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Assessment of sediment toxicity from the Areia Branca off-shore harbor and the Potengi river estuary (RN), Northeastern Brazil

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Abstract. The present study aimed to evaluate the toxic potential of superficial sediments collected in Areia Branca harbor (AB) and Potengi River Estuary (PR). Both areas are situated in Rio Grande do Norte state and are under pressure of anthropogenic activities. Particle size, calcium carbonate content, organic matter, and Fe, Cd, Cu and Zn levels of all samples were analyzed. Acute toxicity assays using the amphipod *Tiburonella viscana* were performed with whole sediment samples, as well as chronic assays using copepods *Tisbe biminiensis* and *Nitocra* sp were conducted with samples from AB and PR, respectively. Principal component analysis (PCA) was performed to observe relationships between sediment variables and toxicity. The sediment analyses showed influence of anthropogenic sources for metals, especially Cu and Zn, while bioassays revealed toxicity in most samples from both areas (acute and chronic effects). PCA results showed the contribution of contamination sources on toxicity and an unexpected relationship between toxicity and $CaCO_3$ rich sediments. These results emphasize the need for further studies to elucidate the relationship of calcium carbonates with contaminants in sediments, as well as the necessity of an integrated assessment using different lines of evidence (LOE) in order to subsidize the environmental management of tropical marine environments.

Key words: sediment quality**,** marine pollution, toxicity tests, tropical marine environments

Resumo. Avaliação da toxicidade dos sedimentos do Terminal Portuário offshore de Areia Branca e do Estuário do Rio Potengi (RN), Nordeste do Brasil. O objetivo deste estudo foi avaliar o potencial tóxico de sedimentos coletados no Porto de Areia Branca e no Estuário do Rio Potengi, localizados na zona costeira do estado do Rio Grande do Norte e sujeitas a atividades antrópicas. As amostras de sedimentos foram caracterizadas quanto a granulometria, teores de carbonatos de cálcio e matéria orgânica, além dos metais Fe, Cd, Cu e Z. Testes com sedimento integral foram empregados para avaliar a toxicidade aguda usando o anfípodo *Tiburonella viscana* e para avaliar a toxicidade crônica foram utilizados os copépodos *Tisbe biminiensis* e *Nitocra* sp. Objetivando identificar associações entre as variáveis, foi empregada a Análise de Componentes Principais (PCA). Os resultados mostram a influência de fontes antrópicas para os metais analisados, principalmente Cu e Zn, além da toxicidade observada na maioria das amostras. O uso da PCA evidenciou a contribuição da contaminação para a toxicidade além da associação não esperada dos efeitos biológicos com sedimentos ricos em carbonatos. Estes resultados reafirmam a necessidade estudos complementares para elucidar a relação dos carbonatos de cálcio com contaminantes em sedimento, além da necessidade de avaliações integradas usando diferentes linhas de evidências (LOE) no sentido de subsidiar a gestão ambiental em ambientes marinhos tropicais.

Palavras chave: qualidade de sedimentos, poluição marinha, testes de toxicidade, ambientes marinhos tropicais

Introduction

Most population world lives on the Coastal Zones (Sale *et al.* 2008), triggering or increasing a variety of pressures on natural resources and ecological processes. Fishing, land occupation for different purposes, tourism and recreation are among the main uses of coastal zones, and the consequent economic development causes environmental impacts on the natural resources of coastal areas (Wagener 2005). In Brazil, such development is characterized by the expansion of urban zones and the installation of harbors and industrial areas along the coast of the country, including on the Northeast Region. As environmental consequences, a large amount of contaminants may reach aquatic ecosystems and compromise performance, ecological balance and present risks to human health (Burton 1999).

When contaminants reach aquatic environments, they take part in geochemical processes that, depending on environmental conditions, can regulate residence time, partition, mobility and bioavailability (and consequently the toxicity) resulting also in the deposition of contaminants in sediments (Di Toro *et al*. 1991, Chapman 1999, Riba *et al*. 2003). Thus, sediments act not only as a sink, but also as a secondary source of contaminants to the water column and biota (Burton & Johnston 2010, Buruaem *et al*. 2012).

As sediment contamination represents a main concern issue, the knowledge of the extent of such impacts, including biological effects on benthic organisms, is important to support management actions and pollution control. To deal with such issue, the use of different lines of evidence (LOE) to assess sediment quality has been recommended (Chapman & Holler, 2006). Among them, the use of sediment toxicity tests represents an integrated evaluation approach that considers the effects of yet unknown chemicals and mixture of contaminants in the estimation of the potential toxicity of sediment samples, providing a screening-level assessment of ecological risks of contaminated sediments (Nendza 2002, Schipper *et al*. 2010).

Sediment toxicity studies in Brazilian coast have been mostly performed in harbor, industrial and urban areas of South and Southeast regions, such as those conducted in the Santos-São Vicente Estuarine System (Abessa *et al*. 2005, 2008, Torres *et al*. 2009), Paranaguá Estuarine System (Choueri *et al*. 2009) and Guanabara Bay (Maranho *et al*. 2009, 2010). Regarding other regions of the country, such as the Northeast, the state of Bahia has ecotoxicological studies with sediments from Camamu (Paixão *et al*. 2011) and Todos os Santos Bay (Evangelista *et al*. 2005, Martins *et al*. 2005), however other areas have no ecotoxicological information.

The potential risks for the aquatic environments from Rio Grande do Norte state are related to the coastal zone occupation, especially in the urban area of Natal city, where sewage and effluents are released into the Potengi River Estuary (Souza & Silva, 2011). The state also presents an oil industry and the Industrial Complex in Guamaré city (Silva *et al*. 2003), in addition to the wastes from shrimp farming and processing (Silva *et al*. 2010) and harbor activities in Natal Harbor and the Areia Branca off-shore harbor. Since such activities represent important sources of contaminants for the Rio Grande do Norte coastal zone , performing ecotoxicological studies may be a useful approach to asses ecological risks of contamination and identify priority areas for monitoring and management. In this perspective, the aim of this study was to investigate the potential toxic of sediment from two areas potentially influenced by anthropogenic activities in Rio Grande do Norte state through sediment characterization and sediment toxicity bioassays using benthic species (amphipods and copepods): the first one is the Areia Branca off-shore harbor and the second one is the Potengi River Estuary.

