, showing water acute toxicity only in inner part of estuary SWI showed toxicity to all samples only during the rainy month. The SWI and water toxicity tests with mysids were a valuable assay for the identification of toxicity from substances that can be remobilized from sediment to water column and therefore an important tool for assessing contamination in tropical estuaries.

Keywords: acute toxicity, sediment/water interface, Aracaju, estuarine pollution.

Testes de interface sedimento-água e toxicidade da água com misídeos (Crustacea, Mysidacea) para avaliar a qualidade de estuários tropicais. Resumo: Este estudo teve como objetivo avaliar a qualidade ambiental do estuário do rio Poxim (Sergipe, Brasil) utilizando o teste de toxicidade aguda com água superficial e interface sedimento-água (ISA) como uma abordagem inovadora com o microcrustáceo *Mysidopsis juniae*. Água superficial e sedimentos foram amostrados em três locais ao longo da extensão do estuário do Poxim, desde as regiões internas até a foz do estuário. Os misídeos foram expostos à água e ao sistema ISA para testar a toxicidade aguda (96 h). As amostras de água foram menos tóxicas para os misídeos do que as amostras de ISA, apresentando toxicidade aguda da água apenas na parte interna do estuário. O teste ISA apresentou toxicidade para todas as amostras apenas durante o mês chuvoso. Os testes de ISA e toxicidade da água com misídeos foram um valioso ensaio para a identificação de toxicidade de substâncias que podem ser remobilizadas do sedimento para a coluna d'água e, portanto, uma importante ferramenta para avaliar a contaminação em estuários tropicais.

Palavras-chave: toxicidade aguda, interface sedimento-água, Aracaju, poluição estuarina.

Introduction

The pollution in estuaries can be monitored by means of water analyses reveling recent and specific contamination processes, and by the sediment matrix that reflects continuous and dispersed contamination (Chapman & Wang 2001). The sediments may accumulate higher concentration of contaminants in contrast to water (Beretta *et al.* 2014; Maioli *et al.* 2010; Nilin *et al.* 2013). This environmental matrix should be used to monitor long term impacts, while water is better indicator of short-term ones (Li *et al.* 2018). Intensive nutrient cycling occurs at the interface between sediments and the water column, and the sediments constitute substrate and habitats for benthic organisms that serve as feeding and breeding sites for demersal species (Chapman & Wang, 2001). Therefore, it is important to validate toxicity tests in both environmental matrices in order to achieve an adequate understanding of the effects of contaminants on an aquatic ecosystem. To assess the sediment toxicity, it is necessary to consider the main exposure routes of contaminants in specific habitats, such as the sediment–water interface (SWI) (Anderson *et al.* 2001, Cesar *et al.* 2004). The SWI is an ecologically relevant habitat, because it is a likely location for contamination exposure of infaunal, epibenthic, and water column species. Due to the flux of contaminants from the sediments, the constant deposition of new material from the water column and the remineralization of the organic matter in the sediments, the SWI may contain toxic concentrations of contaminants as well as it may be an important source of water column contamination (Chapman & Wang 2001).

Mysids are used worldwide in ecotoxicological studies due to their high sensitivity to several pollutants (Anderson & Phillips 2016) and easy maintenance in laboratory conditions. Their short life cycle and ecological relevance as a food source for bottom-feeding fish, support their use in marine and estuarine ecotoxicological studies (Lussier *et al.* 1985, Sardo *et al.* 2005). Out of the possible ecotoxicological tests with Mysids, the *Mysidopsis juniae* Silva 1979 acute test is a standardized method and is indicated for use in marine and estuarine water monitoring programs by the Brazilian Association of Technical Standards (ABNT), however, the standardized protocol only refers to the evaluation of acute toxicity in water matrices (ABNT 2011) and just recently begins to be used with sediment–water interface (SWI) (Moreira *et al.* 2019). Mysids are epibenthic crustaceans with a wide distribution in aquatic environments.

The Brazilian Environmental Legislation indicates that physical-chemical, microbiological and ecotoxicological parameters should be considered to assess the potential impacts of effluents in water bodies, as well as for environmental monitoring (Brasil 2005). However, there is no governmental monitoring program that includes integrated ecotoxicological monitoring and, consequently, the national environmental legislation is not being fully applied. Therefore, local governments are being unsuccessful in controlling the sources of pollution that are deteriorating the environmental conditions of estuaries. Moreover, the ecotoxicological tests applied in Brazil are limited in evaluating ecosystem quality, because they mainly consider the quality of the water but not the quality of the sediments (Krull & Barros 2012).

