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Sediment quality assessment of the tributaries of the Santos-São Vicente Estuarine System – Brazil

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Abstract

The Santos-São Vicente Estuarine System has a long history of environmental impacts, and several studies have been performed in this region. However, the quality of its tributaries was not previously studied. This study aimed to investigate the quality of sediments from five rivers (Santana, Mariana, Piaçabuçu, Quilombo, and Diana Rivers) and three sampling sites at Piaçaguera Canal (riverbed, right and left banks), by using an integrated approach involving chemical analyses and whole sediment toxicity tests. Chronic and acute toxicity tests were performed using, respectively, copepods *Nitocra* sp. and amphipods *Tiburonella viscana*. The studied sediments presented moderate to high levels of PAHs, PCBs, metals (Cu, Hg, Ni, Pb, Zn), and As. The majority of sediments produced chronic toxic effects, and samples from Piaçaguera Canal were consistently toxic in all acute and chronic tests. The integration of data through multivariate analyses indicated that the toxicity was closely related to the sediments contamination. Chemical data were compared with sediment quality guidelines, which evidenced that the site-specific values are adequate to predict impacts. The levels of organic chemicals and metals suggest constant inputs from diffuse and point sources, demanding actions to ensure effective control and management of the contamination sources to the estuary.

Key words: Contamination; toxicity; amphipod; copepod; metal, PAH, PCB, multivariate analysis.

INTRODUCTION

Throughout history, humans have used the water bodies for receiving and diluting wastes. Coastal regions and estuaries in particular tend to experience intense human occupation, which puts high pressure on the marine and estuarine ecosystems. In Brazil, for example, 14 of the country's 25 metropolitan regions are located in estuarine regions, as are the country's main petroleum production facilities and port systems (Lamparelli *et al*., 2001). As consequence, these ecosystems are exposed to intense and varied anthropic impacts, which lead to a loss of natural resources and biodiversity, and negative effects to the biota (Long *et al*., 1995).

The deposition of materials such as suspended sediments, organic material, and man-made contaminants is more extensive in estuaries, because these environments exhibit geomorphological and physical-chemical characteristics that favor such deposition (Du Laing *et al*., 2009). The aspects that control the mobility of these contaminants include changes in salinity, temperature, pH, and oxidation/reduction potential, all of which regulate the retention and release of chemicals associated with the finest sediment fraction, organic material, iron oxides, and sulfides (Rae, 1997). The contaminants may be shifted around different compartments of the sediment matrix, which includes interstitial water, the solid phase (sediment, suspended particles, organic matter, and biota), and the sediment-water interface (Jickells & Rae, 1997). Therefore, sediments can be considered one of the most complex matrices in the aquatic ecosystems, which are important indicators of environmental quality (Power & Chapman, 1992).

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Chemical analyses may provide information on the nature and specific levels of contaminants, which, if bioavailable, may lead to toxicity and bioaccumulation. However, different types of associations between contaminants and sediments may reduce their bioavailability to the biota (Araújo *et al*., 2006). For this reason, a sediment contamination assessment performed exclusively through chemical analyses is often insufficient for estimating damages to aquatic organisms. When this approach is performed alone, it only detects the nature of contamination and quantifies the concentrations present in the environment; it does not provide information on the contaminants bioavailability or their possible effects on the biota. Thus, toxicity tests give a biological significance to the chemical data and complement the studies of environmental contamination.

Toxicity tests are standardized methods used to assess deleterious effects on living organisms exposed to a substance, mixture of substances or environmental sample. These procedures are performed under controlled conditions, where previously selected organisms are exposed for a period of time to the sample of interest, and then the response is evaluated. Therefore, toxicity tests are extremely important for evaluating sediment quality, because they provide information on the effects caused by complex mixtures.

One of the most important Brazilian industrial complexes is located in the vicinity of the Santos-São Vicente Estuarine System (SSES), where the biggest port of South America is installed. Several studies performed in this area have evaluated sediment quality though chemical analyses and toxicity tests (Abessa *et al*., 1998; 2008; 2014a; Lamparelli *et al*., 2001; Cesar *et al*., 2006; Choueri *et al*., 2009; Torres *et al*., 2009; 2014; Buruaem *et al*., 2013). Although some of these studies have assessed the sediment quality at the inner parts of the estuary, information on the sediments of the SSES tributaries was not properly investigated. Therefore, in order to evaluate the extent of contamination and toxicity of the SSES, the objective of this study was to assess the quality of the sediments of the SSES tributaries, using an integrated approach that combines sediment chemistry and toxicity tests.

MATERIALS AND METHODS

Sediment sampling

Two sampling campaigns were performed (summer and winter 2006) in which the sampling stations were distributed in
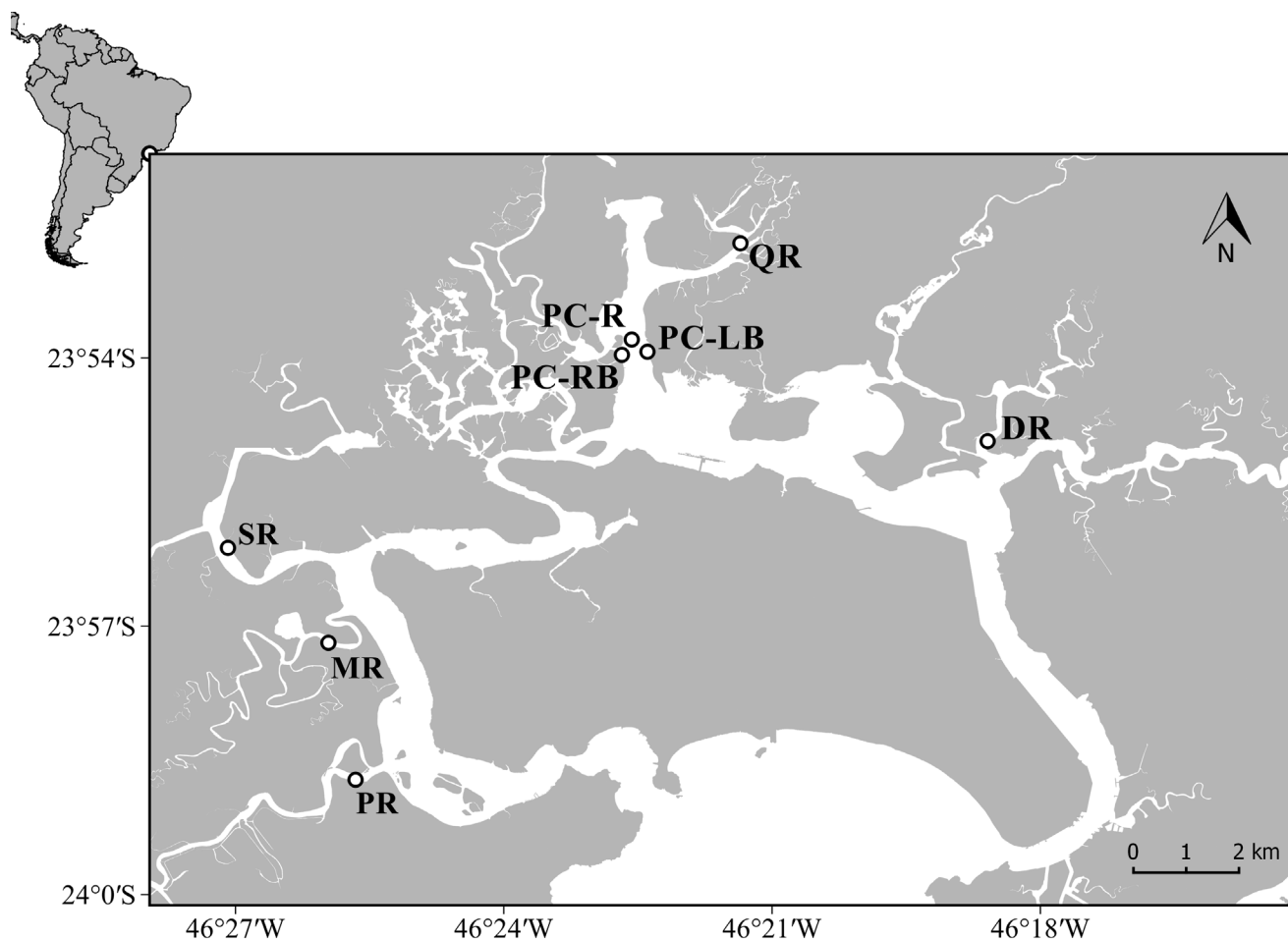