Material and Methods

Study Areas

The Areia Branca harbor (AB) is located offshore, 25.9 km away from the city of Areia Branca (04º 49' 06" S to 37º 02' 43" W). It was planned to support bulk salt commerce, especially the production from Rio Grande do Norte that must supply chemical industries in southeastern Brazil. The harbor terminal is 92 m wide and 166 m long, surrounded by a curtain of sheet piles forming cells, filled with calciferous sediments. The access channel has approximately 15 km of extension and minimum depth of 11 m. The anchoring areas have a length of 400 m and depth from 18 to 23 m, allowing the operation of large ships, with 22 m drafts (CODERN 2010b).

The Potengi River estuary (PR) is located among the cities of Natal (state capital), São Gonçalo do Amarante and Macaíba (5°43′ S to 5°53 ′S and 35°09′W to 35° 21′W), the most populated and urbanized area of the state. The estuary receives discharges of the Potengi, Jundiaí and Doce rivers and is mainly surrounded by mangrove forest, which covers an area of 1.488 ha. The Jundiaí River has a small discharge very influenced by tides and their effects have been observed 30 km from the mouth of the estuary. The Doce River is the smallest freshwater input to the PR estuary (Silva *et al.* 2006, Souza & Silva 2011). Moreover, PR estuary is intensely affected by (1) the urban sprawl of the cities of Natal, São Gonçalo do Amarante and Macaíba, which has increased the release of industrial wastewater and domestic sewage in the estuary; (2) the expansion of shrimp farms, (3) removal of mangrove areas, and (4) the harbor and industrial activities, with an emphasis on textile and tannery. The Natal Harbor is situated 3 km from the mouth of the estuary and covers trades from the states of Rio Grande do Norte, Pernambuco, Ceará and Paraíba, where the main products includes fruits, wheat, fish and seafood, especially shrimp (CODERN 2010a).

The climate in Rio Grande do Norte varies from tropical dry/semi arid, characterized by a dry season in summer and a rainy season in winter, with rainfall levels varying from 1.300 to 2.000 mm/year. Due to the intertropical convergence zone (ITCZ), there is a predominance of trade winds in E-W direction, which determines the sediment transport (Jimenes & Maia 1999, Knoppers *et al*. 1999). The average annual temperature is 26.8°C and the relative humidity is about 80%. The coldest months correspond to the rainy season, being the highest rainfall occurring between April and June, and the warmer months correspond to the dry the season (Souza & Silva, 2011).

The area present typical features of sediments from the Brazilian northeastern region, with two distinctive characteristic in its composition: organogenic and terrigenous facies. Organogenic substrates are derived from calcareous algae *Halimeda* sp. and *Lithothamnium* sp., contributing with some 77 to 96% of calcium carbonate deposition (Nascimento *et al.* 2010). Terrigenous facies are characterized by siliciclastic material, which includes quartz sand, feldspar, heavy minerals (smectite, kaolinite and illite) and clay (Freire *et al*. 2004). Grain size of the outer shelf sediments is covered by gravel and marine bioclastic materials (rodoliths, coralline alga and shell fragments) and sands, while the sediments from the inner shelf are predominantly composed by sand and low amounts of mud (Freire *et al*. 2004, Nascimento *et al.* 2010).

Sediment sampling and handling

In Areia Branca off-shore harbor, samples were collected on August 2011 in 7 sites located around the harbor. The sites AB1, AB3, AB4 and AB5 were placed on the surrounding anchoring areas; AB2 in the east side, upstream of harbor facilities and AB6 and AB7 were placed in the west side, at unsheltered zones (Figure 1). Sediment samples along Potengi River estuary were collected on July 2011 in 4 sites, PR1 to PR3 at the inner estuary and PR4 at the river mouth (Figure 2). Sediment samples for toxicity tests were collected using a *Van Veen* grab, keep up in coolers in aliquots of 2L with ice until arriving at the laboratory and stored at 4°C in the dark. An aliquot of 200 ml was separated, dried at 40°C and packed in plastic containers for sediment characterization analysis (particle size, organic matter, calcium carbonate and metals).

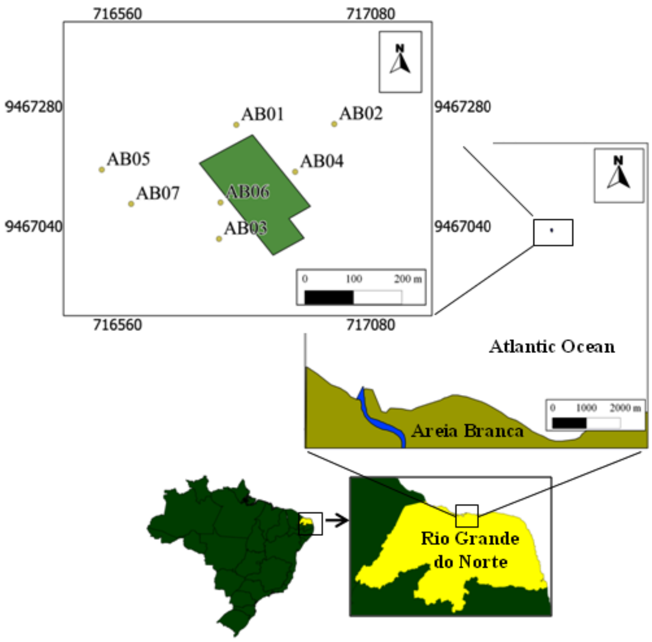

Figure 1. Location of sediment sampling stations at Areia Branca off-shore harbor, RN, Brazil.

