

## Integrated quality assessment of sediments from harbour areas in Santos–São Vicente Estuarine System, Southern Brazil



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### ABSTRACT

Santos–São Vicente Estuarine System is a highly populated coastal zone in Brazil and where it is located the major port of Latin America. Historically, port activities, industrial and domestic effluents discharges have constituted the main sources of contaminants to estuarine system. This study aimed to assess the recent status of sediment quality from 5 zones of Port of Santos by applying a lines-of-evidence approach through integrating results of: (1) acute toxicity of whole sediment and chronic toxicity of liquid phases; (2) grain size, organic matter, organic carbon, nitrogen, phosphorus, trace metals, polycyclic aromatic hydrocarbons, linear alkylbenzenes and butyltins; (3) benthic community descriptors. Results revealed a gradient of increasing contamination for metals and organic compounds, alongside with their geochemical carriers. Sediment liquid phases were more toxic compared to whole sediment. Low number of species and individuals indicated the impoverishment of benthic community. The use of site-specific sediment quality guidelines was more appropriate to predict sediment toxicity. The integration of results through Sediment Quality Triad approach and principal component analysis allowed observing the effects of natural stressors and dredging on sediment quality and benthic distribution. Even with recent governmental efforts to control, pollution is still relevant in Port of Santos and a threat to local ecosystems.

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### 1. Introduction

Human occupation in coastal zones is a cause of several environmental impacts, such as discharge of domestic and industrial effluents into water bodies. This problem is of special concern to developing countries, where population increasing and coastal occupation are combined with industrial activities, representing a challenge for any sustainable development and environmental

management. In several countries, port activities are rapidly developing, demanding more attention, especially in relation to dredging and management of contaminated sediments (Choueri et al., 2009; Torres et al., 2009).

In Brazil, the Santos–São Vicente Estuarine System (SES) comprises a major industrial complex (at Cubatão municipality), as well as the largest port of Latin America (Porto of Santos). Currently, both harbour and industrial activities combined with urban contribution represent the main sources of contaminants to SES (Lamparelli et al., 2001; Abessa et al., 2008). Due to its economic and ecological importance, and environmental pollution, SES has been intensely studied. High concentrations of nutrients and metals (Braga et al., 2000; Hortellani et al., 2008), polychlorinated biphenyls (PCB), aliphatic and aromatic hydrocarbons (PAH) (Bicego et al., 2006;

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Martins et al., 2011), linear alkylbenzenes (LAB) (Martins et al., 2010) and butyltin compounds (Godoi et al., 2003) have been reported in the sediment. In addition, bioaccumulation of several classes of contaminants by estuarine organisms (mussels, oysters, shrimps, crabs and fishes) was also observed (Lamparelli et al., 2001; Torres et al., 2012), whilst ecotoxicological studies evidenced biological responses due to multiple sources of contamination (Cesar et al., 2007; Abessa et al., 2008; Torres et al., 2009). This highlights an ecological risk to the associated biota, with economic implications to dredging operations and management of contaminated sites.

The integrated employment of different lines of evidence has been recommended in the sediment quality assessment (Riba et al., 2004; Chapman and Hollert, 2006; Chapman, 2007), as they provide more reliable information than the use of single techniques. Among those, the Sediment Quality Triad – SQT (Long and Chapman, 1985; Chapman, 1990) comprises an integrated evaluation of benthic community structure, and sediment toxicity and chemistry, providing a better assessment of pollution-induced degradation than the integration of only chemistry and toxicity data (McPherson et al., 2008).

The quality of sediments from SES through SQT was previously investigated by Abessa et al. (2008), who produced a broad diagnostic of the entire system using data collected in 1998, and by Choueri et al. (2009), who generated site-specific guidelines (SQV) for SES sediments from data collected on 1998 and 2005 (Cesar et al., 2007) using similar approaches. Currently, the Port of Santos is expanding, driven by Brazilian economy and the increasing international trade. As consequence dredging has been required to increase or maintain the depth of navigation channel and the remaining areas of mangroves in SES are being substituted by new port facilities. On the other hand, governmental policies for pollution control have been implemented at SES since mid-1980s, when a broad program to restore the environmental quality of Cubatão industrial area was adopted, comprising actions to control 320 contamination sources, from 25 industries (Lamparelli et al., 2001).