The estuary of Poxim river is located in the Brazilian state of Sergipe, in the northeast of Brazil. As a tropical urban estuary, the lack of an adequate basic sanitation network is a constant threat, with most effluents (industrial, domestic and livestock) being discharged untreated or poorly treated into the river (Alves & Garcia 2006). Rapid urbanization, deforestation and aquaculture are also considered threats (Alves *et al.* 2018, Frena *et al.* 2019).

In a study developed to assess the toxicity of water samples from the Poxim River, reduced survival of mysids was observed (Nilin *et al.* 2019). However, no study has reported the toxic effects of pollution on sediments, which is extremely important considering the growing impacts in this study region. In light of this, the aim of this study was to evaluate the environmental quality of the estuary of Poxim river using a standardized method (water acute toxicity assay) and an innovative approach employing a sediment–water interface (SWI) acute toxicity test. We hypothesize that the SWI test will indicate higher levels of toxicity than the water toxicity test, due to the predisposition for the sedimentation of some potential pollutants in this environmental matrix.

Materials and Methods

The Poxim river is about 45 Km long from its source to the estuarine area in the Sergipe River estuarine complex, where it flows into the Atlantic Ocean (Fig. 1). Due to the importance of the Poxim river to Aracaju, the capital of Sergipe State, since is one of the main tributaries of the Sergipe River basin, and contributes to the public water supply of the capital of Aracaju (657,013 inhabitants – 3,140.65 ind./km2), the Poxim Municipal Natural Park (1.8 Km2) was established in 2016 with the purpose to contribute to the preservation and proper management of mangrove remnants (Aracaju 2016). Some studies have demonstrated contamination by metal and hydrocarbons from industrial activity and oil exploration (Garcia *et al.* 2009; Lima *et al.*

2012), and from domestic wastewater in this area (Carreira *et al.* 2015, Quadra *et al.* 2017, Frena *et al.* 2019). Twenty-six percent of the public water supply of Aracaju comes from the Poxim river basin, through the catchment of the Jaime Umbelino de Souza dam, located about 25 Km from the confluence with the Sergipe River. This dam controls the Poxim river flow, and thus affects the estuary dynamics and adjacent areas, including flooding during the rainy season. The lack of suitable hydrological data from the Poxim river is a huge problem for water management (Valério & Negrão 2018); for example, no data on how the dam could affect flooding in Aracaju is available.

According to Koppen's climate classification, this area is classified as climate As, i.e., a warm and humid tropical climate, with a dry season in the Summer (Alvares *et al.* 2013). The rainy season occurs normally between April and July, but this period may vary from year to year (Marengo *et al.* 2017). During this study the total monthly precipitation varied from 26 mm to 203 mm (INMET 2016). On the coast of Sergipe, the tidal regime has a semi-diurnal variation, with tides with a maximum amplitude of 2.6m, which follow the predominant direction of the NE-SW winds and waves. The Poxim estuary presents an average depth of 1.35 m with a maximum of 3.0 m, consequently the sediments are quite susceptible to resuspension both by the increase of the river flow, as resulting from the daily tidal variations (Fonseca *et al.* 2010, Martins *et al.* 2020).

Samples were collected at three sites along the Poxim estuary (Figure 1): P1 (10º57'16.11 "S 37º3'42.6" W) 2.4 km from the river mouth at Inácio Barbosa district pier, located in the inner portion of the estuary near Poxim Municipal Natural Park; P2 (10° 57'33.09 "S 37° 3'13.95" W), located in the middle part of the estuary at Cajueiros Park (Farolândia district); and P3 (10°56'30.89 "S 37°2'58.01" W), located near the confluence with Sergipe river (Treze de Julho district). P3 is close to an artificial canal (Anísio Azevedo avenue) that drains part of the clandestine domestic effluents of the region. All along the estuary it is possible to observe the discharge of untreated effluents directly from houses.