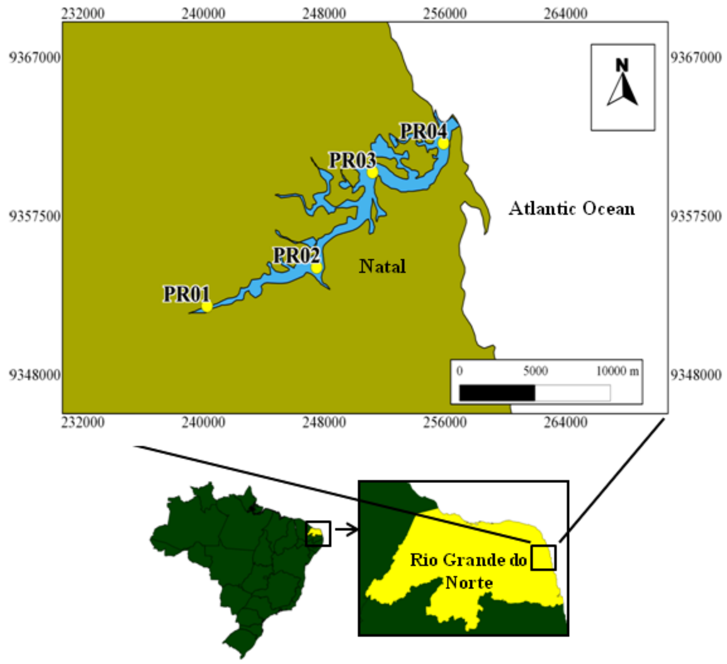

Figure 2. Location of sediment sampling stations at Potengi River Estuary, RN, Brazil.

Sediment characterization analysis

Particle size was determined by the wet sieving method for total fines separation (silt + clay) followed by dry sieving to separate gravel and sand (McCave & Syvitski 1991). Estimation of calcium carbonate contents ($CaCO_3$) was conducted by the gravimetry method following digestion in HCl (Gross 1971), while organic matter content (OM) was determined by the method of ignition loss in a muffle and gravimetry (Luczak *et al*. 1997). Values determined in all methods are expressed in %.

Metals (Fe, Cd, Cu and Zn) were analyzed in sediments according to the EPA 3050B protocol (USEPA 1996). Sediment aliquots were dried at ambient temperature and then sieved to remove gravels; afterwards samples were digested with an acid solution containing HNO_3 and HCl in a volume ratio of 1:3 and extracts were measured using the flame mode of a Fast- Sequential Atomic Absorption Spectroscope Shimadzu, model AA-6800. Validation of this method was performed by analysis of reference material BCR® 667 Estuarine Sediments (Table I). Concentrations of Cd and Cu are expressed in µg/g and Fe content is expressed in %.

Table I. Reference material analysis for method validation. Values expressed in µg/g.

Element (µg/g)	Indicative value (µg/g)	Measured (µg/g)	Recovery (%)
Cd	0.67	0.66	98.14
Cu	60	71.65	119.42
Zn	175	135.94	77.68
Fe*	4.48	3.05	68.08

* = Fe expressed in %.

Sediment toxicity

Acute toxicity was analyzed through whole sediment bioassays (WS) using mortality of the amphipod *Tiburonella viscana* (Barnard 1964) as the endpoint. Acute toxicity tests followed the protocol NBR 15638 (ABNT 2008), where three replicates were assembled in polyethylene chambers and the sediment samples (175 mL) from each sampling stations were placed into the chambers followed by gentle addition of filtered sea-water (750 mL). After an equilibrium period (12h), 10 amphipods were introduced in each test-chamber, exposed for 10 days and at the end of exposure time the content of each chambers were sieved and the number of organisms alive was recorded. Toxicity results are expressed as % of amphipods survival.

Chronic toxicity was assessed through whole sediment bioassays (WS) using the reproduction rate of benthic harpacticoid copepods as endpoint. For samples from PR, the estuarine species *Nitocra* sp. was used as the test-organism, whereas, for AB samples, the marine specie *Tisbe biminiensis* (Volkmann-Rocco 1973) was used. Both species were obtained from cultures maintained under controlled conditions in laboratory.

The bioassays using *Nitocra* sp. were performed according to Lotufo & Abessa (2002). The exposure chambers were prepared in high-density polyethylene vessels and sediment samples (2mL) were placed into the chambers, followed by addition of artificial seawater with salinity of 17 (5mL). After an equilibrium period (12h), ten ovigerous females were added to each test chamber. Supplementary feeding consisted in the addition of 100µL of fish food solution at the start of the assays. The chambers were kept without aeration and under a constant temperature (25 ± 2ºC) with a 16/8 h dark/light photoperiod for 10 days. At the end of the bioassay, the content of each test-chamber was stained with Rose-Bengal dye (0.1%) and fixed with 1ml of formaldehyde (4%). Stained individuals (adults, copepodits and nauplii) were counted using a stereomicroscope. The reproduction rates were evaluated and expressed as the average number of offspring per adults.

The bioassay using *Tisbe biminiensis* was based on the protocol described by ISO (1999). The exposure chambers were prepared in high-density polyethylene vessels and sediment samples (5mL) were placed into the chambers followed by addition of filtered sea-water with a salinity of 35 (20mL). After the equilibrium period (12h), ten ovigerous females were added to each test chamber and supplementary feeding was added, similarly to that done for *Nitocra* sp. The chambers were incubated without aeration at 25 ± 2ºC with a photoperiod of 16/8 light/dark for 7 days. At the end of the bioassay, the content of each test-chamber was stained with Rose-Bengal dye and fixed with formaldehyde. Stained individuals were counted using a stereomicroscope and the reproduction rate was evaluated.

Negative controls were prepared for all treatments using sediments from a reference site where the specimens of *T. viscana* were collected (Engenho D'água beach, Ilha Bela, North coast of São Paulo). Student's t'-test was used to compare each sample with the control (for $p \leq 0.05$). Prior to the hypothesis testing, normality distribution and homogeneity of variances were checked by Chi-square and Fisher tests, respectively, using the software TOXSTAT 3.5® (West & Gulley 1996). Aiming for a better interpretation of the results, data were organized in a matrix and the principal component analysis (PCA) was performed using the program PC-ORD 6.0. All the variables expressed in % were modified using the arcsine transformation.

Results

Sediment characterization analysis

Summarized results of sediment characterization, including the concentrations of metals, are presented in Table II. In AB, all samples presented varied levels of coarse particles and sand, with gravel ranging from 2.63 to 65.75% and sand from 29.63 to 86.86%. The contents of fine particles in samples AB1 (18.62%), AB3 (35.99%), AB4 (19.98%) and AB5 (22.63%) indicate the occurrence of sediment deposition zones induced by the presence of anchoring areas. In PR, sediments exhibited a varied composition with predominance of thicker fractions in the mouth of estuary (PR01, with 27.81% gravel and 54.80% sand), fines particles in estuarine sites (69.05% in PR02 and 40 % in PR03) and predominance of sand in sediments from PR4 (96.46%).