Figure 1. Geographic locations of the sediment collection sites in the Santos-São Vicente Estuarine System tributaries: Piaçabuçu River (PR), Mariana River (MR), Santana River (SR), Quilombo River (QR), Diana River (DR), Piaçaguera Canal riverbed (PC-R), Piaçaguera Canal right bank (PC-RB) and Piaçaguera Canal left bank (PC-LB).

eight different sites within the SSES (Figure 1). Five sampling sites were situated at the rivers Piaçabuçu (PR), Mariana (MR), Santana (SR), Quilombo (QR), and Diana (DR); and other three sampling sites were set up in the Piaçaguera Canal at riverbed (PC-R), right bank (PC-RB) and left bank (PC-LB). Surface sediments were collected with a Van-Veen grab, and kept on ice in thermal boxes. In the laboratory, the aliquots for the ecotoxicological assays were stored at 4°C, while those for the geochemical analyses were stored at -20°C. Aliquots of sediment samples for the sediment texture analyses were separated and left to dry at 60°C for three days before use.

Sediment properties and chemical analyses

Sediment grain size distribution was analyzed based on the protocol proposed by Mudroch & Macknight (1994). This technique consists of wet sieving 100 g of previously dried sediments through a 0.062-mm mesh to separate sandy fractions; followed by settling time determination (to measure silts and clays) with a calibrated hydrometer. Subsequently, the sandy material retained on the 0.062-mm mesh was sieved into different meshes (ϕ scale) in order to separate different classes of sands. The classification method was based on the scale established by Wentworth (1992).

Total organic carbon was determined by combustion at 900°C for total carbon and phosphoric acid addition for inorganic carbon, which are transformed into CO_2. Calcium carbonate was determined according to the protocol described by Hirota & Szyper (1976). This technique consists of separating fractions of 5 g of each sediment sample and then adding 10 ml of hydrochloric acid (5 N HCl) for 24 h to eliminate calcium carbonates. Afterward, samples were washed with distilled water and dried at 60°C. The difference between initial and final weights showed the amount of $CaCO_3$ in the samples.

The elements Ag, As, Cd, Cu, Cr, Hg, Ni, Pb, Se and Zn were extracted from sediments according to USEPA Method 3050B (USEPA, 1996a). Concentrations of Cu, Cr, Ni, Pb, and Zn were determined by flame atomic absorption spectrophotometry (F-AAS). In regard to Ag and Cd the determinations were performed by graphite furnace (GF-AAS). For As and Se the concentration was determined by hydrate generation (HG-AAS). Mercury (Hg) was extracted by combining USEPA Methods 245.5 and 245.6 (USEPA, 1991). Hg concentrations were determined by cold vapor spectrophotometry (CV-AAS).

Polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) were extracted from sediments according to USEPA Method 3550B (USEPA, 1996b), and then passed through a clean-up column with silica gel following the USEPA Method 3630C (USEPA, 1996c). Extracts were analyzed by gas chromatography mass spectrometer (GC–MS) with methods prepared for each class of compounds that were evaluated. PAH were analyzed according to Method 8270C (USEPA, 1996d). The method to quantify PCB was adapted from USEPA Methods 8081B, 8082A, and 8275A (USEPA, 1996e; 1998; 2000; respectively). Detailed descriptions of the chemical analyses of PAHs and PCBs, as well as the respective Quality Assurance / Quality Control (QC/QA) procedures, are available in Torres *et al*. (2009).

Whole sediment toxicity tests

In order to assess whole sediment toxicity, two approaches were carried out: the chronic toxicity test using the benthic copepod *Nitocra* sp. and the acute toxicity test with the burrowing amphipod *Tiburonella viscana*. Physical and chemical parameters (pH, salinity, dissolved oxygen, and temperature) were checked at the beginning and end of each experiment. The reference sediment and dilution water were collected at Engenho d'Água Beach, in the city of Ilhabela, SP, Brazil. This location was chosen due to lack of reference site for the SSES, since the sediments are influenced by anthropogenic activities to varying degrees. Moreover, Engenho D'água Beach presents low levels of contamination (Abessa *et al*., 2008) and has been used as a reference site by several environmental studies (Cesar *et al*., 2006; Buruaem *et al*., 2013; Abessa *et al*., 2014a).

The chronic toxicity assay with the copepod *Nitocra* sp. was performed with whole-sediment following the protocol developed by Lotufo & Abessa (2002). Sediments were introduced into high-density 10-mL polyethylene test chambers, forming a layer of approximately 0.5 cm ; 8 mL of filtered sea water were then added to each test chamber. Four replicates per sample were used; each one received 5 ovigerous females of *Nitocra* sp. The test system was kept at 25 ± 2°C and under a photoperiod of 16:8 light:dark for 10 days. At the end of the experiment, the contents of each test chamber were fixed through the addition of 1 mL formaldehyde (4%) and Rose-Bengal dye (0.1%). The sub-lethal effects on reproduction were estimated by counting the total number of offspring (nauplii and copepodites) divided by the number of surviving females.

The acute toxicity assay with whole-sediment followed the protocol described by Melo & Abessa (2002), using the amphipod *Tiburonella viscana*. Organisms were collected from Engenho d'Água Beach (Ilhabela, SP) by using a manual dredge. The animals were transported to a laboratory and acclimated for 3 days in tanks containing filtered seawater and reference sediment, at 25 ± 2ºC, and under constant lighting and aeration. Tests were conducted in high density polyethylene flasks with 1 L holding capacity. Prior to the test, the sediment samples were thoroughly homogenized, and then a layer of approximately 2 cm (about 175 mL) of sediment was placed in each test chamber together with about 750 mL of filtered seawater. After 24 hours, ten adult and non ovigerous organisms were introduced into each test-chamber. The testing system was kept under constant lightning, aeration and temperature (25 ± 2°C). Four replicates of each sample and control were prepared (sediment from Ilhabela, origin of the amphipods). The number of dead organisms was counted daily. After 10 days, the experiments were concluded and the sediment was sieved (0.5 mm net); the number of survivors was counted in each replicate.

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Statistical analyses

The toxicity tests data were checked by normality and homoscedasticity by Shapiro-Wilks and Bartlett's tests, respectively. Then, the results were compared with their respective controls using the Student's t'-test. Sediments were considered toxic when identified significant differences between the references and the test-samples to each toxicity endpoint. Sediments were classified as toxic, nontoxic, or exhibiting a sign of toxicity (when no significant effect was observed, but exhibit more than 30 % difference between the tested sample and the reference sample).

