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Toxicity of sediments and dredged material from a semi-arid coastal system to marine invertebrates

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Abstract

Dredging involves the sediment excavation in order to increase the bathymetry of harbors by different methods. In urbanized and Industrial areas, dredging pose risks of negative effects on the biota due to sediment contamination. The Brazilian criteria for characterizing dredged material include chemical analyzes and comparison with sediment quality guidelines and toxicity testing, which require the development of novel and different biological models to be used in bioassays. In this study, we aimed to assess the quality of sediments collected during dredging activities of Mucuripe Bay (Fortaleza city, NE Brazil). Sediments were characterized for the concentration of metals and hydrocarbons, in order to establish the contamination status. Whole sediment toxicity was assessed by means of mortality of the amphipod *Tiburonella viscana* and the polychaete worm *Armandia agilis*, while chronic effects were evaluated on the fecundity of the copepod *Tisbe biminiensis*. Liquid phase exposures were determined in the acute toxicity of sediment-water interface (ISA) on the mysid *Mysidopsis juniae*, while chronic toxicity of ISA and elutriates (ELU) assessed by the embryo-larval development of the sea urchin *Lytechinus variegatus*. Results revealed that contaminated samples exhibited both acute and chronic toxicity. An integrative method for integrating different endpoints was employed and classified samples related to dredging activities as the most degraded. Extracts obtained from the material collected within the dredge were also tested for acute and chronic effects and exhibited toxicity as well. Based on our findings we recommend the analysis of material from dredger cistern and application of a set of bioassays in order to properly determine the quality of dredged sediments.

Key words: benthic organisms, contamination, environmental management; marine pollution; tropical environments.

INTRODUCTION

In coastal ecosystems, dredging involves sediment excavation in order to increase the bathymetry of harbor areas by different mechanical and suction-based methods, including trailer hopper suction dredgers (Manap & Voulvoulis, 2016). As a result of such activities, over hundreds of millions of tons of dredged sediments are estimated as being disposed-off in the oceans worldwide every year (OSPAR, 2008; Schipper *et al.,* 2010). Since most of the harbors' terminals are surrounded by urban centers, industrial complexes and other sources of anthropic-related substances, sediments in these areas are often contaminated by typical groups of compounds such as metals and hydrocarbons (Moreira *et al.,* 2017).

The impacts of sediment contamination and dredging in Brazil are regulated by federal resolution #454 (Brasil, 2012), which require a physical and chemical characterization of sediments followed by a comparison with sediment quality guidelines (SQGs). Thus, the toxicity of samples is only evaluated in the case of SQG exceedances. Recent Studies have stated that the benchmarks adopted by resolution #454, which are based on international SQGs, and the threshold

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values are not effective to predict sediment toxicity, which is important to properly establish evidence of ecological risks (Buruaem *et al.,* 2012; Choueri *et al.*, 2009; Moreira *et al.*, 2017).

On the other hand, there are few models covered by official protocols for sediment toxicity testing. Normative for bioassay procedures have been developed for the amphipods involving whole sediment exposure and acute effects, while chronic effects of liquid phase exposures are covered by sea-urchin embryo larval development methods (ABNT, 2006, 2008). Alternative methods have been proposed for the tanaid *Kalliapseudes schubartii* (Mottola *et al.*, 2009), the copepods *Nitocra* sp (Lotufo & Abessa, 2002) and *Tisbe biminiensis* (Araújo-Castro *et al.*, 2009), and recently, the polychaete worm *Armandia agilis* (Saes *et al*., 2018). Usually, these models are employed individually or at least assessed for one acute and one chronic response. A more effective indicator of the potential toxicity of sediments can be provided by the combining different endpoints measured in multiple representative species (Souza *et al.*, 2016).

The Mucuripe bay (MB) is located on the central coast of Ceará State, and is surrounded by the city of Fortaleza, the capital of the State. MB consists of an important semi-arid coastal system in northeast Brazil. The main harbor of the state is situated within the bay and its waters receive substances from urban and industrial activities (e.g. an oil refinery) (Buruaem *et al.,* 2012, 2016). Sediments of MB are mainly sandy but the presence of jetties close to the harbor have caused an increased deposition of fine particles and other materials, including contaminants (Maia *et al.,* 1998; Moreira *et al.,* 2017). As a result, dredging operations for deepening and maintenance of bathymetry are occasionally performed, causing thus impacts of sediment resuspension and release of chemicals that are bound to particles.

In this scenario, the present study aimed to evaluate the toxicity of sediments collected on MB in two surveys during dredging operations to marine invertebrates, in addition to a sample of dredged material, collected within the trailing suction hopper dredger. A set of multi-species bioassays was applied including acute and chronic endpoints evaluated on amphipods, copepods, mysids, sea urchin embryos, and polychaete worms. Results were integrated into a qualitative matrix method in order to estimate the risks of sediment contamination and potential toxicity as relevant lines of evidence of environmental quality influenced by dredging activities in Tropical Environments.