More recently, marine and estuarine protected areas were created in the SES surroundings (São Paulo, 2008) and the regional coastal management plan is being implemented to harmonize economic development and environmental conservation. However, despite efforts to control inputs of contaminants, there are still evidences that human pressure continue to be harmful to local biota and that contamination levels are not decreasing (Cesar et al., 2007; Abessa et al., 2008). In this context, the present investigation aimed to assess the quality of sediments of harbour areas in SES using the classical Sediment Quality Triad. The time-trend of sediment quality and the applicability of site-specific guidelines were also evaluated.

## 2. Materials and methods

In sediment quality evaluations, the use of chemical criteria by comparing chemical measures with sediment quality guidelines has been considered the traditional approach to determinations of contamination levels. However, such comparisons are simplistic considering the complexities of ecosystems and thus an integrated assessment is more appropriate (Burton, 2002). According to Chapman (2007), contamination is the presence of a substance where it should not be or at concentrations above background levels while pollution is the contamination that results in or can result in adverse biological effects to resident communities and therefore, determining when contamination has resulted in pollution requires not only chemical but also biological measurements.

In this study, three lines of evidence were combined: (1) chemical analyses to quantify the compounds present in the environment and the levels of contamination in Port of Santos samples;

(2) sediment toxicity tests to determine whether the interactive toxic effects of complex chemical mixtures in contaminated sediments is potentially harmful to benthic organisms and (3) measures of *in situ* benthic community, which are useful to indicate stress and impacts over the time, taking into account the effects over a species that occupy different niches and responds differently to chemical contamination. The integrative analysis of these components was performed to obtain a realistic estimation of the sediment quality, reducing the uncertainties of single use of each line of evidence.

### 2.1. Study area

SES is located in Southeastern Brazil (23°30′–24°00′S and 46°05′–46°30′W), where climate is classified as a hot humid tropical, and the regime of winds is dominated by the situation of doldrums for more than half of the year (Siqueira et al., 2006). The topography and continental inputs of materials are among the main factors that control the sediment characteristics (Lacerda and Marins, 2005) while, sediment deposition results from a combination of continental and marine hydrodynamics. Continental influence is given by drainage and erosion of wide net of rivers, and may be characterized as a terrigenous facies. On the other hand, marine input is controlled by tidal currents and continental shelf erosion; sediments are transported along the coast and reworked (Fukumoto et al., 2006). Sediments are reworked and transported towards the coast, ranging gradually from silt to sand (Fukumoto et al., 2006). The presence of mangroves has a sedimentary retainer function and contributes to the load of organic matter and fine sediments.

### 2.2. Sediment sampling and handling

The sediment sampling was carried out in November 2007, and comprised five sampling stations in order to observe the estuarine gradient: S1 was positioned at the mouth of navigation channel in the Port of Santos; S2 was located in front of the Containers terminal; S3 was placed at Diana island; S4 was situated in front of Alemoa terminal (petrochemical), and S5 was positioned at Piaçaguera channel, in the vicinity of private terminals of fertilizers and a major steel industry (Fig. 1). Samples were collected at depositional sand or mud banks (except for S1, due to its location in the bay) using a van Veen grab and, during the sampling, the depth of each station was recorded. Dredging occurs all along the centre of the channel (i.e. navigational channel) and in front of terminals and wharfs, but in the channel there are still many sand and mud banks which are not dredged. Thus the sampling was designed to avoid the direct effects of dredging on the results (e.g., removal of sediments and benthic organisms), and stations S5 to S1 were positioned in not dredged sites. However, the constant dredging along the channel promotes the creation of an overflow plume basically composed by a mixture of dredged material (mainly fine particles and the contaminants associated) and water, that eventually may reach the surrounding areas, including the sampling sites. In this sense, sampling stations should be representative of the instantaneous environmental quality in each sector of SES. Following to collection, samples were separated in aliquots.

Sediments for toxicity tests were kept in coolers with ice until the laboratory, where they were stored at 4 °C in the dark for no longer than 10 days until the analyses. For benthic community analysis, three replicates were collected using a van Veen grab sampler (0.026 m<sup>2</sup>) for each station and sediment was carefully sieved through a 0.5 mm mesh and the retained material was fixed with 4% buffered formalin, and then preserved in 70% ethanol. In laboratory, biological material was sorted, identified using guides for identification and quantified under a stereoscopic microscope.