The data were integrated using exploratory techniques. In order to determine the direct association between the variables, a multiple correlation analysis was carried out, using Pearson's product with significance level of 95%. The second approach aimed to understand the indirect associations; to achieve that, the data were integrated through multivariate analysis (Factor Analysis, with the extraction by Principal Component Analysis). Two separate PCAs were conducted on the biological and chemical results, one for summer, and the other one for winter. In the interpretation of PCA data, the eigenvalues higher than 1.0 were considered (Kaiser's criteria). A component loading cutoff of 0.40 was used in selecting variables for inclusion into factors (Tabachnick & Fidell, 1996). Finally, to classify the samples, we used a cluster analysis (Bray Curtis distance; Group Average Link), as proposed by Cesar *et al.* (2009).

RESULTS AND DISCUSSION

Sediment characteristics

Results obtained for sediment properties and chemical analyses are summarized in the Table 1. In summer, sediment samples from the rivers Piaçabuçu, Quilombo, Diana and Piaçaguera Canal (banks) were predominantly sandy (fines percentages ranging between 17 and 29%); whereas sediments from the rivers Mariana and Santana were muddy (88% and 54% of mud particles, respectively). However, all sediment samples were muddy in winter, excepting the samples from Mariana and Diana rivers, where fine sand was predominant (61% and 77%, respectively).

The highest total organic carbon (TOC) quantities were found in the sediment samples from PC-LB and Quilombo River (8.6% and 8.0%, respectively), collected in the summer campaign. Generally, sandy sediments present low amounts of TOC in relation to muddy sediments (Du Laing *et al.*, 2009). However, these samples had small amounts of fine sediments (about 24% for both PC-LB and Quilombo River) in comparison to the other samples. Possibly this aspect may be related to the lower influence of tidal currents in these sites. In winter, the sediment sample from the Santana River showed the highest TOC quantity (6.2%). The sediment from Santana River presented a higher content of $CaCO_3$ (11.6%), while the other samples presented levels ranging from 3.47% to 8.7%.

Comparison with sediment quality guidelines

In Brazil there are no standards for contaminants concentrations in sediments. However, there is a law for dredged sediments established by CONAMA Resolution 454/2012 (Brasil, 2012). This resolution proposes the use of some sediment quality guidelines (SQGs) and establishes that the characterization of sediments prior to dredging should be based on an evaluation of physical, chemical, and ecotoxicological parameters. The SQGs adopted in this resolution consist of a combination of various international sediment quality guidelines, particularly those established by the Canadian Council of Minister of the Environment (CCME, 2002), and the North American Effects Range, which set forth for marine and estuarine environments (Long *et al.*, 1995). Therefore, the results of chemical analyses were compared with the Interim Sediment Quality guidelines (ISQG = TEL - threshold effect Level) and Probable Effect Levels – PEL (CCME, 2002), as well as with the Effects Range Low (ERL) and Effects Range Median (ERM) (Long *et al.*, 1995) in order to provide interpretation in global perspective. Moreover, to provide interpretation of chemical data in a local perspective the results were compared with threshold and probable effects from site-specific sediment quality values (SQVs) derived for the Santos Estuarine System (Choueri *et al.*, 2009), based on sediment physical-chemical, toxicological, and benthic community data integrated through multivariate analysis. The SQVs proposed by Choueri *et al.* (2009) yield two thresholds, termed "SQG-low" and "SQG-high". Chemical concentrations lower than SQG-low were considered not harmful to aquatic biota. On the other hand, concentrations of contaminants above SQG-high are potentially harmful to the ecosystem.

The total PAHs in the sediment from PC-RB (6841 µg Kg^{-1}) exceeded the level 1 of the CONAMA Resolution 454/12 (4000 µg Kg^{-1}, which corresponds to the North American ERL). Also, this value exceeded the site-specific SQG-high (1660 µg Kg^{-1}) (Choueri *et al.*, 2009), thus the sediment of this site is considered as polluted by these contaminants. In this sampling station, 10 of 15 PAHs compound exceeded the TEL. According to standards stated by CONAMA 454/2012, the compounds Benz[a]anthracene, Benzo[a]pyren, Chrysene, Anthracene, Fluoranthene and Pyrene exceeded their corresponding threshold levels (Level 1) which are 280, 230, 85.3, 600 and 665 µg Kg^{-1}, respectively. Moreover, the compounds Fluoranthene and Pyrene exceeded their corresponding PEL values (1494 and 1398 µg Kg^{-1}, respectively). These compounds also violated the TEL in sediments from PC-R, which are 113 µg Kg^{-1} to Fluoranthene and 153 µg Kg^{-1} to Pyrene. Acenaphthene and Acenaphthylene exceeded the corresponding TEL values (6.71 and 5.87, respectively) in the sediments from PC-R and Diana River.

Concerning total PCBs, metals and As, the Figure 2 presents the contaminants concentrations in the tributaries and their respective SQGs adopted in this study. PCBs concentrations were below TEL (21.5 mg Kg^{-1}). In regard of the site-specific SQG, sediment samples from the Piaçabuçu River and

Table 1. Sediment properties and chemical analyses of samples from SSES tributaries.

SUMMER							
Characteristics (%)	PR	MR	SR	PC-RB	PC-R	PC-LB	QR	DR
Medium sand	6.2	2.9	16.4	21.6	31.4	17.9	33.4	11.3
Fine sand	76.5	9.1	30.1	49.0	28.2	57.8	42.3	72.2
Silt	9.2	58.0	34.2	24.1	25.7	14.8	10.1	8.7
Clay	8.1	30.0	19.3	5.3	14.7	9.5	14.2	7.8
TOC	3.2	3.3	7.9	1.2	2.7	8.6	8.0	0.9
CaCO$_3$	NP	NP	NP	NP	NP	NP	NP	NP
Metal and semi-metal (mg Kg^{-1})								
As	7.4	6.4	7.3	7.6	8.0	8.9	9.2	2.5
Cd	< QL	< QL	0.1	0.1	0.1	< QL	0.3	< QL
Cr	28.9	19.1	34.9	45.1	41.7	29.8	53.0	9.0
Cu	8.9	2.9	16.1	22.8	25.8	14.0	28.7	1.1
Hg	0.2	0.6	0.2	1.2	0.7	1.2	0.3	0.2
Ni	10.1	8.0	17.3	23.1	20.0	14.3	26.7	3.8
Pb	23.0	< QL	27.0	31.5	28.0	20.3	63.0	< QL
Zn	57.5	27.3	93.0	110.0	118.8	61.0	189.5	18.7
WINTER								
Characteristics (%)	PR	MR	SR	PC-RB	PC-R	PC-LB	QR	DR
Medium sand	2.4	1.7	7.3	2.7	2.8	6.6	6.6	0.2
Fine sand	44.3	60.6	13.6	27.2	38.6	35.9	18.2	77.4
Silt	21.8	14.3	40.7	52.6	33.6	32.0	41.6	12.9
Clay	31.5	23.3	38.4	17.6	25.0	25.5	33.6	9.5
TOC	2.4	3.3	6.2	4.1	5.4	3.0	2.9	0.8
CaCO$_3$	6.8	5.7	11.6	7.9	8.8	8.7	4.5	3.4
Organic compounds (µg Kg^{-1})								
Benz[a]anthracene	4.9	2.6	2.8	505.6	50.6	16.0	30.5	9.5
Benzo[a]pyrene	5.7	1.5	2.3	475.7	60.7	18.9	36.1	9.0
Chrysene	6.5	1.0	1.7	551.3	61.1	16.5	21.4	8.3
Dibenz[a,h]anthracene	ND	1.0	ND	22.9	3.9	1.4	1.9	2.6
Acenaphthene	1.3	1.2	0.7	12.5	7.2	5.1	1.5	11.4
Acenaphthylene	1.8	1.4	1.0	29.6	7.1	1.6	4.2	10.7
Anthracene	3.4	1.3	1.5	104.5	18.7	3.3	5.4	21.4
Phenanthrene	4.4	2.3	2.2	106.4	28.4	4.6	7.2	22.9
Fluoranthene	11.5	5.0	5.0	2264.9	123.3	34.0	32.7	21.7
Fluorene	ND	ND	ND	13.8	8.4	ND	3.6	14.5
Naphthalene	ND	ND	1.2	7.0	13.1	0.9	2.7	5.5
Pyrene	10.5	5.0	6.6	2166.2	195.2	49.1	125.5	23.3
Benzo[b]fluoranthene	4.5	1.9	2.0	431.6	56.7	18.7	32.5	12.3
Indeno[1,2,3-cd]pyrene	1.3	0.9	ND	81.9	12.7	4.4	7.8	3.4
Benzo[ghi]perylene	1.0	ND	0.6	67.3	11.4	3.8	6.8	2.3
Σ PAH	56.7	25.0	27.6	6841.0	658.4	178.4	319.9	178.8
Σ PCB	1.0	0.3	0.0	1.3	2.1	1.1	0.0	0.0
Metal and semi-metal (mg Kg^{-1})								
As	2.5	10.9	8.9	4.3	6.5	3.7	3.3	1.5
Cd	< QL	< QL	< QL	0.1	0.1	< QL	< QL	< QL
Cr	7.1	33.1	20.1	25.4	34.6	16.6	19.1	9.4
Cu	< QL	5.9	4.4	8.4	18.6	3.1	4.1	< QL
Hg	0.3	0.6	0.3	0.4	0.4	0.4	0.7	0.2
Ni	< QL	11.4	8.8	11.0	17.1	7.7	7.3	< QL
Pb	< QL	17.5	< QL	15.3	31.8	0.0	0.0	38.3
Zn	7.9	52.0	40.6	53.0	98.0	28.6	41.7	11.7