Four sampling campaigns were carried out in October and December of 2015 and February and June of 2016, with precipitation of 32 mm, 26 mm, 51 mm and 203 mm, respectively (INMET 2016). Once a month, one sample of the superficial sediment (<3 cm depth) was collected from the

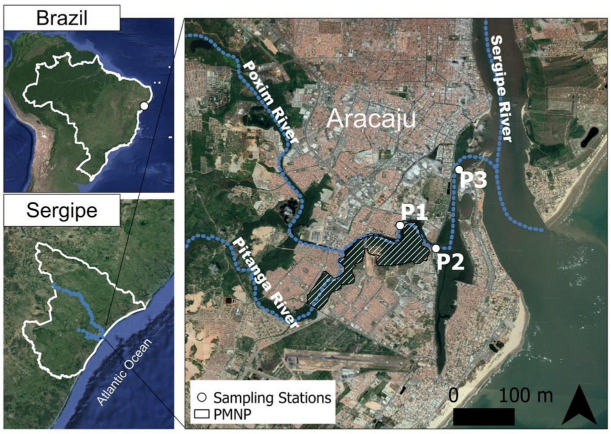

Figure 1. Map of sampling sites in the Poxim river estuary, Aracaju, Sergipe, Brazil. P1 – Inácio Barbosa district; P2 – Farolândia district; P3 – Treze de Julho district;). Poxim Municipal Natural Park (PMNP), (Secretaria de Meio Ambiente, Prefeitura de Aracaju).

exposed mud banks during low tide (0.0 to 0.5m) using a plastic shovel (5 to 8 grabs ~ 1 kg) and stored in plastic bags. Superficial water samples (~20 cm depth) were collected in the same day of the sediment samples and kept in polyethylene bottles (1 L). All samples were stored in a polystyrene box with ice until arrival at the laboratory, then stored in the refrigerator (10ºC) for 48 h (water samples) and up to 60 days (sediment samples), according to ABNT (2015).

The procedures for the culture of mysids and water assays followed the ABNT (2011) standards. Specimens of *M. juniae* were kept in glass aquaria filled with artificial seawater (100 mL/adult animal) made by adding sea salt (Red Sea® salt, Houston) to distilled water until a salinity of 35 ± 2‰ was achieved; this water was also used as the control. The organisms were fed daily *ad libitum* with *Artemia sp. nauplii* (enriched with fish oils and cod liver oil), after partially exchanging the water and separating the juveniles to be used in the experiments. The density of each aquarium was kept at around 60 adult animals (45 females: 15 males). All the aquariums were kept under constant aeration with a controlled photoperiod (12 h light:12 h dark) and temperature (24 ± 2ºC). Zinc ($ZnSO_4.7H_2O$, CAS 7446-20-0, Neon Comercial, São Paulo, Brazil) was used as the reference substance (0.075, 0.15, 0.30 and 0.60 mg. L^{-1} in triplicate) to test the monthly sensitivity of the animals during this study. In each beaker (300 mL), 10 juveniles (6-8 days old) were exposed to zinc for 96 h and survival was assessed every 24 h by counting before feeding (48 h Artemia sp. nauplii). Physical-chemical conditions were controlled (dissolved oxygen ≥ 4.00 mg. $L^{-1,}$ temperature 24 ± 2°C, salinity 35 ± 2‰, pH 7.1–8.3, using a multiparameter Hanna® Instruments, São Paulo, Brazil) (ABNT, 2011).

For the acute toxicity tests of water samples, the previously physical-chemical parameters presented and total ammonia (NH_4^+ + NH_3) of each sample were measured at the end of exposure (96 h) using a colorimetric assay with a limit of quantification of 3 mg. L^{-1} (Labcon® test, Santa Catarina, Brazil). The content of unionized ammonia (NH_3) was expressed according to the data table supplied with the Labcon® test, considering the pH and temperature of each sample. For estuarine water samples with a salinity lower than the acceptable range for *M. juniae* assays (35 ± 2‰), sea salt (Red Sea® salt, Houston) was added until the salinity required by the method was reached, immediately upon arrival at the laboratory. The water samples were tested by exposure of 10 juveniles to 300 mL of samples (in triplicate n=30) and daily survival was checked every 48 h before feeding with *Artemia sp. nauplii* (48 h). The beakers were placed in an incubator with a controlled photoperiod (12 h light:12 h dark) and temperature (24 ± 2ºC). Artificial seawater (35 ± 2‰) used for animal culture was adopted as the control. According to ABNT (2011) to acute test with water, to validation of test the mortality in the control should not exceed 10%.

