Bulletin of Environmental Contamination and Toxicology (2019) 102:310–315
https://doi.org/10.1007/s00128-019-02556-7

Sub-lethal Responses of the Polychaete *Armandia agilis* in Whole-sediment Toxicity Testing

Renan Vandre da Silva Toscano Saes[1] · **Lucas Buruaem Moreira**[1,2] · **Tiago Farias Peres**[1] · **Satie Taniguchi**[3] · **Marcia Caruso Bícego**[3] · **Rozane Valente Marins**[1] · **Denis Moledo de Souza Abessa**[1,2]

Received: 27 July 2018 / Accepted: 20 January 2019 / Published online: 28 January 2019

Abstract

The present study assessed biochemical responses as sublethal endpoints in the polychaete *Armandia agilis* exposed to contaminated sediments to in order to assess its potential use as a test organism. Sediment samples from several locations at a dredging site were obtained and used in whole-sediment exposures. Samples were tested with *A. agilis* to determine the 10-day toxicity of the 100% sample and the enzymatic activity of catalase (CAT), glutathione-S-transferase (GST) and acetylcholinesterase (AChE) biochemical measurements made in whole-body homogenates of a subset of the surviving organisms. Biochemical responses reported in *A. agilis* were not statistically different from the reference site sediment, however, the integrated analysis demonstrated that contaminants bound to sediment samples influenced the sublethal effects.

Keywords Annelida · Benthic organisms · Biomarkers · Dredging · Environmental monitoring · Test organism

Polychaete worms constitute one of the most diverse and abundant groups of marine ecosystems. They occupy a large variety of benthic habitats from sandy to muddy bottoms, exhibit different reproductive patterns and feeding strategies, and are dominant in many aquatic environments. They are also key links of the marine food web, contributing thus to the secondary production of benthic environments by acting as an important prey for invertebrates and demersal fish (Hutchings 1998; Giangrande 1997). Thus, they are important route for the transfer of contaminants from sediments to higher trophic level organisms.

One important trait of polychaetes is that some species can tolerate and colonize impacted environments by either natural or anthropogenic stressors, which allow them to be considered relevant indicators of ecological status in marine and estuarine ecosystems (Ugland et al. 2008). Many species burrow into the sediments for foraging and protection against predation; and by doing that, these animals actively ingest particles in order to obtain their nutrients. Since sediments are considered the sink compartment of different materials transported in the aquatic systems, the bioturbation caused by polychaetes results in their contact with contaminants by dermal tissues and digestive tract producing thus, adverse biological effects (Hutchings 1998).

Some species of polychaetes are considered sensitive to contaminants and have been used in whole-sediment toxicity tests (Yang and Zhang 2013; Maranho et al. 2014) but investigations using them as models for tropical environments such as South Atlantic are limited. In Brazil for example, the estuarine species from muddy sediments *Laeonereis acuta* has been employed as test organism in ecotoxicological studies of nanoparticles (Nunes et al. 2017) and brominated flame retardants (Díaz-Jaramillo et al. 2016), demonstrating the potential of polychaetes as a relevant target model. Recently the polychaete *Armandia agilis*, which is widely distributed along the Brazilian coast including the beaches of São Paulo state (Amaral et al. 2010), has been proposed as a test organism for assessing the acute toxicity of muddy and sandy sediments (Saes et al. 2018).

✉ Lucas Buruaem Moreira
lburuaem@gmail.com

[1] Instituto de Ciências do Mar, Universidade Federal do Ceará (UFC), Meireles – Fortaleza, Av. da Abolição, Ceara 3207, 60165-08, Brazil

[2] Núcleo de Estudos em Poluição e Ecotoxicologia Aquática, Universidade Estadual Paulista (UNESP), Praça Infante Dom Henrique, s/n. São Vicente, Sao Paulo 11330-900, Brazil

[3] Instituto Oceanográfico, Universidade de São Paulo (USP), Praça do Oceanográfico, 191. São Paulo, Sao Paulo 05508-120, Brazil