PR: Piaçabuçu River, MR: Mariana, SR: Santana River, QR: Quilombo River, DR: Diana River, PC-R: Piaçaguera Canal riverbed, PC-RB Piaçaguera Canal right bank and PC-LB: Piaçaguera Canal left bank.

NP = not performed.

ND = not detected.

QL = quantification limit (Cd: 0.02 mg kg^{-1}; Ni: 2.5 mg kg^{-1}; Pb: 5.0 mg kg^{-1}).

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Piaçaguera Canal (riverbed and banks) exceeded SQG-low (0.94 µg Kg^{-1}), but did not violate SQG-high (2.61 µg Kg^{-1}). According to Choueri *et al.* (2009), biological responses are not predictable in the concentration range in between these two thresholds, and then the sediments containing PCBs into this interval are considered as moderately polluted.

The São Paulo's State Environmental Regulatory Agency (CETESB) presented an inventory of contamination sources to the SSES, indicating several major industries, point and diffuse sewage inputs, and landfills (Lamparelli *et al.*, 2001). According to these authors, the sources of PAHs to the estuary may be the substances used or produced by the industrial processes, together with port activities, combustion of coal and petroleum fuels and landfills; whereas the PCBs concentrations in SSES suggest a diffuse contribution of these pollutants (atmospheric deposition of PCBs released by factories.

The chemical analyzes revealed that all sediments from the SSES tributaries presented metals contamination at any degree. In summer, concentrations above the TEL were found for Cr (Quilombo River); Cu (PC-RB, PC-R and Quilombo River); Pb (PC-RB, and Quilombo River), Zn (Quilombo River). Regarding the site-specific SQGs, sediment samples did not exceed the thresholds for Cd, Cr and Cu. On the other hand, concentration above SQG-low was observed for Ni in all sediment samples, except for the Diana River. Concentrations above the SQG-low also were found for Zn at PC-LB and Quilombo River. Sediments from Piaçaguera Canal (riverbed and banks), and Piaçabuçu, Santana and Quilombo Rivers presented Pb exceeding the SQG-high established for SSES.

In the winter campaign, concentrations of Pb in the sediments from PC-R and Diana River exceeded the TEL and the SQG-high. Moreover, this metal was above the SQG-low in the sediments from PC-RB and Mariana River. In PC-R sediment was also found Zn above the SQG-low value. Most of sediment samples, with exception of those from Piaçabuçu and Diana Rivers, presented Ni concentration above SQG-low value.

Considering the two sampling campaigns, sediments collected near the industrial complex presented higher concentration of Cr. From this region towards the bay of Santos, there is an apparent decrease in the concentration of this metal. Overall, the sampling sites present Cu levels below the SQGs, with the exception of the Quilombo River and two sites from Piaçaguera Canal, where a previous study reported concentrations of Cu at 164.0 mg Kg^{-1} (Luiz-Silva & Machado, 2012) and 157.7 mg Kg^{-1} (Cesar *et al.*, 2006), respectively. According to CETESB, the sources of Cu can be industrial effluents and some port terminals of liquid bulk. Regarding Ni, among the inputs of this metal to the estuary there are the point sources (industrial effluents) and diffuse sources (port terminals and atmospheric pollution). The Pb levels measured in this study are related to industrial sources, with the highest contamination observed in the sampling sites closer to a large steel plant and to the industrial complex of

Cubatão. Furthermore, there is a port terminal of a chemistry factory near the Diana River, which could be a possible source of Pb. Although the other rivers have shown Pb levels below the TEL, these values are above the site-specific SQG-high, which are evidence of diffuse sources of this metal. The sediments of the studied SSES tributaries were also somewhat contaminated with Zn, especially the sites near industrial sources. According to the State Environmental Regulatory Agency (Lamparelli *et al.*, 2001), the main source of Zn in the estuary is the steel industry.

The Hg was the main contaminant found in the sediments of SSES tributaries. Hg concentrations in all sediments exceeded the TEL and the site-specific SQG-low in both summer and winter campaigns. The highest Hg concentrations were observed in the sediments from PC-RB and PC-LB, exceeding the PEL in summer. Furthermore, the SQG-high was exceeded at Piaçaguera Canal (riverbed and banks), Mariana and Quilombo rivers in both seasons. Since Hg sources are not known for the Piaçabuçu, Mariana and Santana Rivers (Lamparelli *et al.*, 2001), the presence of this metal seems to depend not only on point sources (industrial complex and some port terminals), but also on diffuse sources. Indeed, Hortellani *et al.* (2005; 2008) and Abessa et al. (2008) suggested that the broad spatial distribution of Hg across the SSES may be due to multiple sources, such as the industrial effluents, landfills and atmospheric deposition.

Concerning the metalloid As, the sediment collected in the Quilombo River presented higher concentration in summer (9.2 mg Kg^{-1}), which corroborate with literature, since Luiz-Silva *et al.* (2006) reported levels of As ranged from 5 to 13 mg Kg^{-1} in the Morrão River (a tributary of the Quilombo River). Moreover, only the sediments from the Mariana and Diana rivers did not exceed the Canadian TEL in the summer campaign. However, in winter, the sediments of Mariana and Santana Rivers presented As concentrations above the TEL. But when it is compared with the site-specific SQVs, As did not exceed threshold levels. According to CETESB, there are no specific industrial sources of As in the SSES, thus its presence may be due to diffuse sources, such as raw materials or wastes (Lamparelli *et al.*, 2001). Potential sources of this element in the region are domestic and industrial dumping sites located on the banks of the Cubatão River and Piaçabuçu River. These landfills, scattered around the estuary, consist of legal and illegal industrial and domestic wastes disposal sites and were installed along the mid and late 20th century. They still represent a considerable source of contamination to the internal areas of the estuarine system (Fortunato *et al.*, 2012; Schepis *et al.*, 2016).