The experimental design for the sediment–water interface (SWI) test for mysids was adapted from Cesar *et al.* (2004) (Fig. 2), that was designed to sea urchin embryos exposure in small test tube (30mL) without contact between embryos and sediment, to determine whether potential pollutants present in the sediment may be transferred to the water column by diffusion. In our experimental design, the mysids were in direct contact with the surface of sediment and water column. Sediment samples (100 mL) were put in beakers (400 mL – four replicates per sample/site), which were then filled carefully with artificial seawater (300 mL) (Fig. 2), with no barriers between the compartments that could prevent contact with the sediment. The beakers were maintained in a room with a controlled photoperiod (12 h light:12 h dark), under constant aeration and temperature (24±2ºC). The beakers were set up 24 h before adding five juveniles (6 –8 days old) per replicate (n=20) to stabilize the system. During the exposure time (96 h), animals were fed using the same procedure described above. Physical-chemical variables (including ammonia) were

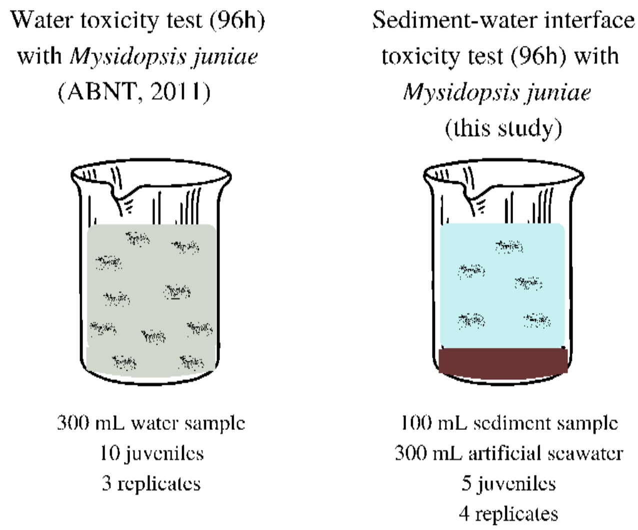

Figure 2. Experimental design of water toxicity test (96 h) with *Mysidopsis juniae* according to ABNT (2011) and sediment–water interface test (96 h) with *M. juniae* adapted from Cesar *et al.* (2004).

measured at the beginning and at the end of the exposure, using a sub-sample taken from the water column. As previously mentioned, this was the first study using a sediment toxicity approach in the Sergipe estuary, so no sediment control was available. Therefore, the artificial seawater added to the SWI system was used as the control.

Sediment analysis was performed by the determination of organic matter (%OM), calcium carbonate (%CaCO3) and the silt and clay fraction (%Mud). The sediment sub-samples were dried in an oven (60°C) for approximately five days. Organic matter (%OM) was estimated using the ignition method (Luczak *et al.* 1997). The dry sediment (10 g) was incinerated in a muffle furnace (500°C) for 4 h, and then organic matter quantities were determined by calculating the difference between the initial and final weights. Calcium carbonate was determined according to the protocol described by Nelson & Sommers (1982), which consists of sediment combustion (10 g) for 1 h in a muffle furnace (1000ºC), and then weighing the samples to calculate the difference between the initial and final weights. The mud fraction was quantified by wet sieving 100 g of dried sediments through a 0.062 mm mesh to separate sand from the silt and clay fractions, and the difference between the initial and the final weights represented the mud fraction (Mudroch & MacKnight 1994).

For reference substance (zinc) assays, mysid mortality was analyzed by determining the average concentration causing 50% mortality (LC50 96 h), using the Trimmed Spearman-Karber method

(Hamilton *et al.* 1977). Differences between temporal and spatial impacts on mysid survival (96 h) in the water and SWI exposure data (mean ± standard deviation) were compared with the control (artificial seawater). Normally distributed data were analyzed with the Shapiro-Wilks test, and non-parametric data using the Kruskal-Wallis test followed Dunn's test, using GraphPad Prism version 7 (GraphPad Software, California, USA). The field and laboratory data were integrated using principal component analysis (PCA) after data transformation to the z-score, using the PAST software (version 4.02).

Results

The physical-chemical variables of the water samples and sediment characteristics are presented in Table I. The pH values (7.60–8.03), temperature (24.1–26.2ºC) and dissolved oxygen (4.12–5.90 mg.L-1) in water samples showed low variation between sites and sampling days. As expected, the salinity varied widely (0–35), with values increasing towards the river mouth (P3). In the sediment samples, the percentage of calcium carbonate showed low temporal variation, but in all campaigns the highest values were observed at P1. The organic matter content varied from 0.92% to 12.47%, also being higher at P1. Similarly, the mud fraction indicated that fine particles tend to deposit in the inner portion of the estuary at P1 and P2 (>70%), while P3 is more sandy, probably due to its proximity with the oceanic zone.