MATERIAL AND METHODS

Study Area and sediment sampling

The climate in coastal zone of Brazilian semi-arid is influenced by the Intertropical Convergence Zone (ITCZ) conditions, with average temperatures ranging from 24°C to 30°C and the predominance of trade winds blowing from the E-SE, controlling thus the sediment transport in the region (Maia *et al.*, 1998; Paula *et al.,* 2013). The sedimentology of Ceará coast is characterized by organogenic facies with elevated deposition of calcium carbonate from calcareous algae and terrigenous facies marked by clastic rocks and clay (Lacerda & Marins, 2006; Marques *et al.*, 2008). A predominance of coarser sediments is found in the outer shelf contrasting with sandy particles with low amounts of mud that cover the inner shelf (Freire *et al.*, 2004).

As mentioned before, Mucuripe harbor is situated within the MB and its facilities comprise the access channel, anchorage areas, an oil terminal and a jetty with 1,900 m long, which reduces the energy of waves but also changes the sediment transport, causing the deposition of sediments and thus leading to a reduction of bathymetry. In this study, the Harbor area of MB was assessed in two surveys, at the beginning (a; January 24, 2011), and at the end of dredging activities (b; July 29, 2011). Three sites affected directly by sediment removal were selected: the site MD1 is located at the center of the commercial docks of Mucuripe harbor, MD2 is placed by the pillar of the terminal oil pier, and MD3 located at the entrance to the navigation channel (Fig. 1). A reference site was selected at Requenguela beach, in the city of Icapuí, Ceará state (4°40’54.7”S, 37°20’13.9”W).

Sediments were collected with a van Veen grab (0.026 m^2) and for sediment analysis, two subsamples were obtained. The first one was dried at 25°C by in a desiccator cabinet and stored in plastic containers designated for the analysis of particle size and trace elements. The second was placed in aluminum foil and stored at -20°C for the analysis of hydrocarbons. For sediment toxicity, an aliquot from the original sample was kept refrigerated in coolers until the transport to the laboratory and stored at 4°C in plastic containers.

Another sample was obtained from the cistern of the trailing suction hopper dredger (OF) in order to characterize

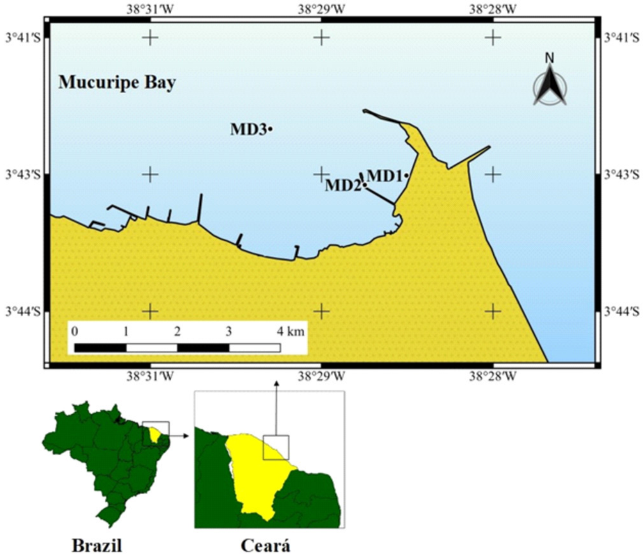


Figure 1: Sediment sampling sites during dredging operations of Mucuripe Bay, Ceará State, Northeast Brazil: dredged sites 1 (MD1), 2 (MD2) and 3(MD3).

the toxicity of the overflow which is formed by the mixture of suspended sediments and the water pumped through the hopper into the dredge cistern. The overflow is pumped out to the sea, in order to facilitate the cistern filling with dredged sediments, contributing thus to the formation of a plume along the water column (Torres *et al.*, 2009). The sample OF was collected directly from the dredger cistern by the use of a stainless steel bottle. The fluid sample was incubated at 25ºC overnight (12h) for the decantation of particles, which were characterized as a sediment sample. The supernatant was collected and tested for acute and chronic toxicity of liquid phase using mysids and sea urchin embryos.

Sediment analysis

Samples were characterized for particle size distribution according to the wet sieving method to separate mud (silt+clay, < 63 µm particle diameter) from sand (McCave & Syvitski, 1991). Total organic carbon (TOC) quantities were estimated using an element analyzer model TOC-VTOC SSM-5000A (Shimadzu). Trace elements (Cd, Cr, Cu, Ni, Pb, and Zn) were extracted from sediments by the digestion in HNO_3, H_2O_2 and HCl (3:1:1) (USEPA, 1996). Then, elements were quantified by Flame Atomic Absorption Spectrometry (FAAS) (Shimadzu AA 6200). Concentrations of total polycyclic aromatic hydrocarbons (PAHs) were analyzed in sediment extracts using a mixture of n-hexane and dichloromethane (1:1) following the protocol described by UNEP (1992). The compounds were quantitatively analyzed using an Agilent 6890 gas chromatograph coupled to a 5973N mass spectrometer (GC/MS). More details of sediment analysis are described in previous studies (Buruaem *et al.*, 2016; Saes *et al.*, 2019). The validation of analytical methods was assured by the analysis of surrogates, blank samples, and the reference materials of the National Institute of Standards and Technology for metals (NIST SRM 1646a), and hydrocarbons (NIST SRM 1944). The detection limits were 0.01% for TOC, 0.01 mg Kg^{-1} for Cd, 0.5 mg Kg^{-1} for Zn, 0.1 mg Kg^{-1} for Cu, 1 mg Kg^{-1} for Pb, 2 mg Kg^{-1} for Cr and Ni, and 1 µg Kg^{-1} for PAHs.