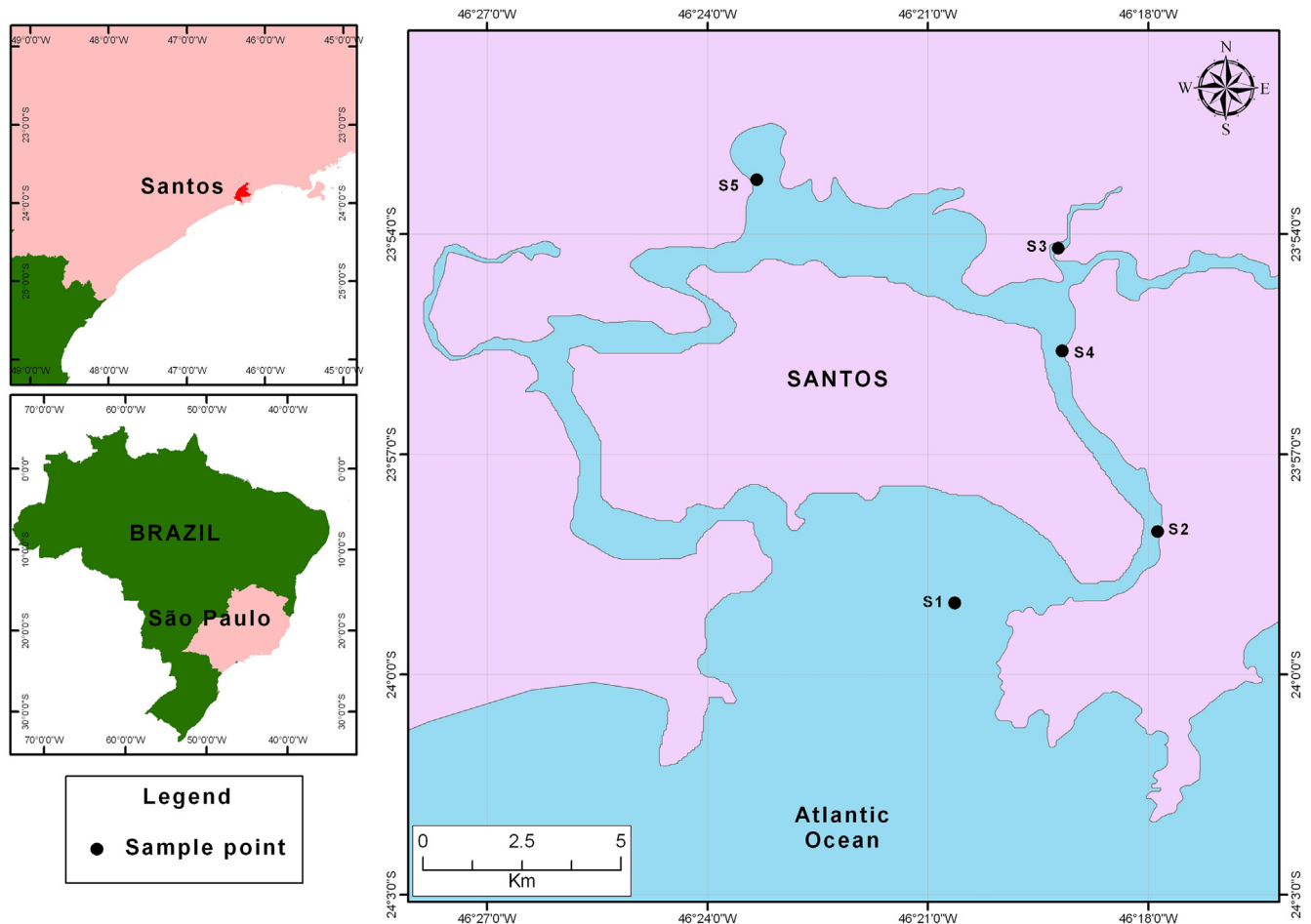


Fig.1. Stations of sediment sampling in Santos-São Vicente Estuarine System.

One aliquot was separated, dried at 40 °C and packed in plastic containers for physical and chemical analysis (grain size, organic matter, carbonate, nutrients and metals) and another one was placed into pre-cleaned aluminium foils and stored at –20 °C for chemical analysis (organic compounds).