Regarding $CaCO_3$, the results showed a typical difference between marine and estuarine environments: samples collected in AB presented levels of $CaCO_3$ ranging from 29.23 to 95.65%, whereas samples collected in PR presented concentrations ranging from 2.57 to 16.61%. The contents of OM ranged from 0.95 to 11.90 % in AB samples and from 3.78 to 16.73% in PR samples.

Concerning metals concentrations, the concentrations of Fe in AB ranged from 0.12 to 1.67% and, in PR, the levels were slightly higher, ranging from 0.29 to 2.20%. Concentrations of Cd ranged from 0.03 to 1.12 µg/g in AB samples, and from 0.02 to 0.28 µg/g in PR samples. The concentrations of Cu in AB ranged from 33.67 to 94.88 µg/g and, in sediment samples from PR, the results varied from 23.80 to 61.14 µg/g. Finally, Zn concentrations for AB (5.02 to 104.45 µg/g) were low compared to those observed in PR (45.99 to 171.11 µg/g). Table II also presents the sediment quality values of level 1 (threshold effect) and level 2 (probable effect) recommended by Brazilian guidelines (Brasil, 2012), as well as the probable effect level of site-specific values (SQVs) for Santos Estuarine System proposed by Choueri *et al.* (2009b). The comparison of the obtained results and the Brazilian guidelines indicates the exceedences of Level 1 for Cu in AB02, AB03, AB04, AB05, PR02, PR03 and PR04, in addition to AB04, which also exceeded the SQVs value. Zn concentrations were above Level 1 only in PR3, while, according to the SQVs, Zn has exceeding values in AB04 and PR01 to PR03. Such results indicate potential risks to the biota exposed to those samples, as toxicity may occur.

Table II. Results of sediment characterization analysis. Particle size, calcium carbonates, organic matter and Fe contents are expressed in %. Concentrations of Cd, Cu are expressed and Zn in µg/g. Levels 1 and 2 = Brazilian SQGs for dredging activities. SQVs = site-specific SQVs for Santos Estuarine System.

Sample	Gravel (%)	Sand (%)	Fines (%)	$CaCO_3$ (%)	OM (%)	Fe (%)	Cd (µg/g)	Cu (µg/g)	Zn (µg/g)
AB01	35.54	45.93	18.62	90.86	10.39	0.26	0.05	21.73	48.63
AB02	10.4	86.86	3.17	77.92	4.34	0.11	0.08	47.72	6.46
AB03	2.63	61.35	35.99	60.32	2.97	0.77	0.06	65.89	4.89
AB04	15.71	64.25	19.98	29.23	0.95	1.41	0.03	79.92	87.98
AB05	35.46	42.07	22.63	85.01	8.5	0.28	0.23	34.62	31.95
AB06	60.04	34.57	5.55	95.65	11.9	0.14	0.45	17.48	2.09
AB07	65.75	29.63	4.79	95.33	8.68	0.15	0.08	12.74	9.54
PR1	27.81	54.8	17.55	5.12	3.78	0.24	0.17	17.22	121.75
PR2	3.74	27.11	69.05	16.61	8.24	2.12	0.05	46.93	64.87
PR3	2.11	58.03	40	13.46	6.03	2.11	0.27	46.35	167.74
PR4	1.09	96.46	2.44	2.57	16.73	0.29	0.02	60.47	45.48
Reference	0	91.64	8.36	8.23	1.3	2.57	0.2	15.35	31.7
Level 1	-	-	-	-	-	-	1.2	34	150
Level 2	-	-	-	-	-	-	7.2	270	410
SQVs	-	-	-	-	-	-	0.75	69	61.7

Sediment toxicity

The acute toxicity bioassay of WS with samples from AB showed that most samples (except AB03) produced significant mortalities when compared to the control ($p<0.05$), as shown in Figure 3. High mortalities were observed in AB01 (63%), AB05 (100%) and AB06 (83%). Among PR sediments, samples from PR01 and PR02 were significantly toxic for *T. viscana* ($p<0.05$). Regarding the results of chronic toxicity bioassays (Figure 4), sediment samples from AB01, AB05, AB06 and AB07 were toxic to *T. biminiensis*. In addition, sediments from PR02 and PR03 were toxic for *Nitocra* sp.

Multivariate Analysis

Results of PCA are presented in table IV. For AB samples, the first three axes explained 83.90% of variances. Negative correlations in Axis 1 indicate associations of gravel, $CaCO_3$, OM, Cd and toxicity for both *T. viscana* and *T. biminiensis*. Positive correlations represent sand sediments and concentrations of Fe and Cu. Moreover, negative correlations in Axis 2 indicated sand sediments, with deposition of Cd, while positive correlations were observed for fines, Cu and Zn. Axis 3 represented only concentrations of Cd. For samples from PR estuary, the first three axes explained 87.53% of variances. Negative correlations to Axis 1 represented the occurrence of fines, $CaCO_3$, Cd, Zn and toxicity for *T. viscana* and *Nitocra* sp, while positive correlations were found for sandy fractions. Negative correlations to Axis 2, were observed for Fe and Cd, and positive correlations for OM, Cu and toxicity for *T. viscana*. Negative correlation to Axis 3 was found for Cd, while positive correlations were found for OM and Cu.

Figure 5 shows a bi-dimensional ordination of the two first axes of PCA results for samples from AB. Such distribution clearly separates stations classified as degraded in Table III, which presented the contribution of sediment toxicity associated to gravel, $CaCO_3$, OM and Cd concentrations (AB01, AB05, AB06 and AB07), from those stations classified as moderately degraded (AB02 and AB04) and not degraded (AB03), with contribution of the mixture of $CaCO_3$, sand and fine particles in sediment composition (Figure 6). A similar distribution can be observed in the ordination of PCA results from samples from PR estuary presented in the Figure 7, which grouped stations from inner estuary classified as degraded (PR01 and PR02) and moderately degraded (PR03), and presented the contribution of fines, $CaCO_3$, Cd, Zn and toxicity (acute and chronic). Those stations were separated from that collected at the river mouth (PR04), which was classified as moderately degraded, with relevant contribution of sand, in addition to OM, Cu and acute toxicity (Figure 8).