Lethality is considered an ecologically relevant endpoint in sediment toxicity assessment but the application of sublethal responses as a metric can properly address the adverse effects with the causal stressors, once other factors such as sediment physical features can influence in the performance of the test, resulting in false-positive based conclusions (Chapman et al. 2002). However, contamination levels often occurs at chronic effects level and in this case, biomarker measurements have been considered as more sensitive, indicating a ‘‘early warning’’ indicator of deviation from the normal status of the organism exposed to chemicals (Martín-Díaz et al. 2004). In Brazil, contaminated sediments and dredged materials are regulated by the Resolution No. 454 (Brasil 2012), which recommends toxicity tests as a complementary line of evidence of sediment quality considering lethal and sublethal effects. Thus, the main objective of this study is to evaluate biochemical responses as sublethal endpoints (e.g. stress indicators) and its correspondence to acute effects in *A. agilis* aiming to assess its potential use as a test organism. Sediment samples from a dredging site were obtained and used in whole-sediment test exposures. Exposure biomarkers were monitored in whole-body homogenates and the results matched with sediment physical and chemical profile.

Materials and Methods

Adults of *A. agilis* (15 to 20 mm total length) were collected at Engenho D’água Beach (Ilhabela, north coast of São Paulo state) during the low tide by using a manual dredge coupled with a 0.4 mm sieving mesh. Sediment samples were obtained from 3 sites from the Harbor area of Mucuripe Bay, located in Fortaleza city (Ceará state, northeast Brazil) in two surveys: beginning (a; January 24, 2011) and at the end of dredging operations (b; July 29, 2011) (Fig. 1). A ‘pristine’ site located in Icapuí (Ceará state) was used as reference. The selection of these sites was based on the potential use of *A. agilis* as a model to assess impacts of dredging according recommendations of the Brazilian federal normative CONAMA no 454/12, which regulates the disposal of dredged sediments (Brasil 2012).

The bathymetry (underwater depth) of each site was determined and sediment samples were analyzed for particle size according to the wet sieving method to separate mud (fine particles, silt + clay < 63 µm particle diameter) from sand fractions (McCave and Syvitski 1991) and total organic carbon (TOC) were obtained in an analyzer model TOC-VTOC model SSM-5000A (Shimadzu). Major elements (Al and Fe) and trace elements (Cd, Cr, Cu, Ni, Pb, and Zn) were analyzed in sediment digested in HNO_3, H_2O_2