The total ammonia (NH_4^+ + NH_3) in water samples varied from 0.25 to above 3.0 mg. L^{-1} (limit of quantification), with higher values found at P1 and P2 in all months (Table II). The unionized ammonia (NH3) showed a similar pattern with an increase towards the inner portion of the estuary (P1≈P2>P3). The physical-chemical parameters measured in water sample assays (Supplementary data 1A) did not differ significantly between sites and campaigns.

The sensitivity of *M. juniae* was assessed through the average zinc toxicity values obtained with an LC_{50} (96 h) of 0.33 ± 0.05 mg.L-1 (n= 9). The ecotoxicological tests of water samples showed that the mysid mortality was significantly higher ($p<0.05$) in Dec/15 (P1 – 66.7%) (Fig. 3). Furthermore, there was a significant increase in mysid mortality after exposure to the SWI system (Table III), mainly in June/16 when high levels of precipitation were recorded, which could have contributed to the runoff and consequent sedimentation of toxic chemicals. The SWI of P1

Table I. Physical-chemical variables of water samples (in situ) from three sampling sites (P1 – Inácio Barbosa district; P2 – Farolândia district; P3 – Treze de Julho district) in the Poxim river estuary (Sergipe, Brazil).

	Sites	pH	Salinity	Temperature (°C)	DO (mg.L^{-1})	OM (%)	Carbonate (%)	Fines particles (%)
Oct/15	P1	8.00	5	24.7	5.75	7.76	2.21	90
	P2	7.93	5	24.6	5.70	1.50	1.05	75
	P3	7.99	15	24.4	5.90	2.86	1.11	27
Dec/15	P1	7.98	10	25.0	4.70	6.31	1.78	90
	P2	8.00	20	25.2	4.50	0.92	0.91	83
	P3	8.03	33	25.2	4.65	1.07	0.39	15
Feb/16	P1	7.88	0	26.3	4.45	6.38	2.11	89
	P2	7.82	5	26.0	4.65	2.54	0.18	78
	P3	7.95	20	26.2	4.70	3.53	1.46	4
Jun/16	P1	7.54	17	25.1	4.12	10.49	5.49	96
	P2	7.60	22	25.3	4.20	5.31	1.89	84
	P3	7.63	35	25.5	4.60	3.65	2.28	15

Table II. Total ammonia and toxic ammonia at the end of the toxicity test in superficial water and sediment–water interface samples from three sampling sites: P1 – Inácio Barbosa district; P2 – Farolândia district; P3 – Treze de Julho district in the Poxim river estuary (Sergipe, Brazil). Artificial seawater was used as the control.

	Oct/15	Dec/15	Feb/16	Jun/16	Oct/15	Dec/15	Feb/16	Jun/16
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P1	3.0	3.0	3.0	3.0	0.067	0.133	0.164	0.108
P2	3.0	3.0	3.0	3.0	0.108	0.133	0.164	0.108
P3	1.5	0.25	0.5	1.5	0.054	0.011	0.027	0.054
Sediment-water interface								
Samples	Total ammonia (ppm)				Unionized ammonia (ppm)			
	Oct/15	Dec/15	Feb/16	Jun/16	Oct/15	Dec/15	Feb/16	Jun/16
Control	0.0	0.0	0.25	0.0	0.0	0.0	0.009	0.0
P1	3.0	1.5	3.0	3.0	0.164	0.082	0.108	0.0167
P2	1.0	0.5	1.0	1.5	0.055	0.027	0.036	0.034
P3	0.25	0.0	1.5	1.0	0.014	0.0	0.055	0.022