In order to establish the risks of toxicity related to contamination status as a chemical criterion, results of the sediment analyses were compared to the threshold (Levels 1) and probable effect (level 2) of the international criteria adopted by the revised version of Brazilian Federal legislation for dredged sediments (Brasil, 2012) and site-specific values proposed by Choueri *et al.* (2009). In these comparisons, in values above level 1, the toxicity may occur or not but above level 2, the effects are often observed.

Set of toxicity tests

In this study, a set of toxicity tests was employed to characterize the toxicity of samples using different invertebrates as model organisms. Whole-sediment exposures (WS) were performed to analyze the acute effects of sediment samples on the amphipod *Tiburonella viscana* and the polychaete worm *Armandia Agilis*, while chronic effects were evaluated on the copepod *Tisbe biminiensis*. Liquid phase exposures were set up to determine the acute toxicity of sediment-water interface (ISA) on the mysid *Mysidopsis juniae*, while chronic toxicity of ISA and elutriates (ELU) assessed by the embryo-larval development of the sea urchin *Lytechinus variegatus*. Samples of the supernatant obtained from the overflow (OF) were diluted in clean and filtered seawater (at 5, 10, 25, and 50%) and tested as a waterborne sample for the determination of toxicity on the *Mysidopsis juniae* and *Lytechinus variegatus* embryos (acute and chronic effects, respectively).

The bioassays with *T. viscana* consisted in the exposure of organisms 10 per replicate (4 replicates) for 10 days in chambers containing 0.25 Kg of sediment sample and 750 mL of clean sea water (salinity 35), under constant lighting, controlled both aeration and temperature (25 ± 2 ° C) (Melo & Abessa, 2002; ABNT, 2008). At the end of the bioassay, living organisms were counted. Similarly, the experiments with *A. agilis* were performed following the protocol proposed by Saes *et al.* (2018). In this experiment, 15 organisms were exposed for 10 days per replicate (3 replicates) in chambers containing 0.15 Kg of sample and 300 mL of sea water (salinity 30), maintained under constant light, and controlled aeration and temperature (25 ± 2°C). At the end of the test the remaining organisms were counted.

Chronic toxicity bioassays using the benthic copepod *T. biminiensis* were performed according to the protocol proposed by Lotufo & Abessa (2002) and Araújo-Castro *et al.* (2009). Replicates were assembled in clean plastic vials as exposure chambers containing 4 g of sediment and 20 mL of clan seawater (salinity 35). Then, 10 ovigerous females were exposed per replicate for 7 days, maintained at a constant temperature (25 ± 2°C). At the end of the experiment, the contents of each chamber were fixed with formaldehyde (4%) and dyed with Rose-Bengal (0.1%). Offspring (nauplii and copepodites) and adults were counted and used to determine the fertility rate.

Sediment-water interface (SWI) exposures were set up and tested for juvenile mysids and sea urchin embryos in order to assess the effects of chemicals that are transferred from the superficial sediment layers and which may be available to the organisms in the adjacent water column, causing thus toxicity (Anderson *et al.*, 2001; Cesar *et al.,* 2004). In this procedure, the test chambers were assembled in beakers for the mysids' tests and in test tubes for the tests with sea urchin embryos, both containing 1:4 sediment and water (v:v). Elutriates (ELU) samples were also prepared in order to simulate the impacts of sediment resuspension (USEPA, 2003). Sediments were mixed with clean seawater at a ratio of 1:4 (v:v) in a jar test apparatus for 30 minutes and then incubated at 4°C for sedimentation. After that, supernatants were separated and tested for the toxicity, based on the embryo-larval development of the sea urchin *L. variegatus*. After an equilibrium period, organisms were exposed following the specific protocol for waterborne exposures as described below.

The acute toxicity test for the *Mysidopsis juniae* was performed following the protocol ABNT NBR No. 15308 (ABNT, 2011). In the experiment, 10 mysid juveniles of 6–8 days old were exposed for 96h (4 replicates). The chambers were maintained under controlled photoperiod (12h light:12h dark) and temperature (24 ± 2 °C) and animals fed daily with *Artemia* sp. nauplii (48h old). In every 24 h, living organisms were counted and dead ones removed. At the end of the exposure, the mortality rate was estimated in %. The toxicity test with embryo-larval development of the sea urchin *Lytechinus variegatus* was conducted according to the protocol NBR No. 15350 (ABNT, 2006). Sea urchin spawning was induced by 9-volt electric shock, and gametes were separated and examined for viability. Then, in vitro fertilization was made, according to the protocol, and fertilization success was confirmed by examination of eggs. The test was conducted by exposing approximately 400 embryos in each exposure chamber (5 replicates), including negative control (filtered seawater). After 24h of exposure, pluteus larvae were analyzed microscopically for morphological anomalies and delayed development.

Negative controls were prepared for all of the bioassays. Filtered and uncontaminated seawater was used as control or dilution water, while sediments from Engenho D'água Beach, northern coast of São Paulo State (local where amphipods and polychaetes were collected) were used as a negative control. Salinity was controlled, and pH and dissolved oxygen were monitored during experiments. Levels of total ammonia were measured using a specific probe and unionized ammonia (NH_3) estimated (Whitfield, 1974) in order to assess the contribution of ammonia to toxicity, once levels above 0.05 mg/L may affect the toxicity of samples for *L. variegatus*, for example (Abessa *et al.*, 2008).