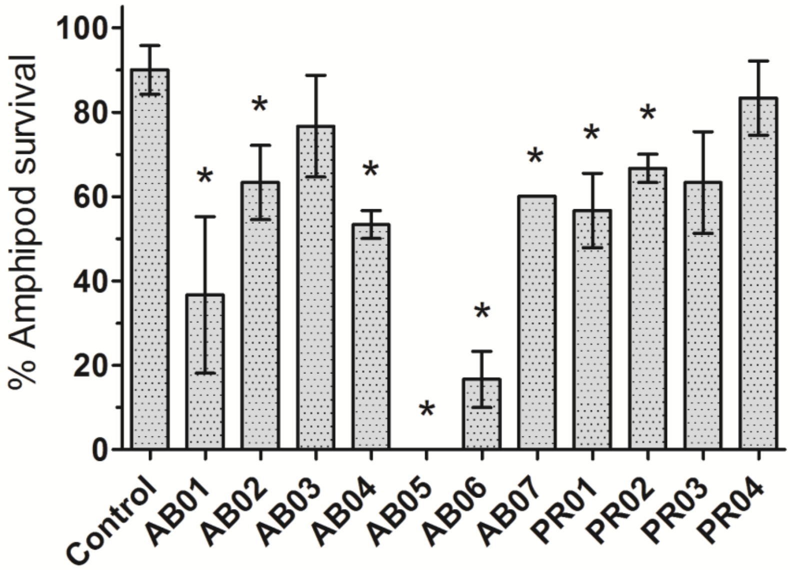

Figure 3. Results of acute toxicity on amphipods *Tiburonella. viscana* exposed to sediment samples from AB and PR, expressed as means ± standard deviation (* = significant differences to the control for p≤0.05).

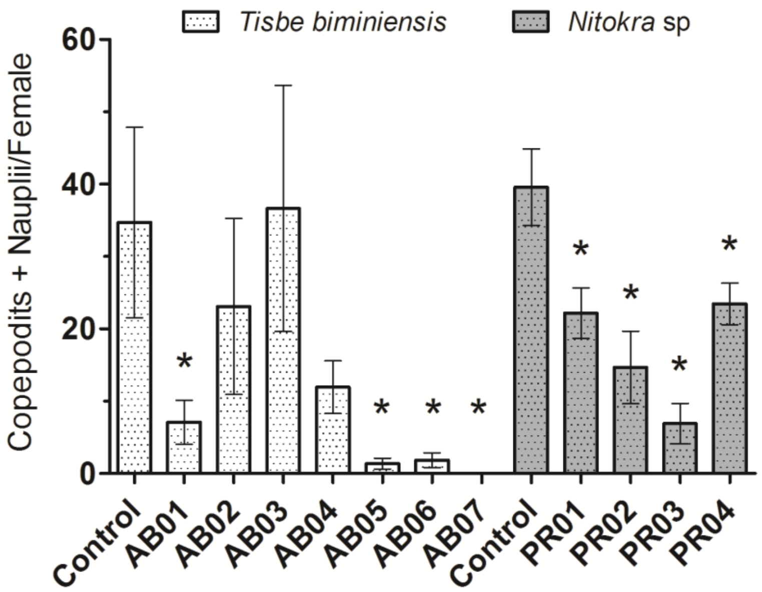

Figure 4. Results of acute toxicity on copepods *Tisbe biminiensis* and *Nitocra* sp. exposed to sediment samples from AB and PR, expressed as means ± standard deviation (* = significant differences to the control for p≤0.05).

A qualitative comparison of results based on toxicity data is shown in Table III. Samples from sites AB01, AB05 to AB07, PR01 and PR02 were considered degraded. Samples from PR01 and PR02 were considered moderately degraded because they only presented acute toxicity. Samples from AB03, PR03 and PR04 were classified as not degraded.

Table III. Conclusive results based on toxicity data of AB and PR samples.

Sample	Acute	Chronic	Conclusion
AB01	Toxic	Toxic	Degraded
AB02	Toxic	Not Toxic	Moderately degraded
AB03	Not Toxic	Not Toxic	Not degraded
AB04	Toxic	Not Toxic	Moderately degraded
AB05	Toxic	Toxic	Degraded
AB06	Toxic	Toxic	Degraded
AB07	Toxic	Toxic	Degraded
PR1	Toxic	Toxic	Degraded
PR2	Toxic	Toxic	Degraded
PR3	Not Toxic	Toxic	Moderately degraded
PR4	Not Toxic	Toxic	Moderately degraded

Discussion

Grain size composition of samples from AB presented high contents of gravel and sand particles and such results were in accordance to previous information regarding the region (Freire *et al*. 2004). However, according to Nascimento *et al.* (2010), sediments from northeastern Brazilian continental shelf consisted mainly of sands with a low contribution of gravel. These differences can be explained by the action of hydrodynamic processes on sediments (as harbor structures form specific zones where sediment transport is altered), thus creating zones with high and low hydrodynamic energy. This phenomenon is evidenced by the presence of sediments with higher percentage of fines at AB01, AB03, AB04 and AB05, opposed to stations AB02, AB06 and AB07, which have low content of fine sediments. Samples from PR presented a varied textural composition, and those from the inner estuary (PR02 and PR03) presented high % of fines, explained by the presence of mangroves (Silva *et al*. 2006), which have a sedimentary material retainer function. High levels of $CaCO_3$ observed in AB samples indicate their organogenic origin, as such substrate is derived from calcareous algae from the genus *Halimeda* sp and *Lithothamnium* sp, rodoliths, and shell fragments (Nascimento *et al.* 2010); on the other hand, sediments from PR were characteristic of terrigenous facies (Freire *et al.* 2004). In this sense, the calcareous nature of sediments from AB and the high contents of fines and OM in many samples from both areas suggest that deposition and retention of contaminants may be favored in sediments from AB and PR.