Metals in estuaries may be originated from natural sources involving both local geological composition and the leaching of soils and rocks present in the drainage basin. Nevertheless, the greatest contribution comes from human sources, such as domestic and industrial effluents (Du Laing *et al.*, 2009), which is strongly influenced by rainfall intensity (Pereira *et al.*, 2006). The results indicated that in general the sediments from the SSES tributaries exhibited higher concentrations of metals
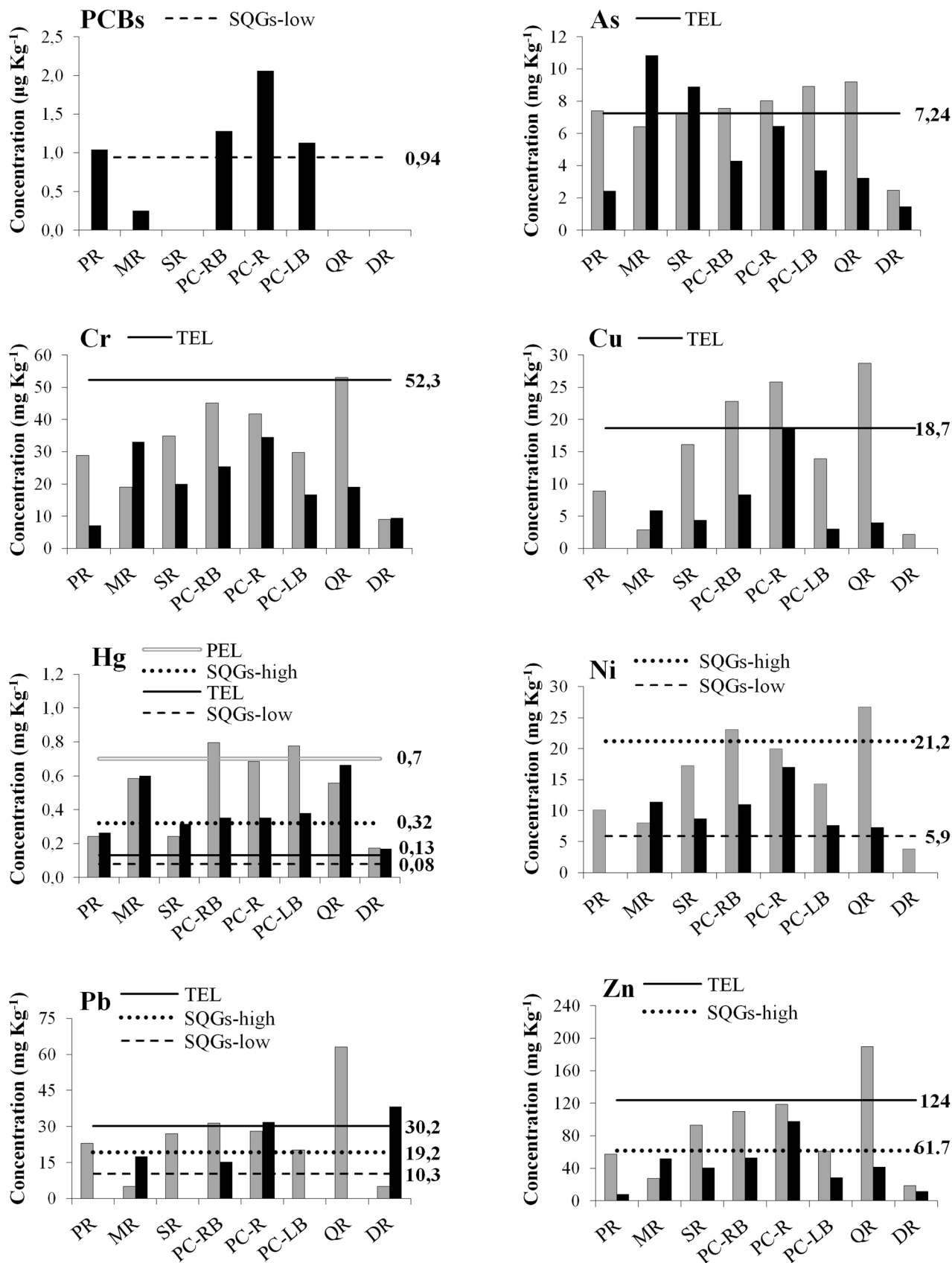
Figure 2. Concentrations of total PCBs, arsenic and metals in sediments from SSES tributaries, collected in summer (grey bars) and winter (black bars); and their respective sediment quality guidelines values adopted in this study: TEL and PEL (CCME, 2002); SQG-low and SQG-high (Choueri *et al.*, 2009). PR: Piaçabuçu River, MR: Mariana, SR: Santana River, QR: Quilombo River, DR: Diana River, PC-R: Piaçaguera Canal riverbed, PC-RB Piaçaguera Canal right bank and PC-LB: Piaçaguera Canal left bank.

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during the summer, which is the rainier season; this pattern was more evident in the Quilombo River. Similar results were also observed by Luiz-Silva *et al.* (2006), who investigate the spatial and seasonal variability of metals in the northern portion of SSES. In the summer campaign the precipitation rate accumulated in the 15 days prior to sampling was 226.5 mm, whereas in the winter campaign it was 87.7 mm. In fact, studies have shown that concentrations of metals increase in river sediments during the rainy season (Cunha *et al.*, 2007; Abessa *et al.*, 2014b). Abessa *et al.* (2014b) detected eventual differences in the geochemistry of sediments after periods of moderate and extreme rainfalls. Additionally, Cunha *et al.* (2007) stated that extreme rainfalls were related to an increase in the adsorption and complexation reactions between fines, organic matter, metals and nutrients.

The rainwater runoff may drag a series of contaminants deposited on soils or associate to sediment particles, as pesticides and metals, among others (Bay *et al.*, 1996). The contaminants tend to be carried to low energy areas, as estuaries, where contaminated particles set, producing environmental contamination (Carr *et al.*, 2000). As a matter of fact, there is a decrease in the concentration of metals from the northern portion of SSES towards the bay of Santos, mainly in the summer (rainy season). This gradient of contamination from the inner to the outer portions of the SSES was previously reported (Lamparelli *et al.*, 2001; Abessa *et al.*, 2008; Torres *et al.*, 2009). According to Hortellani *et al.* (2005), besides the presence of metal sources, the inner portions of the SSES are also affected by tides restricting water circulation, which hinders the dispersion of the contaminants and their accumulation in the sediments.

Whole sediment toxicity tests

The physical and chemical parameters of the overlying water in the test-chambers during the bioassay with *T. viscana* were considered appropriate (Melo & Abessa, 2002). Temperatures ranged between 24.3 and 25.4°C; dissolved oxygen ranged from 6.2 to 9.8 mg L^{-1}; pH levels were between 7.6 and 8.8; and salinities ranged from 34 to 35.

In the summer campaign, the amphipods exposed to sediments from Piaçaguera Canal (both banks and riverbed) and Quilombo River exhibited significant mortalities (Figure 3a). Moreover, sediments from the rivers Mariana and Santana showed a sign of toxicity. In winter, the mortalities of amphipod were significant in the sediments from the three sampling stations in the Piaçaguera Canal (riverbed and banks), and Mariana River (Figure 3a). In this season, signs of toxicity were observed in the sediments from Piaçabuçu, Santana and Quilombo rivers.

In the chronic toxicity test, the measured parameters of overlying waters were within acceptable levels (Lotufo & Abessa, 2002). Dissolved oxygen levels ranged from 4.1 to 5.4 mg L^{-1}; pH ranged between 7.20 and 8.26; and salinities ranged from 17 to 20. In the summer campaign, only the copepods

exposed to sediment from Diana River did not produced fecundity rates significantly lower than those animals exposed to the reference sediment. On the other hand, fecundity rates of copepods exposed to sediments collected in winter were significant lower in all stations samples; thus, these sediments were considered toxic to *Nitocra* sp. (Figure 3b).