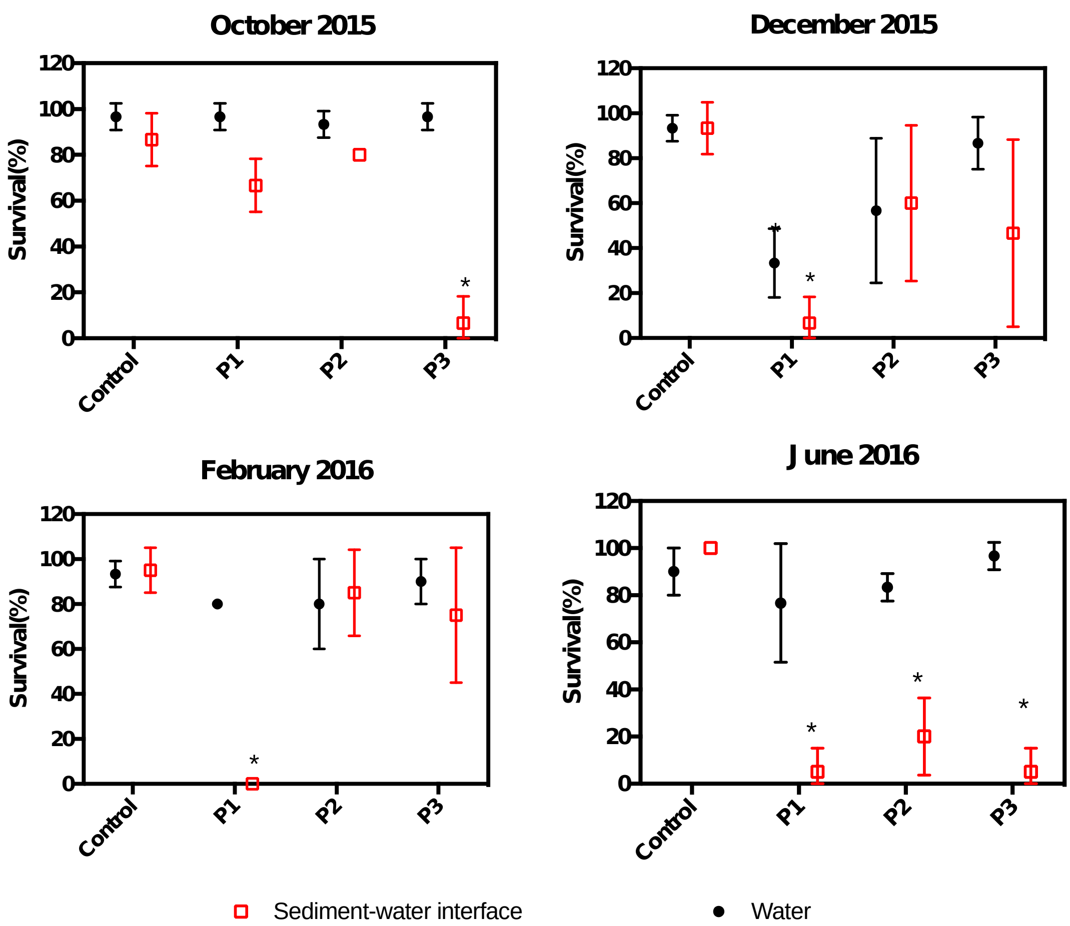

Figure 3. Mysids (*Mysidopsis juniae*) survival (%) after exposure to superficial water and sediment-water interface with samples from three sampling sites (P1-Inácio Barbosa; P2- Farolândia; P3-Treze de Julho) at the Poxim River estuary (Sergipe, Brazil). Artificial seawater was used as control. *indicates significance difference from control ($p<0.05$).

sediment samples was the most toxic almost all sampling occasions (except Oct/15), with mortality varying between 93.3% and 100%, followed by P3. During the SWI experiments the physical-chemical parameters varied within the limits acceptable for mysid assays (Supplementary data 1B). However, higher levels of total and unionized ammonia were observed at P1 in all months (Table II), compared to other sites.

The principal components analysis (PCA) of field and laboratory data (Fig. 4) revealed that P1 was distinct from the other sites, driven mainly by sediment variables (organic matter, carbonates, fine particles), unionized ammonia (water and SWI), and mysid toxicity (water and SWI). The first two PCA components explained 60, 1% of data variability, suggesting that the variables analyzed contribute partially to the toxicity observed. The integrated analyses showed clear differences between the three sites, highlighting the worst conditions in the inner site (P1). According to loadings coefficients, unionized ammonia in both water and SWI were positively related to fine particles and negatively related with salinity (component 1), revealing the relationship with the inner river output, while SWI toxicity was positively related to carbonates and OM (component 2) that can be associated to toxic substances.

Discussion

Our toxicity assays showed that sediments from the Poxim river estuary were more toxic to mysids than water samples, mainly at the inner site (P1), which was associated with higher levels of organic matter and carbonates (Fig. 4). That may act as ligands to several substances like metals and hydrocarbons (Garcia *et al.* 2009, Lima *et al.* 2012, He *et al.*, 2019). It is important to observe that mysids were in directly contact to the surface of sediment, so it can be considered another route of exposure to pollutants, in addition to the contact to the substances present in the water column. The water and sediment present distinct physical-chemical conditions, which influence the bioavailability of pollutants, driven mainly by salinity (Chapman & Wang 2001).