According to Buruaem *et al.* (2012), natural concentrations of metals are influenced by the nature of the inorganic matter resultant from physical and chemical weathering, in addition to the input of contaminants from anthropogenic sources, which also promotes changes on the distribution of those elements. Thus, concentrations of Fe and Cd in all samples can be associated to natural levels, whereas Cu and Zn levels above threshold effect values may be a consequence of anthropogenic sources. Buruaem *et al*. (2012) reported a significant correlation of Cu and Zn concentrations with $CaCO_3$ and OM in sediments from harbors of Ceará state (Northeast coast of Brazil), which may indicate that such carriers also play an important role in the deposition of metals in AR and PR. Besides, Aguiar *et al*. (2007) studied the metal distribution of sediments from Ceará continental shelf, and identified two groups through multivariate analysis: one formed by Fe, Al, Mn, Cr, and Zn with continental origin, and the other composed by $CaCO_3$, OM, Ni, Pb, Ba and Cu, which was influenced by marine deposition. Such results corroborate the trend observed in the present study, where, besides the natural factors, there is also an anthropogenic contribution to the distribution of metals.

Table IV. Eigenvectors and correlation coefficient of principal component analysis (PCA) based on sediment characterization analysis and sediment toxicity from samples of Areia Branca harbor and Potengi River estuary.

	Areia Branca harbor			Potengi River estuary		
Variables	PC1	PC2	PC3	PC1	PC2	PC3
Gravel	**-0.91**	0.18	0.05	-0.33	**0.57**	**-0.70**
Sand	**0.81**	-0.44	0.03	**0.87**	-0.22	-0.06
Fines	0.32	**0.75**	-0.14	**-0.85**	0.05	0.39
$CaCO_3$	**-0.91**	0.15	0.37	**-0.81**	-0.34	0.43
OM	**-0.96**	0.02	0.11	0.29	**0.76**	**0.55**
Fe	**0.82**	-0.12	-0.48	-0.42	**-0.78**	0.46
Cd	**-0.56**	-0.41	**-0.65**	**-0.51**	**-0.53**	-0.44
Cu	**0.57**	**0.60**	0.10	0.07	**0.56**	**0.78**
Zn	0.45	**0.57**	-0.32	**-0.77**	0.27	-0.29
Acute	**-0.71**	0.44	-0.37	**-0.78**	**0.55**	-0.29
Chronic	**-0.88**	-0.04	-0.43	**-0.79**	0.09	0.25
Eigenvalue	6.10	1.87	1.25	4.59	2.67	2.37
% of variance	55.49	17.01	11.40	41.72	24.24	21.57
Total variance	55.49	72.49	83.90	41.72	65.96	87.53

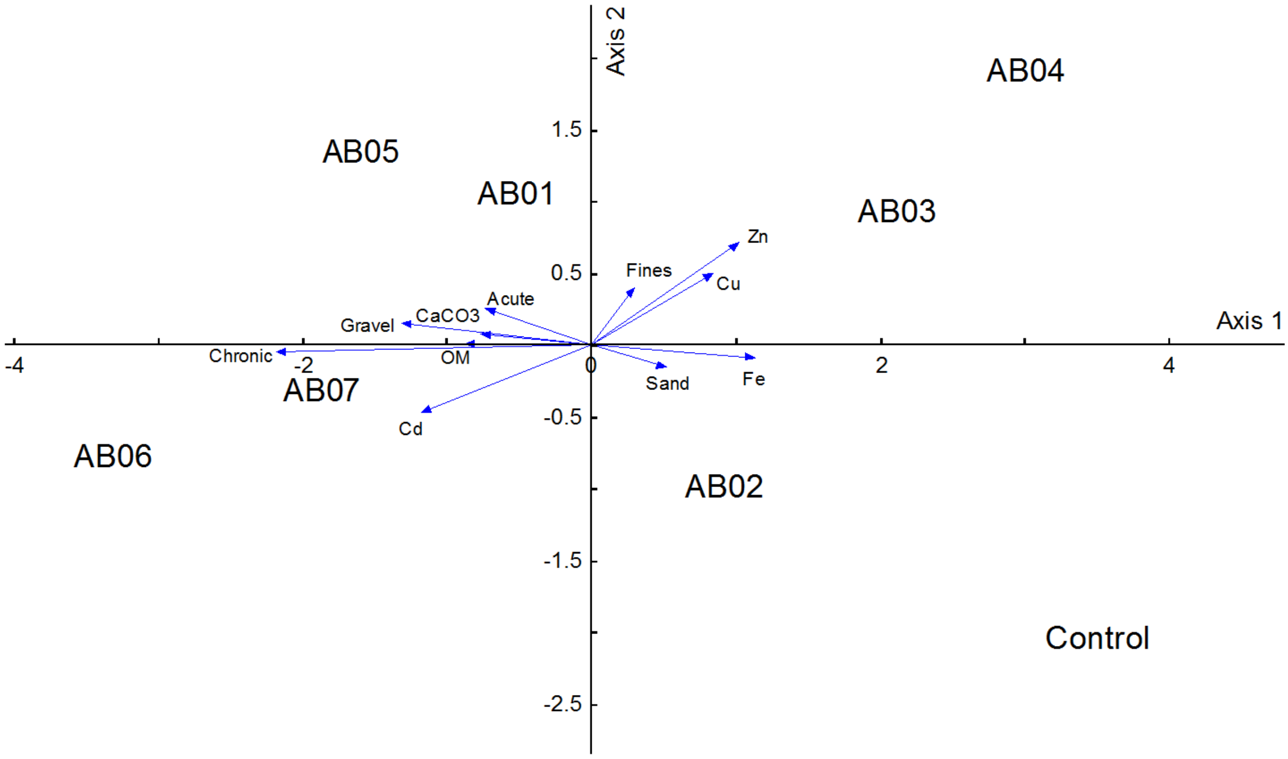

Figure 5. Bi-dimensional ordination of PCA results from axis 1 and 2 of samples from AB.