When the results of both seasons are compared, it is possible to notice that all sediment samples presented toxicity in at least one assay (table 2). Based on the results, it is evident that the sediments from the Piaçaguera Canal (PC-RB, PC-R and PC-LB) presented poor quality, and all samples collected in that area produced adverse effects. Sediments from Quilombo and Mariana rivers presented acute toxicity in summer and winter, respectively. Diana River sediments caused adverse effects only in the chronic toxicity assay in winter; moreover, sediment from this site showed no sign of acute toxicity in both seasons.

The bioavailability of the contaminants in the studied sediments was evidenced by the ecotoxicological tests. The sediments with the highest levels of As in summer (Quilombo River) and winter (Mariana River) showed acute toxicity to amphipods. The sediments from Diana River did not show chronic toxicity in summer, but when this site presented the highest level of Pb, fecundity rates were significantly different from the reference sediment.

In order to verify connections among variables, correlation analyses were performed using the Pearson linear correlation coefficients (p<0.05). In summer, acute toxicity presented strong correlations with Hg (coefficient calculated at 0.88); and moderate correlation with As and Ni (0.58 and 0.50, respectively). The chronic toxicity correlated positively high with As (coefficient calculated at 0.79); and presented moderate correlation with Cr and Ni (0.59 and 0.51, respectively). These results suggested that probably these contaminants influenced directly the sediment toxicity. In fact, Hg concentrations exceeded the site-specific SQG-high in sediments from Piaçaguera Canal, Quilombo and Mariana rivers, and the same samples presented acute toxicity. Although the survival rates of amphipods exposed to sediment from Mariana River were statistically similar to those observed in the reference sediment, the mean of amphipod survival was less than 40%. Moreover, with the exception of Diana River sediment, all samples presented chronic toxicity in summer and these sediments exceeded the site-specific SQG-low for Ni. In addition, excepting the sample from Mariana River, all the sediments presented concentrations of As exceeding the TEL.

The table 3 presents correlation analyses made to verify connections among the variables in the winter. Amphipod survival presented a negative correlation with Ni, PAHs and PCBs; whereas copepod fecundity was negatively correlated with Cr, Cu, Ni and Zn. Mud presents very strong correlation with As, strong correlation with Cr, and moderate correlation with Ni. Moderate to strong correlation was observed between TOC content and As, Cr, Cu, Ni and Zn concentrations (correlation coefficient of around 0.7).

Multivariate Analysis Approach

The use of factor analysis provided a deeper insight into data, and revealed associations of chemical concentrations in sediment and the biological effects. PCA results are presented in Tables 4 and 5. In summer, three factors explained 88.4% of the total variance (table 4). The first factor (PC1) explained 53.3% of the variances; it showed positive correlations to the concentrations of As, Cd, Cu, Ni, Pb, Zn and TOC; these variables were negatively correlated with the amphipod survival and copepod fecundity (indicators of absence of toxicity). This factor represented the association of organic carbon, metals and biological responses. Apart from Cd, all these metals presented high concentrations, at potentially toxic levels to the test-organisms. The second factor (PC2), accounted for 22.4% of the variances and combined mud and concentration of Hg, which were inversely correlated with amphipod survival and copepod fecundity. This factor suggested that the biological effects could be related to

the concentrations of Hg. As previously presented, Hg concentrations are above the SQGs. The third factor (PC3) accounted for the 12.7% variances and again associated Hg and mud, but with positive loadings, which were negatively correlated with amphipod survival. Such correlation suggests that this contaminant was strongly associated with the fine particles and was an important contributor to the acute toxicity. This metal was found in levels above the site-specific SQG-high in the sediments that presented acute toxicity to amphipods (all the sampling sites situated at Piaçaguera Canal and Quilombo River).

In winter, three factors explained 85.9% of the total variances (table 5). The first factor (PC1) accounted for 49.6% of variances. Positive loadings were evidenced to PCBs, all the PAH compounds, Cu, Ni, and Zn, and silt contents; amphipod survival and copepod fecundity were negatively correlated with this factor. These results suggested that probably these contaminants were associated to fines particles and influenced
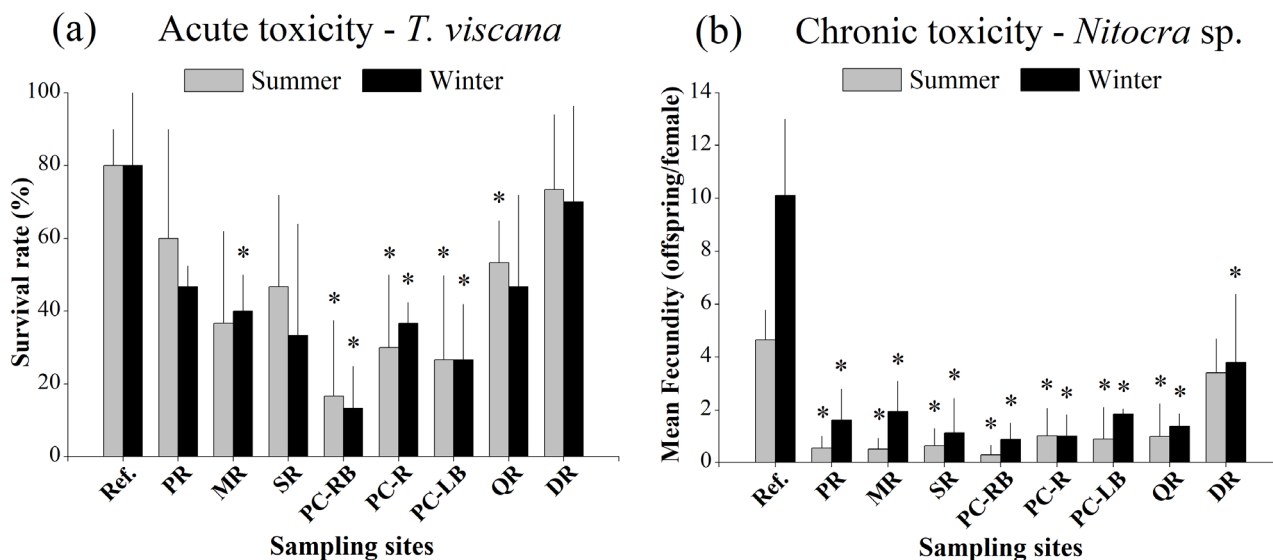

Figure 3. Whole sediment toxicity: (a) Mean survival rates of the amphipod *Tiburonella viscana* and (b) Mean fecundity rates of *Nitocra* sp. exposed to sediments from the SSES tributaries. Asterisks (*) indicate significant differences relative to the reference (p<0.05). Results are presented as means ± standard deviations (error bars).

Table 2. Comparative results obtained in sediment toxicity tests for samples collected in SSES tributaries.