Data analysis and interpretation

The results of sediment chemistry were analyzed by means of the sediment quality guideline quotients (SQGQs) (Fairey *et al.*, 2001). In this approach, the concentrations of metals and PAHs were normalized by the probable effect values of both guidelines, followed by the calculation of its mean quotients. Then, samples were classified according to the contamination level as follows: (a) minimal: SQGQ and SQVQ ranging from 0 to 0.1; (b) moderate: contaminants may produce toxicity, SQGQ and SQVQ ranging from 0.1 to 0.25; and (c) strong: contaminants causing toxicity, SQGQ and SQVQ values exceeding 0.25.

Results of toxicity bioassays were analyzed by means of Student's t-test to compare responses from each sample with the respective controls; bioequivalence was used for the liquid phase treatments (Bertoletti *et al.*, 2007). Samples that differed significantly from the control were considered toxic. For the OF results, both lethal and inhibitor effect concentrations to 50% of exposed organisms (LC_{50} and IC_{50}, respectively) were calculated followed the nonlinear model using GraphPad Prism version 6 (GraphPad Software, La Jolla California USA, www.graphpad.com).

The results of all endpoints assessed in the toxicity bioassays compiled in a qualitative table following classification proposed by Souza *et al.* (2016) (Table 1). For WS toxicities, non-toxic samples were considered good, and toxic ones were classified as poor (up to 50% difference from the control) or very poor (≥50% in common with the control). For liquid-phase experiments with sea urchin embryos, four classes were considered as follows: good (non-toxic); moderate (development inhibition rates ranging from 30 to 49%); poor (development inhibition rates between 50% and 74%); very poor (development inhibition equal or greater than 75%). These classifications were integrated to determine the environmental quality of each site based on the combination of toxicity tests, generating five possible sediment quality classes (Fig. 2). In this approach, we focused on a conservative criterion by weighting mainly the acute effects, once they are related to severe responses compared to chronic ones.

Results

Results of sediment characterization for physical and chemical variables are shown in Table 2. Samples collected in MB during dredging activities (survey 1) presented elevated levels of mud (in about 60%) in MD1, followed by MD2 and MD3. During survey 2, at the end of dredging, levels of fine particles also increased in MD2 (in about 40%). Sediments obtained from the overflow (OF) exhibited higher contents of mud (in about 74%) while reference site presented the lowest value (in about 4%). A similar distribution was found for levels of TOC, except in OF sample, which presented a lower quantity compared to MD1 in both surveys.

Regarding levels of contaminants, low concentrations were found in the reference site (Icapuí). Elevated concentrations of Cd, Ni, and total PAHs were found in MD1, exceeding the threshold effect value of site-specific SQVs. In the case of PAHs, the concentration found during the first survey was above the probable effect level. Samples from MD2 and MD3 exhibited exceedances of threshold effect values for Pb (both surveys) and Ni (survey 2). Probable effect value exceedances were found for Pb in MD1 (both surveys) and MD3 (survey 2). Sediments obtained from the overflow (OF) presented concentrations of Cu, and Ni and above the threshold effect value; the concentration of Pb was also above the probable effect level.

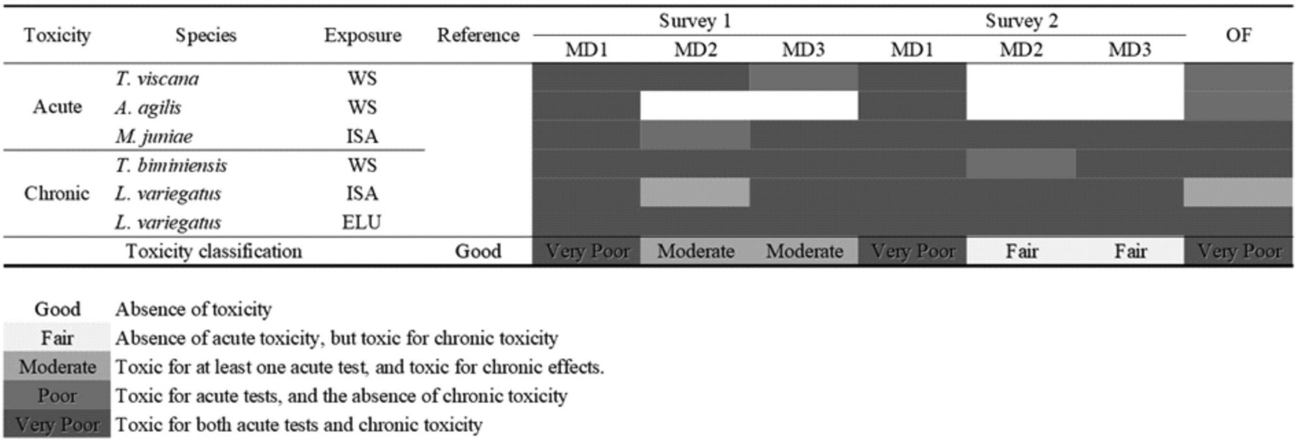
Results of sediment toxicity on marine invertebrates of samples collected in Mucuripe Bay are presented in Tables 3 to 6, including chemical parameters monitored during the experiments, which not affected the results, excepting ammonia. Acute toxicity of WS samples on the amphipod *T. viscana* was observed in MD1 to MD3 (survey 1), MD1 (survey 2), and OF. Acute effects on the polychaete *A. agilis* were reported in samples from MD1 (both surveys) and OF. Acute toxicity on *M. juniae* in ISA exposures was observed in samples from MD1 (both surveys) and MD2 (survey 2). Regarding chronic toxicity, WS exposures from all samples (except reference site) caused significant effects on the

Table 1: The criterion for classifying the toxicity of sediment samples from Mucuripe Bays to marine/estuarine invertebrates.