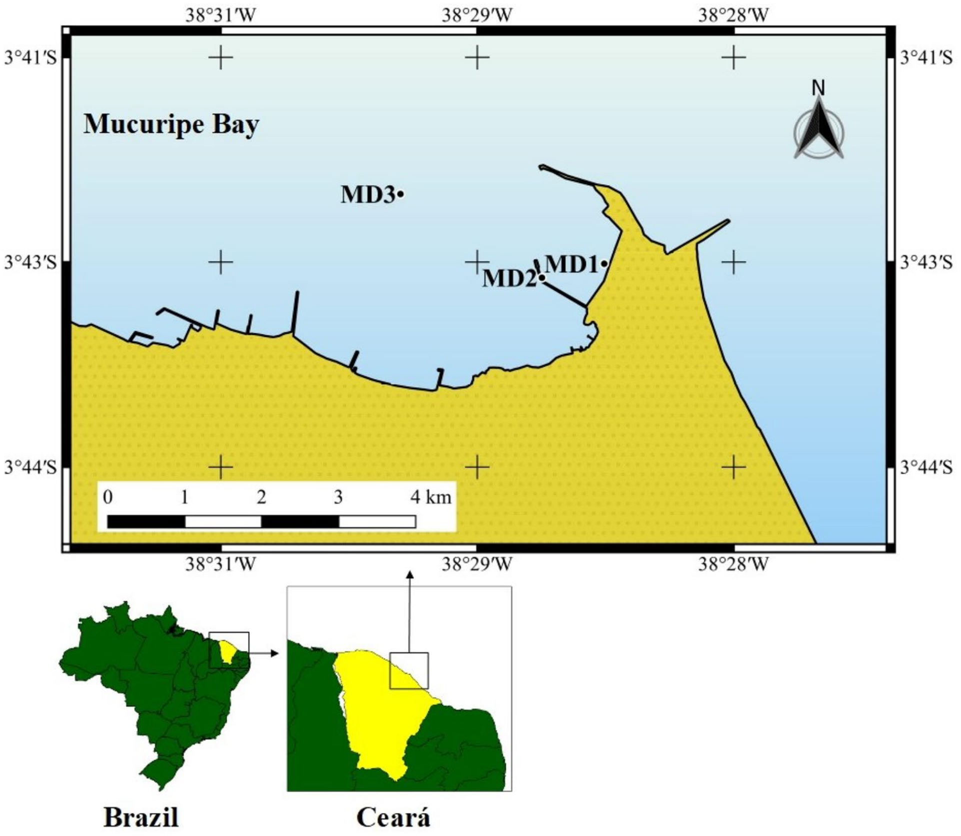

Fig. 1 Sediment sampling surveys during dredging operations of Mucuripe Bay, Fortaleza in January and July 2011: Mucuripe dredging sites 1 (MD1), 2 (MD2) and 3(MD3)

and HCl (3:1:1) following the EPA 3050B protocol (USEPA 1996). Elements were detected by Flame Atomic Absorption Spectrometry (FAAS) (Shimadzu AA 6200) and a background correction with a deuterium lamp. Concentrations of aliphatic hydrocarbons (AHs) and polycyclic aromatic hydrocarbons (PAHs) were determined in extracts using a mixture of n-hexane and dichloromethane (1:1) (UNEP 1992). Samples were fractionated and compounds quantitatively analyzed using an Agilent 6890 gas chromatograph coupled to a 5973N mass spectrometer (GC/MS). Analyses of surrogates standards, reference materials, and blank samples were carried out to validate methods. The detection limits were 0.01% for Al, Fe and TOC, 0.01 µg/g for Cd, 0.5 µg/g for Zn, 0.1 µg/g for Cu, 1 µg/g for Pb, 2 µg/g for Cr and Ni, and 1 ng/g for PAHs.

Whole-sediment exposures were based in method developed for the species (Saes et al. 2018). The chambers were set up in 3 replicates of polyethylene containers (1L) containing 100 mL of sediment sample and 400 mL of filtered seawater (35‰ salinity, 0.45 µm pore size filters). After the equilibrium period, 10 organisms per replicate were exposed for 10 days at 25 ± 2°C with photoperiod of 24 h light in order to induce negative phototaxis and ensure the interaction of worms with the samples. At the end of exposure time, lethal effects were estimated and the surviving organisms euthanized on ice and kept at − 80°C for biochemical analyses. Whole-body homogenates were prepared by homogenizing tissue samples composed by the surviving animals which were pooled into 3 to 5 subset of samples in a buffer solution (pH 7.6) containing TRIS (50 mM), EDTA (1 mM), dithiothreitol (DTT, 1 mM), Sacarose (50 mM), KCl (150 mM), and phenylmethylsulfonyl fluoride (PMSF, 1 mM). Extracts were centrifuged at 4°C and a 9000 g for 45 min, then separated in aliquots for the analysis of enzymatic activity and determination of total proteins (Bradford 1976).

Catalase activity of the extracts (CAT) was monitored at 240 nm in a buffer solution containing Tris–HCl (0.05 M), EDTA (0.025 mM), and H_2O_2 (10 mM) (Monserrat et al. 2006). Conjugation activity of glutathione-S-transferases (GST) was also analyzed in the extracts at 34 nm in a potassium phosphate buffer containing 1-Chloro-2,4-Dinitrobenzene Solution (CDNB, 42 mM), glutathione (GSH, 1 mM) (Martín-Díaz et al. 2009). Enzymatic activity of acetylcholinesterase (AChE) was monitored in the homogenates at 412 nm in a potassium phosphate buffer (0.1 M, pH 7.6) containing acetylcholine iodide (0.075 M) and 5,5-dithiobisnitrobenzene acid (DTNB, 10 mM) (Monserrat et al. 2006). Results are expressed in µmol/mL/mg of protein. Statistical differences in the responses of *A. agilis* exposed to samples from those of reference site were determined using one-way ANOVA followed by Dunn's comparisons test ($p < 0.05$). Biological responses were integrated with the chemical profile using multiple correlations analysis. After that, a matrix of data was constructed using the median (50th percentile) of each variable per site and then, lethal and sublethal responses were integrated with physical–chemical profile of samples by means of principal component analysis (PCA). Statistical analyses were performed using package Past 3.20 (Hammer et al.2001).