### 2.3. Sediment properties and chemical analyses

Particle size distribution was measured by the wet sieving (0.063 mm mesh) method for total mud (silt + clay) separation followed by dry sieving to separate gravel (>2 mm) and sand (>0.062–2 mm) fractions according to Wentworth scale (Wentworth, 1992). Estimation of carbonate contents in sediments (CaCO<sub>3</sub>) was conducted following digestion in HCl and gravimetry method (Gross, 1971) while organic matter content (OM) was determined by adapting the method of loss by ignition in a muffle and gravimetry (Luczak et al., 1997). Total organic carbon (TOC) was determined by oxidation and titration according to Strickland and Parsons (1972). Total nitrogen (N) and total phosphorus (P) were estimated by extraction and oxidation in persulfate followed by spectrophotometers measures (Grasshoff et al., 1983). Values determined in all methods are expressed in %.

Metals (Al, Fe, Hg, Cd, Cr, Cu, Ni, Pb and Zn) were analyzed according to the EPA 3051A protocol (USEPA, 1996). Sediments were digested with an acid solution containing 9 mL of HNO<sub>3</sub> and 3 mL of HCl, in high pressure microwave system (CEM Corporation, model MDS–2000). Extracts were measured using the flame mode of a

Fast-Sequential Atomic Absorption Spectroscopy Varian, model Spectr-AAS-220-FS, with a deuterium lamp background correction for Pb, Ni and Hg. Levels of Hg were measured by cold vapour generation through coupling the spectrometer to a typical FIA (Flow Analysis Injection) manifold with a manual injection valve that injects 500 µL of sample at a flow of Milli-Q water (10 mL min<sup>-1</sup>). The validation of this method was performed by analysis of two Standard Reference Materials (SRM 2704 – Buffalo River Sediment and SRM-1646a – Estuarine sediments), in triplicates and a detailed interpretation of the Quality Assurance and Quality Control (QA/QC) is discussed by Buruaem et al. (2012).

For the Polycyclic aromatic hydrocarbons (PAH) and Linear alkylbenzenes (LAB), freeze-dried samples were homogenized in a mortar with pestle. An amount of 20 g was Soxhlet-extracted with a 50% mixture of residue grade n-hexane and dichloromethane for 8 h (UNEP, 1992). Then, extracts were fractionated into F<sub>1</sub> (LAB) and F<sub>2</sub> (PAH) by silica gel-alumina column chromatography and quantitatively analysed by an Agilent 6890 gas chromatograph coupled to a 5973N mass spectrometer (GC/MS) in a selected ion mode (SIM). Certified standards from AccuStandard, USA, at five concentrations were used to build analytical curve and blanks. Reference material from National Institute of Standards and Technology – NIST (SRM 1944) was used as surrogates. PAH and LAB identification was based on GC retention times of certified standards and individual quantitation ion (*m/z*).

QA/QC procedures were based on the analysis of blank, blank spike, matrix spike, matrix duplicate and standard reference

material that were processed with the samples. The mean recoveries for surrogates and target compounds were within 65 and 102%. The precision ranged from 1.0 to 17% for LAB and 1.6 and 19% for PAH. Method detection limit (MDL) was based on the standard deviation ( $3 \times \sigma$ ) of seven replicates of a sediment sample containing target compounds at a level of one to five times the expected MDL. All solvents were for residue analysis grade and the blanks were checked under the same conditions as those of the analyses. All concentrations were reported as  $\text{ng g}^{-1}$  (dry weight).

Butyltins (TBT, DBT and MBT) compounds were analyzed according to Castro et al. (2012). Briefly, 5 g of dry sediments were placed into 40 mL vials, spiked with 100 ng of tripropyltin as surrogate standard. Afterwards, organotins were extracted with 0.05% tropolone solution (w/v) and concentrated HCl (37%) in ultrasonic bath. After derivatization, the pentylated butyltins were recovered by a liquid–liquid extraction with hexane. Those extracts were evaporated and eluted with hexane/toluene solution (1:1) and then, concentrated under gently nitrogen flow. Extracts were analyzed in a Perkin Elmer Clarus 500MS (GC/MS).