Sampling sites	Acute toxicity		Chronic toxicity	
	Summer	Winter	Summer	Winter
PR	not toxic	sign of toxicity	toxic	toxic
MR	sign of toxicity	toxic	toxic	toxic
SR	sign of toxicity	sign of toxicity	toxic	toxic
PC-RB	toxic	toxic	toxic	toxic
PC-R	toxic	toxic	toxic	toxic
PC-LB	toxic	toxic	toxic	toxic
QR	toxic	sign of toxicity	toxic	toxic
DR	not toxic	not toxic	not toxic	toxic

PR: Piaçabuçu River, MR: Mariana, SR: Santana River, QR: Quilombo River, DR: Diana River, PC-R: Piaçaguera Canal riverbed, PC-RB Piaçaguera Canal right bank and PC-LB: Piaçaguera Canal left bank.

directly the sediment toxicity. The second factor (PC2) explained 23.5% of variances. In this factor, both indicators of absence of toxicity were inversely correlated with all metals, excepting the Pb, which were associated with clay, $CaCO_3$, and TOC in the sediment. Hence, this factor tended to associate contaminants with geochemical carriers (clay, TOC and $CaCO_3$) and toxicity to organisms tested. This factor showed negative correlation for sand, since these particles are reported to be almost nonreactive compared to fine particles (Sageman & Lyons, 2003). The Factor 3 explained 12.8% of variances, showing positive correlations for Cr, Cu, Pb, Zn, some PAH compounds (Fluorene and Naphthalene), and negative correlations to fines.

In order to separate the SSES tributaries with different degrees of sediments quality, according to geochemical and ecotoxicological properties, cluster analysis were conducted with each data set (Figures 4 and 5, to summer and winter, respectively), as proposed by Cesar *et al.* (2009). In summer, due to the absence of data (such as $CaCO_3$, PAHs and PCBs), the cluster analysis was unclear concerning degradation degrees and relationship among the sites. However, Diana River was placed isolated from the others sites; this sediment showed absence of toxicity and the lowest levels of metals, although it presented moderate levels of Hg; hence this site could be classified as low or not degraded. In the sequence appears the Mariana River, which could be classified as low degraded. Then, there is a larger group, subdivided according to the degree of degradation; where Piaçabuçu River was associated with PC-LB; in the sequence was linked two different groups. The first one contemplates the Santana and Quilombo Rivers. Finally, the last group, containing PC-R and PC-RB, which could be classified as degraded sites.

In winter, Diana River also was placed isolated from the others sampling sites; although its sediments presented Pb above the SQG-high, moderate contamination by some PAHs, and chronic toxicity. Therefore, this site could be classified as low to moderately degraded. A second group was formed by the rivers Mariana and Santana, which showed evidence of acute toxicity, contamination by As, Hg and Ni (concentrations above TEL and site-specific SQG) and could be classified as moderately degraded. Piaçabuçu River (chronic toxicity, signal of acute toxicity, contamination by Hg and PCBs) could be classified as moderately degraded as well. The Quilombo River and the PC-LB showed more evidences of degradation (Hg concentration exceeding SQG-high, Ni values above SQG-low) and could be classified as degraded. In this analysis, PC-R and PC-RB were again placed isolated from the other sampling stations; besides in this season the percentage of similarity with the other samples was lower; probably it is due to concentrations of PCBs, the high levels of PAHs and the stronger toxicity to amphipods. Therefore, PC-R and PC-RB could be classified as highly degraded. In the winter, the clusters formed seemed to be indicating not only the different degrees of contamination among sites, but also the influence of different pollution sources in each estuarine region. These aspects are further discussed in the next section of this paper.

Sediment quality of the Santos-São Vicente Estuarine System tributaries

Overall, sediment from Diana River was classified as low to moderately degraded. The chronic toxicity of these sediments in the winter campaign may be related to the port operations. Acenaphthene, Acenaphthylene, and Pb (the highest value of this metal in winter) were found in these sediments. Despite the absence of acute toxicity observed, previous studies reported high mortality of amphipods exposed to sediments from Diana Island (collected near our

Table 3. Correlation analysis of sediment properties, chemical contaminants and toxicity tests results of samples from SSES tributaries. Variables significantly correlated and their respective Pearson linear correlation coefficients (p = 0.05) are highlighted.

	Amphipod survival	Copepod fecundity	Mud	CaCO₃	TOC	Σ PCB	Σ PAH	Zn	Pb	Ni	Hg	Cu	Cr	Cd	As
As	-0.34	-0.38	0.96	0.45	0.65	-0.09	-0.12	0.52	-0.04	0.64	0.41	0.40	0.74	0.10	—
Cd	-0.36	-0.46	0.00	0.29	0.49	0.81	0.41	0.84	0.50	0.72	-0.12	0.93	0.63	—	
Cr	-0.48	**-0.50**	0.71	0.27	0.61	0.36	0.23	0.91	0.28	0.95	0.49	0.84	—		
Cu	-0.42	**-0.56**	0.29	0.37	0.65	0.68	0.26	0.98	0.39	0.91	0.16	—			
Hg	-0.20	-0.36	0.47	-0.20	0.11	-0.22	-0.08	0.32	-0.35	0.42	—				
Ni	**-0.61**	**-0.65**	0.54	0.47	0.74	0.49	0.27	0.96	0.16	—					
Pb	0.40	0.49	0.05	-0.37	-0.21	0.18	0.10	0.32	—						
Zn	-0.44	**-0.58**	0.43	0.35	0.69	0.54	0.23	—							
Σ PAH	**-0.63**	-0.38	-0.14	0.11	0.16	0.35	—								
Σ PCB	**-0.50**	-0.46	-0.18	0.37	0.31	—									
TOC	**-0.64**	**-0.79**	0.44	0.86	—										
CaCO₃	**-0.68**	**-0.66**	0.21	—											
Mud	-0.21	-0.21	—												
Fecundity	0.80	—													
Survival	—														

Values marked in bold are negatively correlated with amphipod survival and copepod fecundity.

Sediment quality assessment of the tributaries...

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Table 4. Principal component analysis (PCA) based on chemical contamination and effects on amphipod survival and copepod fecundity exposed to sediments of Santos-São Vicente Estuarine collected in the summer.

Variables	PC 1	PC 2	PC 3
As	**0.86**	-0.30	0.01
Cd	**0.80**	0.35	-0.37
Cr	**0.99**	0.02	0.02
Cu	**0.95**	0.11	0.09
Hg	0.32	**-0.52**	**0.73**
Ni	**0.98**	0.01	0.03
Pb	**0.93**	0.32	-0.12
Zn	**0.95**	0.23	-0.14
TOC	**0.50**	-0.03	-0.38
Sand	0.15	**0.80**	**0.54**
Mud	-0.15	**-0.80**	**-0.54**
Amphipod survival	-0.48	0.74	-0.44
Copepod Fecundity	**-0.58**	**0.65**	0.05
Eigenvalue	6.93	2.91	1.65
Total of variance (%)	53.3	22.4	12.7
Cumulative (%)	53.3	75.7	88.4

Bold indicates significant correlation.

Table 5 Principal component analysis (PCA) based on chemical contamination and effects on amphipod survival and copepod fecundity exposed to sediments of Santos-São Vicente Estuarine collected in the winter.