Results and Discussion

Sediment samples from Mucuripe Bay were sandy, but muddy particles occurred in MD1, which is related to the low energy zone induced by the jetties (Maia et al. 1998). As a result, different materials and chemicals were found at high levels, especially Cr, Cu, Pb, AH, and PAHs. Samples of MD1 were also toxic considering acute effects, indicating an effect of contaminants on *A. agilis*. These results corroborate those produced in previous studies by using similar analytical methods. Enriched levels of Hg, Cd, Cu, Ni and Zn were found and elevated concentrations of AHs, PAHs and tributyltin (TBT) reported in deposits within the Bay, originated from harbor activities and industrial effluent discharges (oil refinery) causing toxicity in amphipods from the species *Tiburonella viscana* and also effects on the macrobenthic organisms (Moreira et al. 2017). However, concentrations of metals and PAHs in Mucuripe Bay were found below the threshold levels of sediment quality guidelines applied in Brazil for the management and disposal of dredging materials (Brasil 2012).

The antioxidant activity of the enzyme CAT increased twofold (average) in animals exposed to MD1 compared to Icapui (reference site), but no statistical difference compared to reference site was observed. The exposure to contaminants can induce the formation of reactive oxygen and nitrogen species (RONS), causing oxidative stress (Di Giulio and Meyer 2008). Changes in the antioxidant defense responses of the polychaete *L. acuta* treated with Cd were observed, but the activity of CAT was not affected (Sandrini et al. 2008). An increased activity of CAT in the polychaete *Nereis diversicolor* was documented as a response to trace metals such as Cu (Sole et al. 2009). Another study also reported the increased activity of CAT in the species *Perinereis nuntia* correlated with Cu exposure over the time, leading to an inhibitory effect at higher concentrations (Won et al. 2012) (Table 1).

The activity of GST was also elevated in MD1 in both samples but again, no significant difference was reported in comparison to reference site. This enzyme plays role in phase II in the detoxification mechanism by catalyzing the conjugation of GSH with contaminant substrates originated in phase I, related to the biotransformation of organic compounds of (Stegeman and Livingstone 1998). In polychaetes, GST activity may be induced or inhibited in response to a

Table 1 Profile of physical and chemical characteristics of sediments from Mucuripe Bay, Fortaleza in January (a) and July 2011 (b) and biological responses measured in *A. agilis* expressed as mean values ± standard deviation. Reference site located at Icapuí

Variable		Reference	MD1 a	MD2 a	MD3 a	MD1 b	MD2 b	MD3 b
Physical and chemical characteristics	Bathymetry (m)	0	11	6	5	13	6	8
	Sand (%)	95.9	37.9	82.7	91.6	36.6	57.6	87.9
	Fine particles (%)	4.6	62.2	19.1	10.0	63.5	44.4	13.4
	TOC (%)	0.10	0.49	0.07	0.06	0.69	0.04	0.06
	Al (%)	0.09	0.96	0.24	0.25	0.96	0.06	0.08
	Fe (%)	0.13	1.06	0.25	0.26	1.08	0.14	0.21
	Cd (µg/g)	0.19	0.16	0.16	0.15	0.15	0.14	0.14
	Cr (µg/g)	< 2.0	25.9	15.5	13.4	2.5	< 2.0	< 2.0
	Cu (µg/g)	0.4	13.6	1.4	1.6	11.1	2.0	2.2
	Ni (µg/g)	2.1	8.0	1.6	2.2	10.1	4.2	3.5
	Pb (µg/g)	9.8	22.0	15.9	19.4	22.9	13.9	22.4
	Zn (µg/g)	0.7	25.8	2.5	2.8	21.0	1.1	2.1
	AH (µgg)	0.6	655	1.0	0.8	408	0.5	0.5
	PAH (ng/g)	< 1.0	1160	3.0	< 1.0	691	< 1.0	< 1.0
Biological responses	CAT (µmol/mL/mg of protein)	45.77 ± 33.48	111.15 ± 69.39	116.70 ± 32.86	79.62 ± 75.40	136.78 ± 126.62	81.38 ± 15.15	70.35 ± 48.62
	GST (µmol/mL/mg of protein)	0.98 ± 0.31	1.58 ± 0.12	0.64 ± 0.15	1.14 ± 0.50	1.56 ± 0.71	0.94 ± 0.22	0.83 ± 0.15
	AChE (µmol/mL/mg of protein)	1.0 ± 0.06	1.57 ± 0.73	1.14 ± 0.27	1.51 ± 0.17	0.83 ± 0.71	0.93 ± 0.16	0.62 ± 0.25
	Toxicity (% survival)	73 ± 25	40±24*[a]	93 ± 7	100 ± 0*	44±8*[a]	96 ± 4	96 ± 4