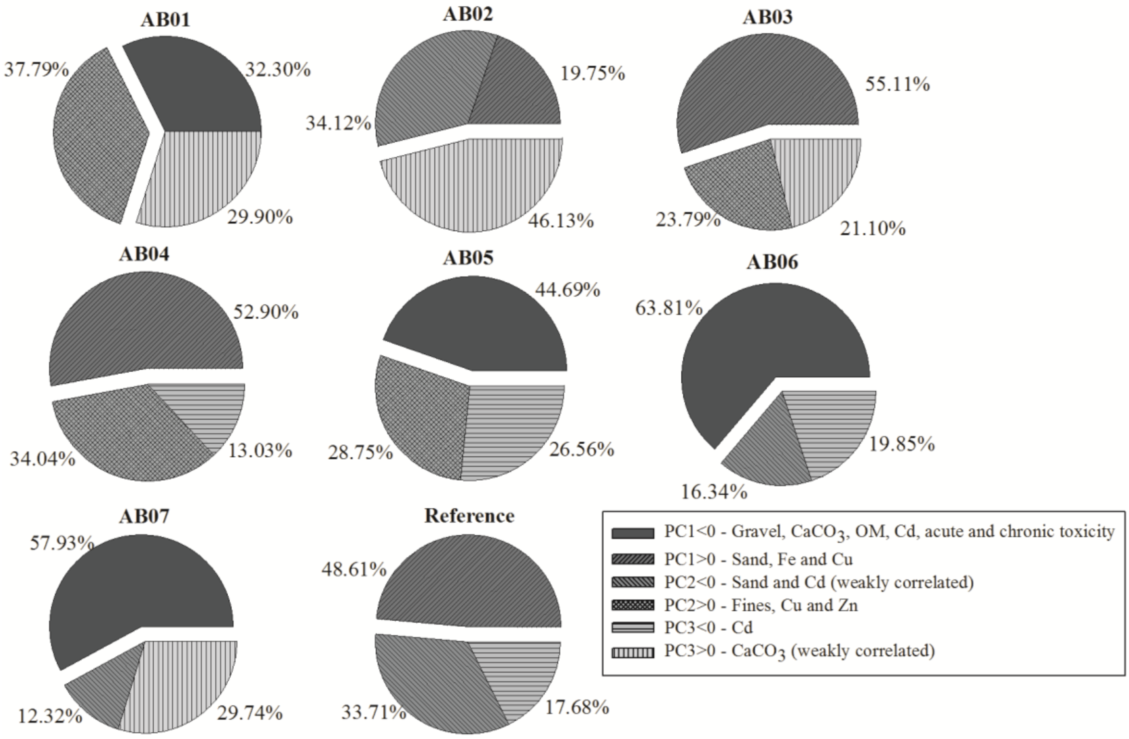

Figure 6. Contribution in % of each axis to the total variance of PCA results from samples of AB.

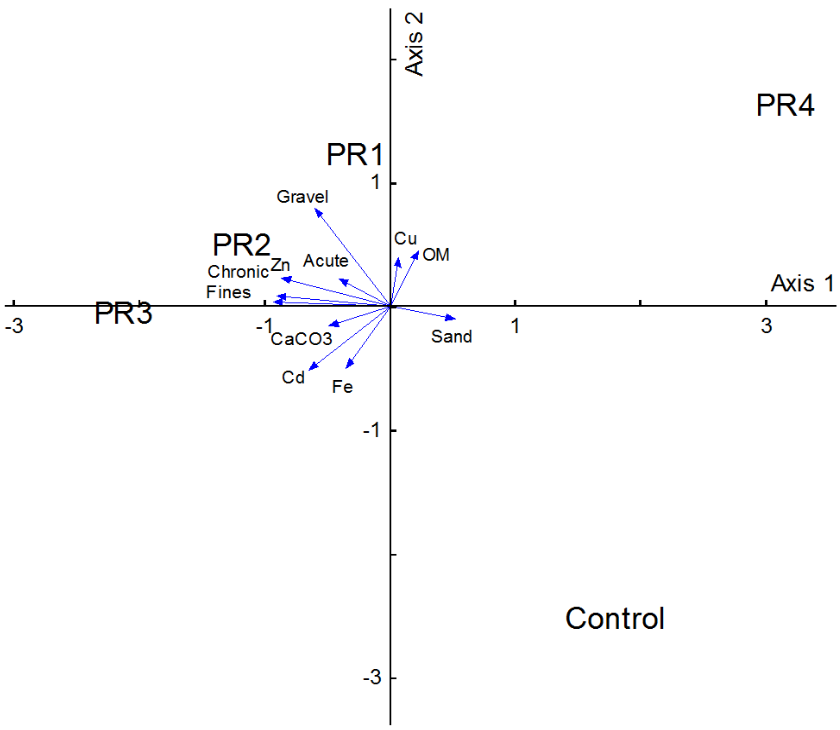

Figure 7. Bi-dimensional ordination of PCA results from axis 1 and 2 of samples from PR estuary.