Toxicity	Species	Endpoint	Sediment classification based on toxicity			
			Good	Moderate	Poor	Very Poor
Acute	*T. viscana*	% mortality	non-toxic	-	$<50\%^{a}$	≥50%
	A. agilis	% mortality	non-toxic	-	$<50\%^{a}$	≥50%
	M. juniae	% mortality	non-toxic	-	$<50\%^{a}$	≥50%
Chronic	*T. biminiensis*	fecundity ((nauplii + copepodits)/female)	non-toxic		$<50\%^{b}$	≥50%
	L. variegatus	% abnormal development	non-toxic	30–49%	50–74%	>75%

a Mortality below 50%, but significantly different from negative control

b Significantly toxic and different from the control group is less than 50%.



Toxicity	Species	Exposure	Reference	Survey 1 MD1	Survey 1 MD2	Survey 1 MD3	Survey 2 MD1	Survey 2 MD2	Survey 2 MD3	OF
Acute	*T. viscana*	WS		■	■	▨	■			▨
	A. agilis	WS		■			■			▨
	M. juniae	ISA		■	▨	■	■	■	■	■
Chronic	*T. biminiensis*	WS		■	■	■	■	▨	■	■
	L. variegatus	ISA		■	░	■	■	■	■	░
	L. variegatus	ELU		■	■	■	■	■	■	■
	Toxicity classification		Good	Very Poor	Moderate	Moderate	Very Poor	Fair	Fair	Very Poor

Good	Absence of toxicity
Fair	Absence of acute toxicity, but toxic for chronic toxicity
Moderate	Toxic for at least one acute test, and toxic for chronic effects.
Poor	Toxic for acute tests, and the absence of chronic toxicity
Very Poor	Toxic for both acute tests and chronic toxicity

Figure 2: Classification of Mucuripe Bay sites at the beginning (Survey 1), and at the end of dredging activities (Survey 2) based on the combination of the conclusions obtained for each sediment toxicity bioassay. Reference – Sediments from Icapuí

fecundity of copepod *T. biminiensis*. Similarly, samples from all the sites, except the reference, caused toxicity to embryos of *L. variegatus* in ISA and ELU exposures. The combination of the different criterion for classifying samples is presented in Fig 2. The sediment quality of the site MD1 was classified as very poor in both surveys, while MD2 and MD3 were classified as presenting Moderate quality in survey 1 and Fair in survey 2. Sediments from OF were classified as very poor, while reference site presented good quality.

Discussion

The presence of jetties in Mucuripe harbor has been recognized as a relevant factor influencing on the local sedimentology (Maia *et al.*, 1998; Paula *et al.*, 2013). The higher levels of mud within the bay reported in our study corroborate previous findings (Maia *et al.*, 1998), and indicate the occurrence of deposition zones of sediments and other materials in the bay, caused by the reduction of the energy due to the decrease of waves and currents intensities. Elevated levels of metals (Hg, Cd, Cu, Ni, and Zn) and PAHs were reported in sediments of MB before the dredging operations (Buruaem *et al.*, 2012; 2016). It was demonstrated later that such levels of contamination were associated with acute toxicity to amphipods (*T. viscana*) after whole-sediment exposure and chronic effects on the larval development of sea urchins (*L. variegatus*) in bioassays using liquid phases (porewater, ISA and ELU) (Moreira *et al.*, 2017).

Chemical-specific criteria were applied by means of SQGs comparisons and no exceedances of resolution #454 values were observed for contamination levels of MB sediments. However, Site-specific SQGs indicated that concentrations of Cd, Ni, Pb, and PAHs pose risks to induce adverse biological effects (Choueri *et al.*, 2009). The SQGQs calculated based on values from CONAMA 454/12 indicated that all samples presented minimal contamination, except MD1 in (survey 1), which presented moderate contamination. Site-specific values classified samples from reference (Icapui), MD2 and MD3

Table 2: Physical and chemical characterization of sediment samples collected in Mucuripe Bay at the beginning (a; January 24, 2011), and at the end of dredging activities (b; July 29, 2011). Concentrations above guidelines marked in bold. NP – not performed.