Bathymetry (m) underwater depth of site

TOC total organic carbon, *AH* aliphatic hydrocarbons, *PAHs* polycyclic aromatic hydrocarbon, *CAT* catalase enzymatic activity, *GST* glutathione-S-transferases enzymatic activity, *AChE* acetylcholinesterase enzymatic activity

* significant difference from reference site ($p < 0.05$)

[a] toxic sample

mixture of contaminants in sediments as observed in the species *Perinereis gualpensis* and *L. acuta* (Leão et al. 2008; Díaz-Jaramillo et al. 2016). However the activity of GST was induced in *P. nuntia* and *Hediste diversicolor* exposed to Cu and pharmaceutical products, respectively (Won et al. 2012; Maranho et al. 2014). Increased activity of GST correlated with dichlorodiphenyltrichloroethane (DDT) in tissues of *N. diversicolor* was reported in animals collected in sites affected by multiple sources of contaminants, suggesting a detoxification activation of lipophilic compounds including polychlorinated biphenyls (PCBs), DDTs, and PAHs (Solé et al. 2009). For our results, a moderate stress and response on GST activity in *A. agilis* are considered, since elevated levels of AH and PAH were present at MD1.

The activity of the neurotoxicity marker AChE was also evaluated and no statistical changes were observed in animals exposed to sediments from Mucuripe Bay compared to the reference site. This enzyme acts at the nerve endings by catalyzing the hydrolysis of the neurotransmitter acetylcholine forming thus acetate and thiocholine, allowing the cholinergic receptor to return to an initial condition (Andreescu and Marty 2006). It is also known that neurotoxic compounds such as pesticides can inhibit Ache activity and in polychaetes, such effects have been reported in *N. diversicolor* (Douhri and Sayah 2009; Solé et al. 2009). Thus, our results indicated that no effects are related to neurotoxic substances in *A. agilis*.

Significant positive correlations ($p < 0.05$) were found for toxicity and GST activity with TOC, Cu, Ni, Zn, AH, and PAHs. The activity of AChE was correlated positively with Cr. CAT activity was not significantly correlated with any of the chemicals (Table 2). The results including physical properties were also integrated with sediment variables in a PCA analysis, in order to observe associations and correlations

Table 2 A matrix of correlation coefficient between physical and chemical characteristic sediments (Mucuripe Bay and reference) and biological responses measured in *A. agilis*

	Toxicity	AChE	GST	CAT	PAH	AH	Zn	Pb	Ni	Cu	Cr	Cd
TOC	***0.87**	0.08	***0.88**	0.72	***0.87**	0.88	****0.93**	0.57	****0.94**	****0.92**	0.20	0.00
Cd	0.42	0.23	−0.02	−0.33	0.02	0.02	−0.04	−0.61	−0.24	−0.11	0.09	
Cr	0.24	***0.86**	0.25	0.39	0.56	0.55	0.49	0.29	0.11	0.46		
Cu	***0.82**	0.27	***0.87**	0.70	****0.98**	****0.99**	****0.99**	0.65	****0.93**			
Ni	***0.78**	−0.02	***0.87**	0.67	***0.85**	***0.86**	***0.90**	0.62				
Pb	0.23	0.04	0.52	0.59	0.56	0.57	0.63					
Zn	***0.85**	0.32	***0.88**	0.70	****0.98**	***0.99**						
AH	***0.86**	0.37	***0.85**	0.62	****1.00**							
PAH	***0.85**	0.39	***0.84**	0.61								