Tetrabutyltin solution was added as internal standard and the QA/QC were based on regular analyses of blanks, spiked matrices and certified reference material (PACS-2/National Research Council of Canada, Ottawa, Canada). Results obtained for the PACS-2 were in good agreement with the certified values. The samples recoveries ranged between 88.5% and 109% and the relative standard deviations were below 20%. Additionally, the analytical curves were prepared using standard addition in order to avoid matrix effects during analyses. The quantification limits were 2.5, 3.0 and 3.0  $\text{ng Sn g}^{-1}$  for TBT, DBT and MBT respectively. All concentrations were reported as  $\text{ng Sn g}^{-1}$  (dry weight).

#### 2.4. Sediment toxicity

The toxicity of sediments samples from the Port of Santos was assessed by analyzing the mortality of amphipod *Tiburonella viscana* (Barnard, 1964) and embryo-larval development of sea urchin *Lytechinus variegatus* (Lamarck, 1816). For better characterization of toxicity, four types of exposure were employed: Whole sediment, pore water, sediment–water interface and sediment elutriate.

- Whole sediment (WS) toxicity test followed the protocol ABNT NBR 15638 described in ABNT (2008). Mortality of amphipods *Tiburonella viscana* was used to assess the acute effects related to whole sediment. The bioassay was conducted in polyethylene chambers, in triplicate; the sediment samples were placed into the test chambers in aliquots of about 175 mL, as well as 750 mL of filtered seawater. After an equilibrium period, the test was carried out by exposing the amphipods (10 organisms per replicate) to the samples for 10 days and estimating lethal effects at the end of exposure time. At the end of the test, contents of each test chambers were sieved and the number of organisms alive was recorded.
- Pore water (PW) was extracted by suction method (Winger and Lasier, 1991) and tested for waterborne toxicity using sea urchin *Lytechinus variegatus* embryo-larval development according to ABNT NBR 15350 protocol (ABNT, 2006) at 100, 50 and 25% of dilution, in 4 replicates per sample. To achieve that, sea urchin spawning was induced and subsequent *in vitro* fertilization was made. The test was conducted by introducing approximately 400 embryos in each of four replicates. After the end of the test (24 h), embryos were analyzed microscopically for morphological anomalies and development delays. The dilutions were used to help the interpretation of the data since unionized ammonia can contribute to the toxicity of pore water samples (Chapman et al., 2002; Losso et al., 2007).

- Sediment–Water Interface test (SWI) was conducted following the method described by Cesar et al. (2004) for small volumes. This treatment assesses the effects of contamination which arising from sediment may affect organisms from the adjacent water column. In this procedure the test system was set up in test tubes (4 replicates per sample), containing sediment and water 1:4 (v:v). Afterwards, sea urchin embryos were exposed following the protocol described above for PW.
- Elutriates treatment (ELU) was set according to USEPA (2003). Homogenized sediments were mixed with dilution seawater 1:4 (v:v) in a mechanic shaker during 30 min. After sedimentation, the supernatant was used to test the toxicity using the *Lytechinus variegatus* embryo-larval development test in 4 replicates per sample.

Negative controls were prepared for all the treatments by using filtered and uncontaminated seawater in liquid phase tests and sediments from amphipods collection site (Engenho D'água Beach, Ilha Bela, North coast of São Paulo) in the whole sediment test. This location was chosen due to lack of reference site for the SES in addition to the low levels of contamination reported by (Abessa et al., 2008). Salinity (35), temperature ( $25 \pm 2$  °C) and dissolved oxygen ( $>5$  mg/L) were controlled during the tests. For liquid phases, total ammonia concentration was measured by phenate Method 4500-NH<sub>3</sub>C (APHA, 1999) and the un-ionized ammonia contents were estimated using the model proposed by Whitfield (1974). Student's *t*-test was used to compare responses for each sample and their respective controls: 10% of amphipod mortality for whole sediment tests and 4% of abnormal larvae for liquid phase tests. False positive and negative results may occur by using such statistical tool and for liquid phase tests, natural effects can contribute to it (e.g. % of embryos which do not develop naturally). Thus, in order to consider such natural effects on embryos development along results interpretation, the bioequivalence hypothesis test was used for the liquid phase treatments using the constant of 0.91 (Bertoletti et al., 2007). Samples statistically different from the control were considered toxic. From dilutions of pore water, the linear interpolation method (Norberg-King, 1993) was used to calculate the inhibitory concentration to 10% of embryos (IC<sub>10</sub>) and then, results were transformed into toxic units (TU = 100/IC<sub>10</sub>).