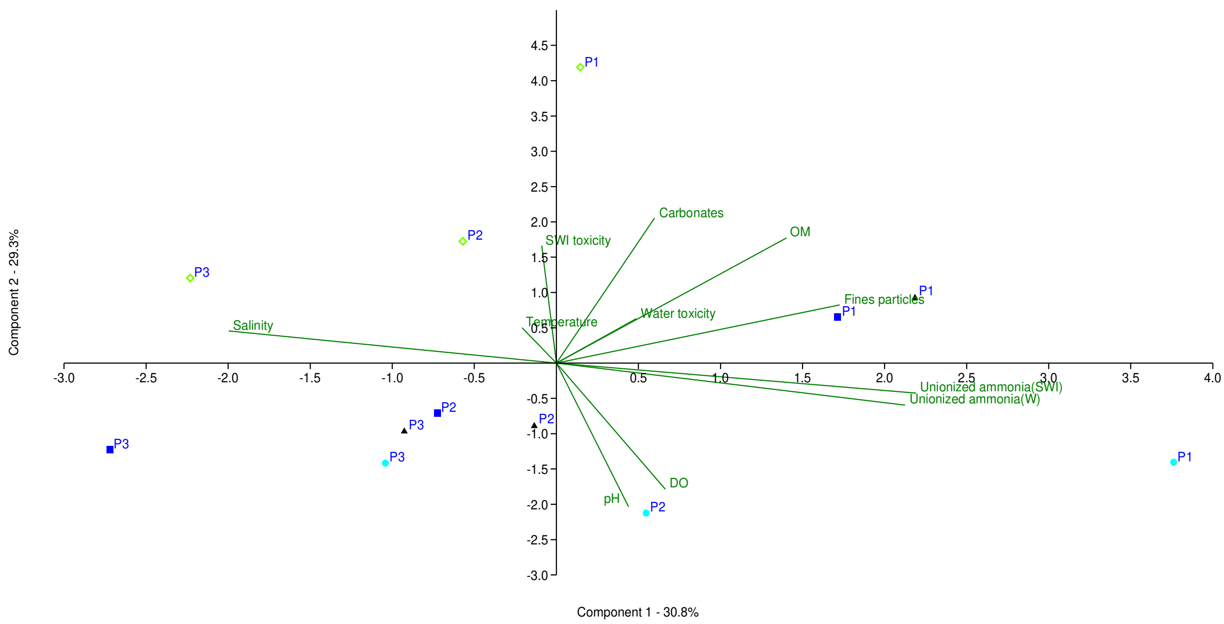

Figure 4. Principal component analysis (PCA) of field data as measured in water samples (pH, salinity, dissolved oxygen – DO, temperature). Laboratory data as measured in sediment samples (organic matter – OM, carbonates and fine particles) and acute tests with mysids (*Mysidopsis juniae*) after exposure to superficial water (W) and sediment–water interface (SWI) samples and their unionized ammonia. Sampling sites (P1 – Inácio Barbosa district; P2 – Farolândia district; P3 – Treze de Julho district) in the Poxim river estuary (Sergipe, Brazil). Symbols: Dot – October/15; Square – December/15; Triangle – February/16; and Star – June/16).

This clear difference between the observed toxicity in water and sediment shows the importance of evaluating the two matrices in continuous monitoring programs.

A previous study in this region found that toxicity in water samples had a seasonal pattern (Nilin *et al.*, 2019), with the worst water quality during the dry months, mainly in the inner sites, and with the toxicity associated with high concentrations of pollutants due to the reduced river flow and, subsequently, the reduced potential for dilution and dispersion of effluents discharged into the river, but this pattern was not observed in the currrent study. Although P3 is close to the channel that drains untreated sewage, no toxicity was observed in water samples from this site, probably due to its proximity to the ocean and the high hydrodynamic flow that may promote pollutant dispersion. In contrast, the SWI test with sediment collected in a rainy month showed toxicity at all collection sites, suggesting that the pollutants released into the water may have settled out into the sediment due to contact between the freshwater and seawater flow (Mhashhash *et al.* 2018). There are several studies on the influence of precipitation on pollutant sedimentation and toxicity (Biati *et al.* 2010, Casadio *et al.* 2010, Hassani *et al.* 2017, Li *et al.* 2010). However, the hydrodynamics of each location should be considered for a better understanding of the results to improve assessment.