Significant correlations marked in bold (* $p<0.05$, ** $p<0.01$)

between biological effects and contaminants based on the maximum variance of data projected in a multidimensional space. The first two axis (eigenvectors) explained 86.58% of variability and negative correlations in Axis 1 were found for bathymetry (−0.89), mud (−0.90), TOC (−0.92), Cu (−0.99), Ni (−0.94), Pb (−0.72), Zn (−0.98), AH(−0.96), and PAH (−0.95). Acute toxicity (−0.76), CAT (−0.75) and GST (−0.85) activities were also correlated with Axis 1. Axis 2 represent positive correlations for bathymetry (0.40) and Pb (0.50) contrasting with negative correlation found for Cd (−0.92), acute toxicity (−0.52) and Ache (−0.51). The bi-dimensional ordination clearly separates the most contaminated and toxic site (MD1) from uncontaminated ones (MD2, MD3 and reference), with axis 2 separating samples by sampling campaign (Fig. 2). A good representation of data in PCA is obtained by 2D ordination with samples separated far from the origin (zero) as observed for MD1. In this case, the results suggest an influence of sediment contamination in the responses observed *A. agilis*. Pereira et al. (2014) transplanted the oyster *Crassostrea rhizophorae* and the mussel *Perna perna* to transplanted contaminated sites at Santos Port Channel and Bay, and reported a change in biochemical status correlated with sediment toxicity and reduced richness and diversity of benthic community using multivariate techniques, indicating a correspondence between sediment contamination and the effects at the different level of biological organization.

The establishment of a relationship between effects at lower levels of biological organization such as biochemical responses with those at higher levels including