Samples	Depth (m)	Mud (%)	TOC (%)	Cd (mg Kg^{-1})	Cr (mg Kg^{-1})	Cu (mg Kg^{-1})	Ni (mg Kg^{-1})	Pb (mg Kg^{-1})	Zn (mg Kg^{-1})	PAHs (µg Kg^{-1})
Icapuí	0	4.59	0.10	0.19	2.94	0.36	2.07	9.84	0.71	<1.00
MD1a	11	62.16	1.49	0.16	25.94	**13.64**	**8.04**	**21.99**	25.79	**1160.43**
MD2a	6	19.08	0.70	0.16	15.51	1.41	1.57	**15.93**	2.45	2.96
MD3a	5	10.02	0.60	0.15	13.43	1.56	2.23	**19.44**	2.84	<1.00
MD1b	13	63.51	1.69	0.15	2.51	**11.11**	**10.06**	**22.92**	20.97	**690.88**
MD2b	6	44.40	0.40	0.14	2.73	2.03	**4.24**	**13.89**	1.10	<1.00
MD3b	10	13.36	0.60	0.14	3.49	2.18	**3.52**	**22.43**	2.14	<1.00
OF	-	74.16	0.81	0.17	26.05	**9.31**	**14.16**	**26.41**	16.31	15.89
Resolution #454 (Brasil, 2012)		Level 1	-	1.2	81	34	20.9	46.7	150	4000
		Level 2	-	7.2	370	270	51.6	218	410	-
Site specific SQGs (Choueri et al. 2009)		Level 1	-	-	27.8	6.5	3.9	10.3	37.9	163
		Level 2	-	0.75	65.8	69	21.2	22.1	110.4	950

(both surveys) as moderately contaminated, while samples from MD1 (both surveys) and OF as strongly contaminated (Table 8). These results indicated that site-specific values are more accurate in distinguishing different degrees of contamination that can be related to toxicity, as demonstrated by previous studies (Choueri *et al.*, 2009; Moreira *et al.*, 2017).

In this study, the potential toxicity of sediments was assessed in a set of bioassays involving solid and liquid phases of sediment samples. Multiple endpoints were evaluated in benthic marine invertebrates in order to estimate the effects at the context of different trophic levels and ecological niches (Juvonen *et al.,* 2000).

In the acute toxicity tests with whole-sediments, amphipods exhibited toxic effects in low contaminated samples, while polychaete worms exhibited lethal responses only at elevated levels of contaminants. Chronic toxicity tests based on whole-sediment exposure produced toxicity for all samples. Thus, each species clearly responded uniquely to the sediments and the associated contaminants. This difference between the threes species sensitivity has been demonstrated in a comparative analysis of laboratory exposures to reference chemical (potassium dichromate), corroborating our results (Saes *et al.*, 2018). In this case, the pattern of the most sensitive organism according to LC_{50} (48h) is presented as follows: *T.viscana>T. biminiensis>A.agilis.* Differences in response of benthic invertebrates to chemicals bound to sediments in toxicity tests are expected and can be attributed to multiple factors related to their functional traits, including the dietary habit, contact and/or ingestion of sediment particles, foraging behavior, among others (Millward *et al.*, 2005; Melo & Nipper, 2007).

The size of sediment particles is the most relevant confounding factor in whole-sediment exposures. The survival rates of amphipod *T.viscana* and the polychaete *A. agilis* are influenced by pure fine particles (Melo & Nipper, 2007; Saes *et al.*, 2018), while *T. biminiensis* fecundity was not affected significantly by any of sediment fraction tested, ranging from muddy to sandy (Araújo-Castro *et*

Table 3: Levels of pH and dissolved oxygen (DO) monitored during whole-sediments and sediment-water interface exposures. Samples collected at the beginning (a; January 24, 2011), and at the end of dredging activities (b; July 29, 2011).

	WS – *T.viscana*				WS – *T. biminiensis*			
	pH		DO (mg L^{-1})		pH		DO (mg L^{-1})	
Sample	Day 0	Day 10	Day 0	Day 10	Day 0	Day 7	Day 0	Day 7
Control	8	8.08	5.43	8.18	7.45	7.32	5.61	5.01
Icapuí	7.83	8.1	2.4	8.38	8.01	8.08	5.16	5.24
MD1a	7.99	8.17	5.38	8.15	7.69	7.77	4.98	4.73
MD2a	8.04	8.04	5.34	7.92	8.01	7.99	5.12	5.1
MD3a	8.06	8.02	5.43	7.99	8.16	7.98	5	4.98
MD1b	8.05	8.18	5.2	5.04	8.01	7.92	4.99	4.52
MD2b	7.98	7.45	4.67	5.04	7.93	7.99	5.31	5.26
MD3b	8.06	8.1	5.36	5.26	8.1	8.17	5	4.98
OF	7.83	8	5.05	8.08	7.99	7.96	4.98	4.86

	WS – *T. biminiensis*				ISA – *M.juniae*			
	pH		DO (mg L^{-1})		pH		DO (mg L^{-1})	
Sample	Day 0	Day 10	Day 0	Day 10	Day 0	Day 4	Day 0	Day 4
Control	8.18	8	5.54	6.4	7.72	7.92	6.41	6.64
Icapuí	8.01	8.08	5.16	5.24	7.9	7.94	6.33	6.28
MD1a	7.99	8.17	5.38	8.15	7.85	8.11	5.96	5.15
MD2a	8.04	8.04	5.34	7.92	7.88	8	6.15	6.14
MD3a	8.06	8.02	5.43	7.99	7.86	7.95	6.18	6.55
MD1b	8.05	8.18	5.2	5.04	7.91	7.88	6.5	5.57
MD2b	7.98	7.45	4.67	5.04	8	8.01	5.1	4.63
MD3b	8.06	8.1	5.36	5.26	8.01	7.88	6.22	4.91
OF	8.14	7.5	4.52	3.25	8.01	7.89	6.2	5.09

al., 2009). Another investigation stated that amphipods survival may be affected in sediments containing levels of mud above 30% (Bertoletti, 2011). Sediments from MB presented elevated amounts of mud in MD1 in both surveys, and MD2 in the 2nd survey, in addition to the OF. However, sediments from MD2 exhibited 40% mud and was found to be nontoxic, indicating that particle sizes may not be relevant to the results, once sediments in the MB are well sorted.