High levels of fecal coliforms above the Brazilian guideline limits (Brasil 2005) and coprostanol (Carreira *et al.* 2015) have been recorded in water and sediment in this study zone (Frena *et al.* 2019). Emerging contaminants such as steroids were also found in muddy sediments from all coastal zones of Sergipe, indicating a strong contribution of domestic wastewater, mainly through the Sergipe River outflow (Carreira *et al.* 2015, Quadra *et al.* 2017). Despite an increase of 15% in wastewater collection and treatment in Aracaju between 2013 and 2017, only 51% of the water consumed is treated, and the data shows that there has been a reduction in accessibility to wastewater treatment services (Instituto Trata Brasil 2019). Both treated and untreated wastewater from Aracaju and nearby cities is discharged into the estuarine region (Sergipe River basin, including Poxim river), and then transported to the marine environment. In addition to increasing the organic load, domestic wastewater may contain a mixture of pollutants, such as pharmaceuticals and other chemicals (Quadra *et al.* 2017). Furthermore, Batista *et al.* (2021) analyzed the sludges from a water treatment plant near to Poxim estuary, and identified a concentration of aluminum above the allowed in the Brazilian guidelines (910% - decanter sludge and 210% - filter sludge), being the sludges highly toxic to mysids survival. These authors highlighted that the sludges, without any treatment, could potentially be released into the river.

The in situ physical-chemical variables were within the acceptable ranges for estuarine areas, and none of these parameters seemed to be related to mysid mortality. The toxicity of the water samples cannot be attributed only to ammonia (total and unionized), since in Oct/15 samples showed high levels of toxic ammonia (0.054- 0.108 mg.L^{-1}), but low mysid mortality (7% in P1 and 4% in P2) was observed. Ammonia toxicity depends on factors such as salinity, temperature, pH and the developmental stage of crustaceans (Chen & Lin 2001). Crustacean species can be highly tolerant of ammonia, mainly in the adult stage when the animals are less sensitive, with LC50 values at 96 h ranging from 11 to 99 mg. L^{-1} of total ammonia, and 1 to 7 mg. L^{-1} of unionized ammonia (NH_3) (Miller *et al.* 1990, Romano & Zeng 2013), which are higher than the values observed in the current study. Despite the limitation of the method used to measure ammonia (limit of quantification: 3 mg.$L^{-1}NH_3$) in the present study, it is important to realize that there is no data on the toxicity of ammonia for *M. juniae* and further studies are needed to understand the toxicity mechanism involved. However, based on data reported by Burgess *et al.* (2003) that the ammonia LC50 for the mysid *Americamysis bahia* (48h old) in overlaying water varies between 48.6 and 51.9 mg. L^{-1}, it is likely that the ammonia in the Poxim river samples may not affect the mysids. The SWI test was first standardized using embryos of *Lytechinus variegatus* (Anderson *et al.* 2001, Cesar *et al.* 2004), but they are highly sensitive to ammonia, and this could be a limitation to the use of this test (Maranho *et al.* 2010, Nilin *et al.* 2013), since the ammonia concentration can be naturally high in marine and estuarine environments. Therefore, SWI tests with mysids appear to be less affected by ammonia, and therefore would be a good alternative to tests using embryos of *L. variegatus*.

The SWI can provide a better indication of chemical pollutant levels, because the chemical activity at the sediment–water interface directly affects the quality of the adjacent water column (Cesar *et al.*, 2004). The SWI with mysids is a new approach to study toxicity in sediments, as showed by Moreira *et al.* (2019). Although it is a promising method, our data showed a considerable variation between the replicates, that can be reduced by using more replicates, than four used in this study. This variation also observed in water experiments (three replicates), but this was already adjusted by ABNT that increased the replicates to five in the new version of NBR15308 (ABNT, 2017).

The purpose of this research was to integrate different toxicity tests to assess the potential ecotoxicological risk of estuarine management areas, using the Poxim river estuary as a case study. Due to the sensitivity shown by the SWI test, its use is recommended in order to complement the results obtained by the water test. The current study provide evidence about the toxicity of water and sediments of the Poxim river estuary. Probably due to untreated sewage discharge, since chemical markers of the release of domestic effluents have already been recorded, which in turn may contain a mixture of thousands of substances toxic to aquatic organisms. The SWI bioassay with *M. juniae* proved to be a suitable test for revealing the toxicity of sediment samples as hypothesized, and this new approach can be used as a tool for the integrated assessment of estuarine areas. It is also suggested that continuous monitoring of the waters and especially of the sediments should be carried out considering inclusion of others water and sediment toxicity assays.

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