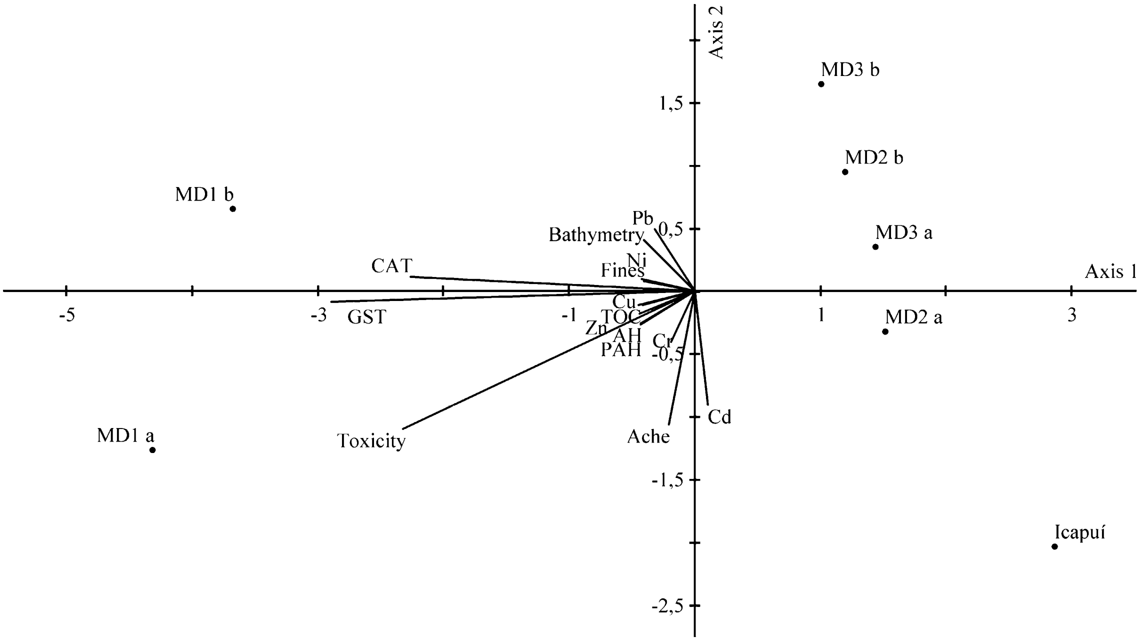

Fig. 2 2D ordination based on PCA results separating MD1 from remaining sites by variables correlated to axis 1 and sampling surveys in January (**a**) and July (**b**) by variables correlated to axis 1, except for MD3 a

individual-level represents a key issue to be addressed in risk assessment and management of contaminated sites (Chapman et al. 2002). Sublethal responses reported in *A. agilis* in our study were slightly changed (not significantly) compared to reference site but the results of PCA analysis demonstrated that contaminants bound to sediment samples influenced such effects and they are associated with acute toxicity. The findings of this study provide relevant information for the development of new models in sediment quality evaluations, and also the polychaete *A.agilis* is a suitable test organism for studies assessing both lethal and sub-lethal responses.

Acknowledgements This study was supported by the port authorities of Ceará State (Companhia Docas do Ceará). The project was also funded by the Foundation for Research Support of Ceará State (FUNCAP, grant number 1571/07 and BMD-0008-00058.01.18/09) and the Brazilian National Research Council (CNPQ) within the Continent-Ocean Materials Transfer program (INCT-TMCOcean, grant number 573.601/2008-9). R.V.S.T. Saes was funded by FAPESP (grant #2010/07605-7). LB. Moreira (grant #142002/2010-0) and DMS Abessa (grants #552299/2010-3 and #311609/2014-7) were sponsored by CNPq.

References

Amaral ACZ, Migotto AE, Turra A, Schaeffer-Novelli Y (2010) Araçá: biodiversidade, impactos e ameaças. Biota Neotrop 10:219–264

Andreescu S, Marty JL (2006) Twenty years research in cholinesterase biosensors: from basic research to practical applications. Biomol Eng 23:1–15

Bradford MB (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:248–254

Brasil (2012) Resolution No. 454/12. Establishing general guidelines to the evaluation of dredging material in brazilian jurisdictional waters, CONAMA, Environmental National Council

Chapman PM, Ho KT, Munns WR, Solomon K, Weinstein MP (2002) Issues in sediment toxicity and ecological risk assessment. Mar Pollut Bull 44:271–278

Di Giulio RT, Meyer JN (2008) Reactive oxygen species and oxidative stress. In: Di Giulio RT, Hinton DE (eds) The toxicology of fishes. CRC Press, New York, pp 273–324

Díaz-Jaramillo M, Miglioranza KSB, Gonzalez M et al (2016) Uptake, metabolism and sub-lethal effects of BDE-47 in two estuarine invertebrates with different trophic positions. Environ Pollut 213:608–617

Douhri H, Sayah F (2009) The use of enzymatic biomarkers in two marine invertebrates *Nereis diversicolor* and *Patella vulgata* for the biomonitoring of Tangier's bay (Morocco). Ecotox Environ Safe 72:394–399

Giangrande A (1997) Polychaete reproductive patterns, life cycle and life histories: an overview. In: Ansel AD, Gibson RN, Barnes M (eds) Oceanography and marine biology: an annual review, vol 35. Aberdeen University Press, London, pp 323–386

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4:1–9

Hutchings P (1998) Biodiversity and functioning of polychaetes in benthic sediments. Biodivers Conserv 7:1133–1145. https://doi.org/10.1023/A:1008871430178

Leão JC, Geracitano LA, Monserrat JM, Amado LL, Yunes JS (2008) Microcystin-induced oxidative stress in *Laeonereis acuta* (Polychaeta, Nereididae). Mar Environ Res 66:92–94

Maia LP, Jimenez JA, Serra J, Morais JO (1998) The coast line of Fortaleza City. A product of environmental impacts caused by the Mucuripe Harbor. Arq Cienc Mar 31:93–100