#### 2.5. Benthic community descriptors

For analyses and interpretation of community descriptors (ecological indices), all measures were expressed as function of mean value for three replicates per site. Number of species, individuals and richness (Margalef index) were estimated and expressed in numbers per 0.026 m<sup>2</sup>. Density of major taxonomic groups was determined and expressed in % for Mollusca, Crustacea, Polychaeta, and Nematoda.

#### 2.6. Comparison with sediment quality guidelines

The results of chemical analyses were compared with threshold (Level 1) and probable effects levels (Level 2) of sediment quality guidelines (SQG) recommended by the Brazilian Federal legislation for dredged sediments (BRASIL, 2004) and with threshold and probable effects from site-specific values (SQV) derived for Santos Estuarine System (Choueri et al., 2009). The guidelines adopted in Brazilian legislation is a reproduction of empirically based range of values by combining the effects range-low and effects range-median (ERL/ERM) with threshold effect level and probable effects level (TEL/PEL), both derived from databases of contaminants concentrations and their



correspondence with observed biological effects (FDEP, 1994; Long et al., 1995; CCME, 2002). On the other hand, the site-specific values were derived from sediment physical–chemical, toxicological, and benthic community data integrated through multivariate analysis (Factor Analysis, with the application of Principal Component Analysis). Anyway, comparisons with both sets of values aimed to provide interpretation of chemical data in global (general) and local (specific) perspectives.

The comparisons involved the use of Sediment Quality Guidelines Quotients (SQGq) approach (Fairey et al., 2001). Such method consists in calculating the quotients of each chemical by dividing the concentrations by their respective probable effect level (both for SQG and SQV). Although other compounds were analyzed, the quotients were calculated only for metals and PAH, due to lack of guidelines and reference values of LAB and TBT for the Brazilian coast. According to the mentioned authors, from the computation of quotients, samples were ranked by the following criterion of contamination:

- Minimal contamination. Uncontaminated sediments: SQGq and SQVq value between 0 and 0.1;
- Moderate contamination. Contamination levels may produce occasional toxicity: SQGq and SQVq value between 0.1 and 0.25;
- Strong contamination. Contamination levels probably will cause negative effects to the biota: SQGq and SQVq value greater than 0.25.

### 2.7. Integrated approach: ratio to mean values and multivariate analysis

This first employed integrative approach is an adaptation proposed by Cesar et al. (2009) of the Ratio-to-Reference (Long and Chapman, 1985) and Ratio-to-Maximum Values methods (DeValls and Chapman, 1998). From the data matrix, values obtained for each variable in all lines of evidence were converted to non-dimensional values by dividing the value obtained by the arithmetic mean obtained for all stations. Then, these values were normalized, by the calculation of a mean, producing the RTM index (Ratio-to-Mean) for chemistry, toxicity tests and benthic descriptors. After, values were plotted in 3-axis graphics forming triangles and the calculated area of each triangle ( $\text{area} = 1/2 * \text{RTM1} * \text{RTM2} * \text{sen}120^\circ$ ) represented the site specific RTM, related to the degree of degradation of sediment sample, which increase as the area of triangles increase. The RTM was employed due to the fact that practically all SES presents some degree of contamination, which makes difficult to establish a reference area. Also, according to Abessa et al. (2008) and Cesar et al. (2009), the use of RTR may produce large differences between the values of each lines of evidence, and thus by applying the mean values such differences are reduced. The second approach aimed to observe relationships among variables by using a principal component analysis (PCA).

## 3. Results

### 3.1. Sediment properties and chemical analysis, toxicity and benthic community descriptors

Results are summarized in Table 1. The higher amounts of mud were observed in S1 and S5, contrasting with the higher percentage of sand in S2–S4. Calcium carbonate contents were high in S1, S3 and S4, whereas, on the other hand, OM and TOC levels tended to be higher in S3–S5. Nitrogen concentrations exhibited small

**Table 1**

Sediment properties, chemical analysis, toxicity tests results and benthic community descriptors of samples from Port of Santos.