In liquid phase exposures, the presence of unionized ammonia originated from natural sources may be considered the main confounding factor, contributing thus to the toxic effects (Chapman *et al.*, 2002). On the other hand, high concentrations of ammonia reported in contaminated sediments or the absence of natural sources of ammonia support the idea of its consideration as a relevant chemical (Losso *et al.*, 2007).

For liquid phase exposures of sea urchin embryos (ELU and ISA), the levels of ammonia detected in samples from MD1 in both surveys were above the threshold toxic level for the species (Abessa *et al.*, 2008). Since the site is affected by effluent discharges and urban runoff containing illegal sewage and other contributions, we considered the effects of unionized ammonia as pollution-related; since other contaminants were detected in such samples, we assumed that the presence of ammonia was originated from anthropogenic sources. Thus, the toxicities observed for both acute and chronic endpoints of liquid-phases indicate a potential transfer of contaminants to the water column through diffusion and resuspension, producing risks to infaunal, planktonic and epibenthic organisms, as was the case of mysids and sea-urchin embryos used as test organisms in our investigation (Anderson *et al.*, 2001, Moreira *et al.*, 2017).

The combination of several endpoints assessed in different exposures allowed distinguishing samples according to surveys. Sediments from MD1, which presented higher levels of contamination, were classified as very poor during and after dredging activities, as well as the sediments from the dredger (OF) which were basically composed by similar materials removed from the navigation channel. Samples from MD2 and MD3 collected in the survey 1 were classified as presenting

Table 4: Levels of pH, dissolved oxygen (DO), ammonia (NH_4) and un-ionized ammonia (NH_3) monitored during sediment-water interface exposures. Samples collected at the beginning (a; January 24, 2011), and at the end of dredging activities (b; July 29, 2011).

ISA – *L. variegatus*				
Sample	pH	OD (mg L^{-1})	NH_4 (mg L^{-1})	NH_3 (mg L^{-1})
Control	6.52	7.88	0.05	<0.01
Icapuí	8.03	5.8	3.58	0.03
MD1a	5.24	7.53	5.78	0.09
MD2a	6.87	7.73	0.52	0.01
MD3a	7.53	7.73	1.78	0.04
MD1b	7.99	4.7	5.98	0.26
MD2b	8.01	6.22	0.12	0.01
MD3b	8.01	6.2	0.28	0.01
OF	6.12	7.44	0.08	0.01

ELU - *L. variegatus*				
Sample	pH	OD (mg L^{-1})	NH_4 (mg L^{-1})	NH_3 (mg L^{-1})
Control	8.23	7.75	0.07	<0.01
Icapuí	7.94	5.61	3.79	0.01
MD1a	7.35	4.91	0.69	0.10
MD2a	7.7	6.31	0.31	0.01
MD3a	7.99	6.8	0.46	0.02
MD1b	7.97	4.82	2.70	0.11
MD2b	7.99	6.1	0.31	0.01
MD3b	8.06	6.95	0.30	0.01
OF	8.23	7.75	<0.04	<0.01

Table 5: Levels of pH, dissolved oxygen (DO), ammonia (NH_4) and un-ionized ammonia (NH_3) monitored during overflow exposures. NM - note measured (24h of exposure). Samples collected at the beginning (a; January 24, 2011), and at the end of dredging activities (b; July 29, 2011).

ISA – *M. juniae*						
	pH		OD (mg L^{-1})		NH_4 (mg L^{-1})	NH_3 (mg L^{-1})
Sample	Day 0	Day 4	Day 0	Day 4	Day 0	Day 0
Control	7.98	8.11	5.22	4.27	<0.04	<0.01
5%	8.07	8.08	5.18	5.47	0.2	<0.01
10%	8.05	8.04	5.16	5.35	<0.04	<0.01
25%	8.06	8.01	5.14	5.23	<0.04	<0.01
50%	8.04	8.02	5.1	5.14	<0.04	<0.01
100%	8.07	8.02	4.94	4.84	0.8	<0.01

ISA – *L. variegatus*						
	pH		OD (mg L^{-1})		NH_4 (mg L^{-1})	NH_3 (mg L^{-1})
Sample	Day 0	Day 0	Day 0	Day 4	Day 0	Day 0
Control	7.88	NM	6.52	NM	0.05	<0.01
5%	7.02	NM	7.64	NM	0.16	<0.01
10%	7.1	NM	7.67	NM	<0.04	<0.01
25%	7.33	NM	7.94	NM	<0.04	<0.01
50%	7.18	NM	8.3	NM	<0.04	<0.01
100%	7.22	NM	8.69	NM	0.78	<0.01

Table 6: Toxicity of sediment samples collected in Mucuripe Bay at the beginning (a; January 24, 2011), and at the end of dredging activities (b; July 29, 2011) to marine invertebrates. Acute toxicity to *T. viscana*, *A. agilis*, and *M. juniae* expressed as % (mean±SD). Chronic toxicity to *T. biminiensis* expressed as fecundity (mean±SD), and effects on *L.variegatus* embryos expressed as % of abnormal development (mean±SD). * - Toxic samples (marked in bold).