Maranho LA, Baena-Nogueras RM, Lara-Martín PA, DelValls TA, Martín-Díaz ML (2014) Bioavailability, oxidative stress, neurotoxicity and genotoxicity of pharmaceuticals bound to marine sediments. The use of the polychaete *Hediste diversicolor* as bioindicator species. Environ Res 134:353–365

Martín-Díaz ML, Blasco J, Sales D, DelValls TA (2004) Biomarkers as tools to assess sediment quality. Laboratory and field surveys. TrAC-Trend Anal Chem 23:10–11

Martín-Díaz ML, Gagné F, Blaise C (2009) The use of biochemical responses to assess ecotoxicological effects of Pharmaceutical and Personal Care Products (PPCPs) after injection in the mussel *Elliptio complanata*. Environ Toxicol Pharmacol 28:237–242

McCave IN, Syvitski JPM (1991) Principles and methods of geological particle size analysis. In: Syvitski JPM (ed) Principles, methods, and application of particle size analysis. Cambridge University Press, Cambridge, pp 3–21

Monserrat JM, Geracitano LA, Assis HCS, Colares EP, Bianchini A (2006) Biomarcadores Bioquímicos. In: Lana PC, Bianchini A, Ribeiro CAO, Niencheski LFH, Fillmann G, Santos CSG (eds) Avaliação Ambiental de Estuários Brasileiros - diretrizes metodológicas. Museu Nacional/UFRJ, Rio de Janeiro, pp 124–131

Moreira LB, Castro IB, Hortellani MA et al (2017) Effects of harbor activities on sediment quality in a semi-arid region in brazil. Ecotoxicol Environ Safe 135:137–151

Nunes SM, Josende ME, Ruas CP et al (2017) Biochemical responses induced by co-exposition to arsenic and titanium dioxide nanoparticles in the estuarine polychaete *Laeonereis acuta*. Toxicology 376:51–58

Pereira CDS, Abessa DMS, Choueri RB et al (2014) Ecological relevance of sentinels' biomarker responses: a multi-level approach. Mar Environ Res 96:118–126

Saes RVST, Moreira LB, Davanso MB, Perina FC, Abessa DMS (2018) Developing a protocol whole sediment toxicity testing with the polychaete Armandia agilis. Ecotoxicol Environ Contam 13:85–97. https://doi.org/10.5132/eec.2018.02.11

Sandrini JZ, Lima JV, Regoli F et al (2008) Antioxidant responses in the nereidid *Laeonereis acuta* (Annelida, Polychaeta) after cadmium exposure. Ecotoxicol Environ Safe 70:115–120

Solé M, Kopecka-Pilarczyk J, Blasco J (2009) Pollution biomarkers in two estuarine invertebrates, *Nereis diversicolor* and *Scrobicularia plana*, from a Marsh ecosystem in SW Spain. Environ Int 35:523–531

Stegeman JJ, Livingstone DR (1998) Forms and functions of cytochrome P450. Comp Biochem Phys C 121:1–3

Ugland KI, Bjørgesæter A, Bakke T, Fredheim B, Gray JS (2008) Assessment of environmental stress with a biological index based on opportunistic species. J Exp Mar Biol Ecol 366:169–174

UNEP (United Nations Environment Programme) (1992) Determinations of petroleum hydrocarbons in sediments. Reference methods for marine pollution studies. http://www.ais.unwater.org/ais/aiscm/getprojectdoc.php?docid=3936. Accessed 20 July 2018

USEPA (United States Environmental Protection Agency) (1996) Method 3050B: Acid digestion of sediments, sludges, and soils. Washington, DC

Won EJ, Rhee JS, Kim RO et al (2012) Susceptibility to oxidative stress and modulated expression of antioxidant genes in the copper-exposed polychaete *Perinereis nuntia*. Comp Biochem Phys C 155:344–351

Yang M, Zhang X (2013) Comparative Developmental toxicity of new aromatic halogenated DBPs in a chlorinated saline sewage effluent to the marine polychaete *Platynereis dumerilii*. Environ Sci Technol 47:10868–10876