Variables	Sampling stations				
	S1	S2	S3	S4	S5
Depth (m)	15	14	2	3	4
Salinity	35	32	32	30	21
Sediment properties					
CaCO <sub>3</sub> (%)	10.96	7.99	10.16	12.46	7.54
Sand (%)	88.61	93.48	91.12	93.40	82.14
Mud (%)	11.37	6.17	8.51	6.60	17.41
OM (%)	9.91	5.35	12.22	13.88	11.41
Chemicals					
TOC (%)	1.75	1.31	3.88	2.88	2.05
N (%)	0.54	0.55	0.58	0.56	0.56
P (%)	0.35	0.77	0.63	0.73	1.17
Al (%)	1.62	1.99	3.01	2.89	3.78
Fe (%)	2.15	1.91	2.14	2.56	3.23
Hg (mg kg <sup>-1</sup> )	0.04	0.15	0.18	0.28	0.64
Cd (mg kg <sup>-1</sup> )	<0.60	<0.60	<0.60	<0.60	<0.60
Cr (mg kg <sup>-1</sup> )	28.17	26.31	29.82	34.26	42.74
Cu (mg kg <sup>-1</sup> )	9.01	15.04	12.02	17.56	27.28
Ni (mg kg <sup>-1</sup> )	10.88	11.42	11.10	15.18	22.28
Pb (mg kg <sup>-1</sup> )	9.09	15.6	7.57	16.7	12.76
Zn (mg kg <sup>-1</sup> )	509.08	621.24	108.93	917.43	1077.33
PAH (ng g <sup>-1</sup> )	193.02	501.61	748.37	1248.64	4803.62
LAB (ng g <sup>-1</sup> )	37.24	38.49	50.61	168.3	52.34
TBT (ng g <sup>-1</sup> )	26.62	13.72	38.08	28.84	159.2
DBT (ng g <sup>-1</sup> )	14.34	14.26	15.66	14.7	15.26
MBT (ng g <sup>-1</sup> )	10	9.98	22.48	16.1	14.98
Sediment toxicity (mean ± SD**)					
WS (% of amphipod mortality)	20 ± 10	7 ± 6	27 ± 6*	33 ± 15	23 ± 23
PW (UT)	12	4	5	7	10
NH <sub>3</sub> (mg/L) in PW 100%	0.26	0.48	1.03	0.44	0.46
SWI (% of abnormal larvae)	100*	100*	39 ± 7*	29 ± 2*	68 ± 16*
NH <sub>3</sub> (mg/L) in SWI	0.03	0.01	0.01	0.01	0.03
ELU (% of abnormal larvae)	98 ± 1*	93 ± 3*	31 ± 8*	38 ± 2*	7 ± 3
NH <sub>3</sub> (mg/L) in ELU	0.24	0.20	0.07	0.08	0.03
Benthic community descriptors/0.026 m <sup>2</sup>					
Number of species	14	7	2	5	5
Number of individuals	48	117	2	5	13
Richness	3.36	1.26	1.44	2.49	1.56
Mollusca (%)	15	0	0	33	7
Polychaeta (%)	83	100	50	33	93
Crustacea (%)	0	0	50	33	0
Nematoda (%)	2	0	0	0	0

CaCO<sub>3</sub> = calcium carbonates; OM = organic matter; TOC = total organic carbon; N = nitrogen; P = phosphorus; Al = aluminium; Fe = iron; Hg = mercury; Cd = cadmium; Cr = chromium; Cu = copper; Ni = nickel; Pb = lead; Zn = zinc; PAH = polycyclic aromatic hydrocarbons; LAB = linear alkylbenzenes; TBT = DBT = dibutyltin; MBT = monobutyltin; WS = whole sediment; PW = pore water; SWI = sediment–water interface; ELU = elutriates; NH<sub>3</sub> = un-ionized ammonia.

\* Toxic ( $p < 0.05$ ).

\*\* Standard deviation.

variation, however, phosphorus concentrations indicated a gradient, with increasing values towards from S1 to S5. Based on these results, including the salinity, we can distinguish the stations in: marine zone (S1), transition zone (S2–S4) and estuarine zone indeed (S5).