Species	*T. viscana*	*A. agilis*	*T. biminiensis*	*M. juniae*	*L.variegatus*	*L.variegatus*
Exposure	WS - Acute	WS - Acute	WS - Chronic	ISA - Acute	ISA - Chronic	ELU - Chronic
Control	13±12	13±12	66±15	3±6	17±3	8±6
Icapuí	23±11	38±27	53±11	3±6	21±9	21±9
MD1a	***93±12**	***60±24**	***11±3**	***40±20**	***99±1**	***100±0**
MD2a	***56±15**	7±7	***37±2**	10±10	***41±12**	***100±0**
MD3a	***36±6**	0±0	***28±11**	20±17	***81±14**	***100±0**
MD1b	***70±17**	***56±8**	***25±5**	***44±11**	***100±0**	***100±0**
MD2b	16±21	4±4	***14±2**	***52±24**	***100±0**	***100±0**
MD3b	27±12	4±4	***4±1**	11±11	***100±0**	***100±0**
OF	***47±12**	***40±7**	***17±2**	33±6	***48±7**	***100±0**

moderate quality, due to the influence of acute toxicity to *T. viscana*, which is also expected since they were affected by the sediment resuspension. In survey 2 both samples were classified as fair and chronic effects were more relevant to such classification.

In general, toxicity classification is consistent with sediment contamination classification based on site-specific values (Table 8). The classification of samples is also consistent with another study that applied a similar approach to producing a fair conclusion weighted mainly in acute

Table 7: Waterborne toxicity of liquid-phase extracted from the overflow (OF), collected in the trailing suction hopper dredger during dredging activities of Mucuripe Bay. Acute toxicity to *M. juniae* expressed as % of mortality (mean±SD), and effects on *L.variegatus* embryos expressed as % of abnormal development (mean±SD). * - Toxic samples (marked in bold). NC- not calculated

Species	Acute - *M. juniae*	Chronic – *L.variegatus*
Control	4±6	18±3
5%	7±6	30±2
10%	11±11	32±3
25%	15±6	***37±8**
50%	26±6	***34±5**
100%	***89±19**	***55±4**
CL_{50} (95% CI)	66.3% (37.7 to 116.6)	NC
IC_{50} (95% CI)	NC	57.9% (39.6 to 84.8)

effects (Souza *et al.*, 2016). In the criterion used in site quality classification, the relevance of acute effects is based on their representation as a population-level endpoint that is linked with initiating events of toxicity pathways (Ankley *et al.*, 2010). In this sense, we recommend the use of different endpoints, especially on multiple species in acute toxicity bioassays in order to properly address the risk assessment of contaminated sediments dredged materials.

As for the toxicity of liquid-phase extracted from the material collected within dredger cistern, both LC_{50} and EC_{50} were estimated up to 60%. This general estimation indicated that impacts of toxicity caused by chemicals are expected not only during the sediment resuspension but also at disposal site of dredging material, downstream the Mucuripe Bay. The potential toxicity of materials collected within the dredge cisterns associated with contaminants was also estimated in a previous study, carried out during dredging activities of the navigation channel of the Port of Santos, in SW Brazil (Torres *et al.*, 2009).

During dredging activities, the resuspension of particles into the water column induces the formation of turbid plumes changing the underwater light and primary productivity (Fisher *et al.*, 2015). In the dredge, the overflow is formed from the water pumping to the sea, which allows its filling with sediments (Torres *et al.*, 2009). The combination of these processes results in the transfer contaminants to the water column or can favor the particles sink to the bottom, causing thus environmental contamination and/or recontamination (Torres *et al.*, 2009; Pineda *et al.*, 2017; Wenger *et al.*, 2017).

In conclusion, we highlight that the sediment toxicity profile was properly provided, by testing samples using different organisms and responses. Contaminated samples from Mucuripe Bay exhibited acute and chronic effects, and the combination into a single criterion ranked sites according to surveys influenced by dredging activities. We recommend the application of similar approaches for sediment toxicity testing. Besides, the assessment of effects induced by the material formed within the dredgers must be included as complementary evidence for monitoring and decision-making process of dredging activities.

Table 8: Summary of sediment chemistry and toxicity analysis of sediment samples from Mucuripe Bay based on sediment quality guidelines and battery of bioassays.

Samples	CONAMA 454/12		Site-specific (SQV)		Toxicity
	SQGQs	Contamination	SQGQs	Contamination	
Icapuí	0.02	Minimal	0.12	Moderate	Good
MD1a	0.11	Moderate	0.52	Strong	Very Poor
MD2a	0.03	Minimal	0.18	Moderate	Moderate
MD3a	0.03	Minimal	0.21	Moderate	Moderate
MD1b	0.08	Minimal	0.40	Strong	Very Poor
MD2b	0.03	Minimal	0.16	Moderate	Fair
MD3b	0.03	Minimal	0.21	Moderate	Fair
OF	0.08	Minimal	0.40	Strong	Very Poor

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