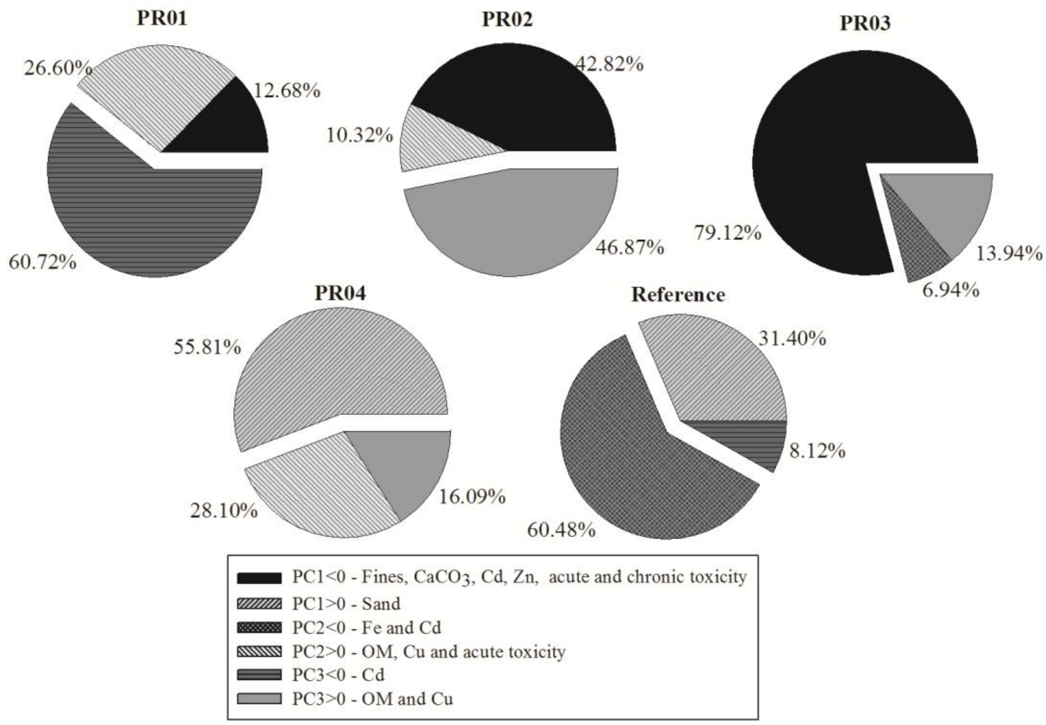

Figure 8. Contribution in % of each axis to the total variance of PCA results from samples of PR estuary.

In PR estuary, several studies had reported contamination for metals along the estuary using bivalve molluscs (*Crassostrea rhizophorae*, *Mytella charruana*, *Anomalocardia brasiliana*, *Anadara ovalis*, *Phacoides pectinata*) and barnacles (*Fistulobalanus citerosum*, *Balanus amphitrite*) as biomonitors (Silva *et al*. 2001, 2003, 2006). These authors argued that anthropogenic activities, such as dredging, agricultural use of fertilizers and pesticides, discharge of untreated sewage and from medical use, textile and leather effluents , are the main sources of Fe, Mn, Zn, Cu, Ni, Ag, Pb and Cr for this estuary. Also, metals have a high binding ability and can be adsorbed on $CaCO_3$ (Correia & Costa 2000) and, thus, being further remobilized by biological activity of benthic organisms, as demonstrated by Papadopoulos & Rowell (1989).

Moreover, both areas are continuously influenced by transport and anchorage of boats and ships and it is well known that Cu and Zn (minority) take part in the composition of anti-fouling paints used on ships and small boats (Yetreberg *et al*. 2010). This could explain the presence of Cu and Zn in the samples from both studied areas. Biggs & D'Anna (2012) reported Cu enrichment in coastal sediments after inauguration of a new marina in San Diego, California. Thus, previous studies corroborate results obtained in the present investigation, which suggests that there is a relationship between Cu and Zn contamination in sediments and the navigation activities may be an effective source of both elements. Moderate to high levels of some contaminants may also indicate the bioavailability of these contaminants in the environment, representing potential risks of biological effects such as acute and chronic toxicity.

Most samples from AB were toxic to *T. viscana* (except AB03), while; for PR, only samples PR01 and PR02 were toxic. The main confounding factors in bioassays using amphipods may be the predominance of fine sediments (Bertoletti 2011). However, Melo & Nipper (2007) showed that a mixed grain size distribution is necessary for *T. viscana* survival. Since very high levels of fines were not observed, the influence of this confounding factor was low or lacking, thus results from the acute toxicity assays were not caused by confounding factors. Burrowing amphipods are directly exposed to contaminants associated to sediment particles and pore water and, consequently, the results from bioassays may be related to the toxic effects resulting from the mixture of pollutants to benthic organisms (Burton 1992, Abessa *et al.* 2006).

Results from the chronic toxicity bioassays results evidenced toxicity in samples PR01, PR05, PR06 and PR07 for *T. biminiensis*, while samples from PR02 and PR03 were toxic for *Nitocra* sp. Copepods are considered highly tolerant to a wide range of sediment grain sizes, as reported by Araújo-Castro *et al*. (2009) and, in this case, the particle size cannot be considered as a confounding factor. According to Lauer & Bianchini (2010), copepods can be bioindicators because they have a great ability to uptake trace metals from contaminated waters. Pinho *et al.* (2011), through autoradiography techniques, emphasized that metal accumulation may occur on all external surfaces of the copepods and such elements can reach internal tissues, which may lead to detrimental physiological effects. According to Decho & Fleeger (1988), the adult stage of copepod *Nitocra lacustris* consumes diatoms, bacteria and sediment particles, which is also an indicative of the direct exposure by consumption of sediments and biofilm to those organisms during the bioassays.

The PCA results for AB harbor samples indicated correlations of toxicity (acute and chronic) with $CaCO_3$, OM and Cd. $CaCO_3$ are known for their chelating ability which involves complexation and/or adsorption of organic or carbon agents (Vera & Pocsidio 1998, Lee *et al.* 2007). In carbonate-rich sediments, since the organisms are exposed directly to these components via epithelium (branchial and digestive) and behavior (foraging and burrowing activities), it is possible that the demand in calcium balance may interact with exposure routes and sequestration of metals in benthic organisms. Ahearn *et al.* (2004) reviewed the detoxification mechanisms of metals in crustaceans and described clearly the transport of calcium and divalent metal cations from the cytoplasm (or other medium) to the endoplasmic reticulum, lysossomes and epithelial cells by calcium-ATPase active transport proteins. However, these interactions of physiological processes of calcium balance in benthic organisms with the mechanisms of toxicity of contaminated sediments are scarcely documented. Regarding to the PCA with samples from PR estuary, results showed a correlation of toxicity with the deposition of fines, $CaCO_3$, OM, Cd and Zn. As discussed above, this association can be explained by the presence of anthropogenic sources of contaminants in the estuary, coupled to the bioavailability of metals (Silva *et al*. 2001, 2003, 2006).

As a conclusion, the results of this investigation reaffirm the evidence of biological effects related to the presence of contaminants in sediments from both areas. Thus, as a recommendation, it is important to perform integrated broader studies in these areas, including several LOEs, as toxicity tests, chemical analyses of different groups of contaminants (e.g. hydrocarbons, pesticides, anti-fouling paints), biomarkers of effect and/or exposure, bioaccumulation and toxicity identification evaluation (TIE), as recommended by Chapman & Hollert (2006). Such approaches can be contributed to establish causal relationship between sources of contamination and biological effects and the implementation of environmental management programs in contaminated areas.

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