Variables	PC 1	PC 2	PC 3
As	-0.09	**-0.78**	0.18
Cr	0.36	**-0.72**	0.49
Cu	0.46	-0.61	0.59
Hg	-0.10	**-0.50**	-0.11
Ni	**0.42**	**-0.79**	0.40
Pb	0.24	0.35	**0.89**
Zn	0.41	**-0.70**	0.55
Benz[a]anthracene	**0.97**	0.07	-0.20
Benzo[a]pyrene	**0.98**	0.06	-0.18
Chrysene	**0.97**	0.07	-0.18
Dibenz[a,h]anthracene	**0.98**	0.11	-0.10
Acenaphthene	0.71	**0.54**	0.36
Acenaphthylene	**0.94**	0.31	0.01
Anthracene	**0.97**	0.20	-0.07
Phenanthrene	**0.98**	0.17	-0.01
Fluoranthene	**0.96**	0.09	-0.21
Fluorene	0.66	0.55	0.44
Naphthalene	**0.56**	-0.05	**0.68**
Pyrene	**0.97**	0.07	-0.20
Benzo[b]fluoranthene	**0.98**	0.07	-0.18
Indeno[1,2,3-cd]pyrene	**0.98**	0.07	-0.16
Benzo[ghi]perylene	0.98	0.06	-0.16
PCBs	**0.48**	-0.26	0.27
TOC	0.26	**-0.86**	-0.01
CaCO$_3$	0.17	**-0.68**	-0.23
Sand	0.13	**0.64**	-0.25
Silt	**0.66**	-0.39	**-0.42**
Clay	-0.39	**-0.67**	**-0.52**
Amphipod survival	**-0.63**	**0.59**	0.37
Copepod Fecundity	**-0.41**	**0.79**	0.33
Eigenvalue	14.9	7.0	3.8
Total of variance (%)	49.6	23.5	12.8
Cumulative (%)	49.6	73.1	85.9

Bold indicates significant correlation.

sampling site) (Cesar *et al.*, 2006; Buruaem *et al.*, 2013). It should be noted that dredging activities in the Santos harbour may be a sporadic source of toxicity and contamination to Diana River. The dredging along the navigation channel promotes an overflow plume composed by a mixture of fine particles and the contaminants associated, that may reach the surrounding areas, including this sampling site.

The sites located in the eastern portion of the SSES (Piaçabuçu, Mariana and Santana Rivers) were classified as moderately degraded. The toxicity of the sediments collected in these sites are probably related to diffuse sources of contamination (i.e., domestic sewage, urban drainage and waste disposal landfills), which may be contributing to inputs of PCBs, As, Hg, Ni and Pb, together with other not measured contaminants. Sewage may receive contribution from rain water and urban drainage waters containing high concentrations of PCBs, metals, as well as ammonia and detergents, which increasing their toxic potential (Bay *et al.*, 1996; Abessa *et al.*, 2014a). Although the latter has been considered a confounding factor in ecotoxicological studies (Ankley *et al.*, 1992; Ward *et al.*, 2011), since it can occur naturally, ammonia can also interact with other contaminants and have additive or synergistic effects that considerably increase toxicity (Ankley *et al.*, 1990). It was shown that in the presence of ammonia, sediments presenting moderate levels of contamination become toxic (Araújo *et al.*, 2013; Camargo *et al.*, 2015). Therefore, even when contamination levels are low to moderate, due to the presence of ammonia and detergents, they are still harmful to biota in many instances (Phillips *et al.*, 1997; Abessa *et al.*, 2014a). However, ammonia levels

were not measured in the present study, which could explain some correlations between the low levels of some metals and the biological effects.

The Piaçabuçu River receives domestic sewage *in natura* and the river basin drains areas affected by former industrial landfills, which are contaminated by a variety of chemicals (Cesar *et al.*, 2006). Furthermore, there was a dumping ground (deactivated in 2003) close to the right bank of this river. The Mariana River receives treated domestic sewage, but the most
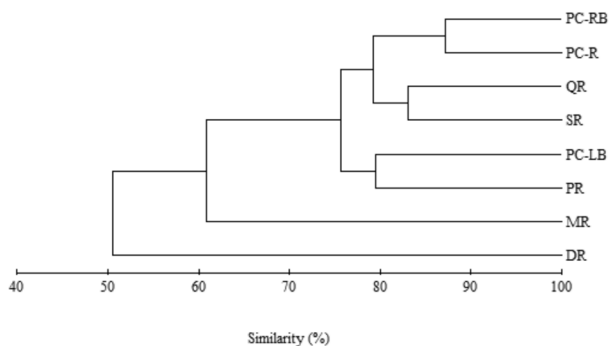
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Figure 4. Cluster analyses showing the groups of Santos-São Vicente Estuarine System tributaries according to the quality of sediment in the summer.
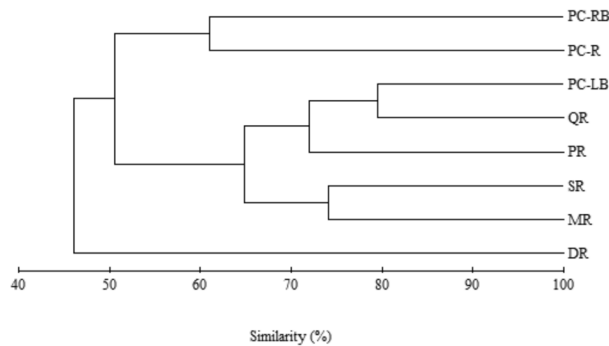

Figure 5. Cluster analyses showing the groups of Santos-São Vicente Estuarine System tributaries according to the quality of sediment in the winter.

important source of contamination to this site seems to be an illegal industrial landfill located close to this site (deactivated in 1993). The main contamination source to the Santana River possibly is a factory which processes metallurgical wastes (slag). In addition, the Branco River, a tributary of the Santana River, also presents an illegal industrial landfill (deactivated in 1993).

The most degraded sediments ware collected in the northern portion of the SSES (PC-RB, PC-R, PC-LB and Quilombo River). According to Lamparelli *et al*. (2001), this part of the estuary is affected by the Cubatão River, which receives most of the effluents from the Cubatão industrial complex (including a petroleum refinery plant and several fertilizer and chemical plants); this area is under influence of a major steel plant (considered the main source of PAHs and some metals to the SSES, according to CETESB) and a fertilizer plant. These results suggest that such levels were due to industrial discharges, since this sampling site is situated in the vicinity of privative terminals of fertilizers and a major steel industry, which are potentially polluting agents. As reported by Luiz-Silva & Machado (2012), anomalous concentration of iron (i. e. above local background) in sediments from Morrão River (tributary of the Quilombo River) was attributed to the inflow from the steel plant; accordingly, contents also anomalous of Cr, Pb, and Zn showed positive correlations with those of Fe and were interpreted as an indication of a common source for these metals.

Sediments from Piaçaguera Canal (PC-RB, PC-R and PC-LB) showed the highest values of PCB, Cr, Cu, Ni and Zn in the winter. Moreover, this region presented the highest levels Hg and PAHs, corroborating the results obtained by other studies carried out at Piaçaguera Canal (Abessa *et al*., 2008; Cesar *et al*., 2006; Torres *et al*., 2015; Buruaem *et al*., 2013). The Piaçaguera Canal was created from the dredging and rectification of the Mogi and Piaçaguera Rivers, in order to enable the installation of port terminals. Since the project was concluded in 1965, it requires periodic dredging to maintain adequate depths for ship navigation. For the disposal of the dredged sediments, a dam was built at right bank of the canal, occupying more than 1.5 million square meters, this dam receives not only dredged material but mainly the residues from the steel plant (Lamparelli *et al*., 2001). Indeed, the sediments of the PC-R (navigation channel of this industry) presented some PAH compounds above the TEL values, but

these levels are lower than those found in PC-RB sediments (with some compounds above the PEL). This difference probably was due to the dredging itself, which removes more contaminated material from the navigation channel.

CONCLUSION

The sediments of the Santos-São Vicente Estuarine System tributaries present a certain degree of degradation, especially those from the northern portion of the estuary (Quilombo River and the three sampling sites situated at Piaçaguera Canal). The evaluation of the metals concentrations revealed that all the sediments from the SSES are contaminated with Hg and Pb, and some sites were somewhat contaminated with As, Cu, Ni and Zn, which showed strong relationship with biological effects, together with PAHs. Sources of contamination seem to differ from site to site across the estuary, indicating local sources tend to be more important to the toxicities, with exception of the Hg, which is spread in the system. This study also shows that degradation is not restricted to the main estuarine channels, evidencing both sediment contamination and toxicity in some tributaries of the SSES. Our results also show that the contamination management policies failed to avoid environmental degradation of the SSES and that more effective policies are necessary.

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