The trend in distribution of metals, phosphorus, PAHs and TBT suggests that concentrations rise towards the inner portions of the estuary. Comparing such values with the SQG, Hg (in S2–S5), Ni and PAH (both in S5) were above level 1, while Zn was above level 2 in all stations. But when they are compared with site-specific SQV, Hg, Cr, Ni (S2–S4), Pb (S2 and S5) and PAH (S1–S3) exceeded Threshold

Levels (relative to Level 1) while Cu, Zn (S1–S5), Hg and Ni (in S5) and PAH (S4 and S5) were found above Probable Levels (relative to Level 2). Cd levels were below the detection limit.

The whole sediment test showed low rates of amphipods mortality, with averages ranging between 7 and 33%. The highest mortality rates occurred in sediments from Diana Island and Ale-moa Terminal (S3 and S4, respectively) but only S3 presented significantly toxic sediments, compared to negative control which presented a mortality rate of  $12 \pm 4\%$ . In liquid phase tests, negative control presented a low % of abnormal larvae ( $4 \pm 2\%$ ). For pore water, all samples were toxic at 100% and, considering the toxic units results, S1 and S5 showed higher toxicity, followed by S4, S3 and S2, respectively. All samples were toxic in the sediment water interface, and, for elutriates, only S5 was not toxic. For pore water and elutriates, unionized ammonia levels ( $\text{NH}_3$ ) were estimated above 0.05 mg/L, the NOEC for *Lytechinus variegatus* embryonic development (Prósperi, 2002).

For the benthic community, 185 individuals were distributed in 25 taxa. Polychaeta was the most abundant group, followed by Crustacea and Mollusca. Nematoda occurred only in S1. Stations S1 and S2 showed a high number of individuals in contrast to internal stations, where few individuals were observed. The number of species (taxa) can be considered as a proxy of diversity and therefore, S1 presented a greater diversity compared to the other stations, with 14 taxa identified. Richness was higher in S1 and S4. The most abundant species, which presented the frequency of occurrence in greater than 50% of sampling stations were: *Glycinde multidentis* > *Timarete* sp > *Kinbergonuphis* cf. *tenuis* > *Ninoe brasiliensis* > *Sigambra grubii* > *Sthenolepis grubei* > *Eurypanopeus* sp.

3.2. Integrated approach: ratio to mean values and multivariate analysis

The graphs containing the RTM projections values are displayed in Fig. 2. In general, the biological variables (toxicity and

benthos) influenced the ranking of the stations, pushing up the values. On the other hand, RTM values calculated for chemical contamination reflected the estuarine gradient and proximity to the sources of contamination, decreasing towards the marine portion of port. The site specific RTM indices integrated revealed the following trend in distribution of stations by sediment quality:  $S1 < S2 < S4 < S5 < S3$ .

PCA results are presented in Table 2 and Figs. 3 and 4, where the first three axes explained 89.20% of variances. Positive correlations to Axis 1 ( $PC1 > 0$ ), which explained 46.57% of variance, represented sediments from deeper zones, with high content of sand, toxicity for elutriates and sediment–water interface and high number of species, individuals and richness, while negative correlations to this axis ( $PC1 < 0$ ) represents organic enrichment, toxicity for whole sediment, contamination for metals (except for Cd and Pb), PAH and butyltins. The Axis 2 accounted for 27.79% of variances, and positive correlations ( $PC2 > 0$ ) indicated phosphorus, metals (Fe, Hg, Cu and Ni), PAH and TBT contamination, associated with Polychaeta occurrence. Negative correlations ( $PC2 < 0$ ) were found for  $\text{CaCO}_3$ , sand, TOC and nitrogen, indicating the contamination by LAB and toxicity for whole sediment associated to such variables. At last, Axis 3 represents 14.84% of variance and positive correlations ( $PC3 > 0$ ) to it were associated to OM and  $\text{CaCO}_3$ , LAB, toxicity for whole sediment and pore water, besides the number of species and richness. Nitrogen and number of individuals were negatively correlated to this axis ( $PC3 < 0$ ).

The bi-dimensional ordination of two first axes (Fig. 3) separated the stations under marine influence, which presented low levels of contamination and toxicity for liquid phases tests (S1 and S2) from estuarine stations, where the levels of contamination were higher; and, in this case, S3 and S4 were separated (low number of individuals and species) from S5 (most contaminated). The joint plot of RTM values corroborated the PCA results, which confirmed these integrative index to observe trends among different degrees of degradation.

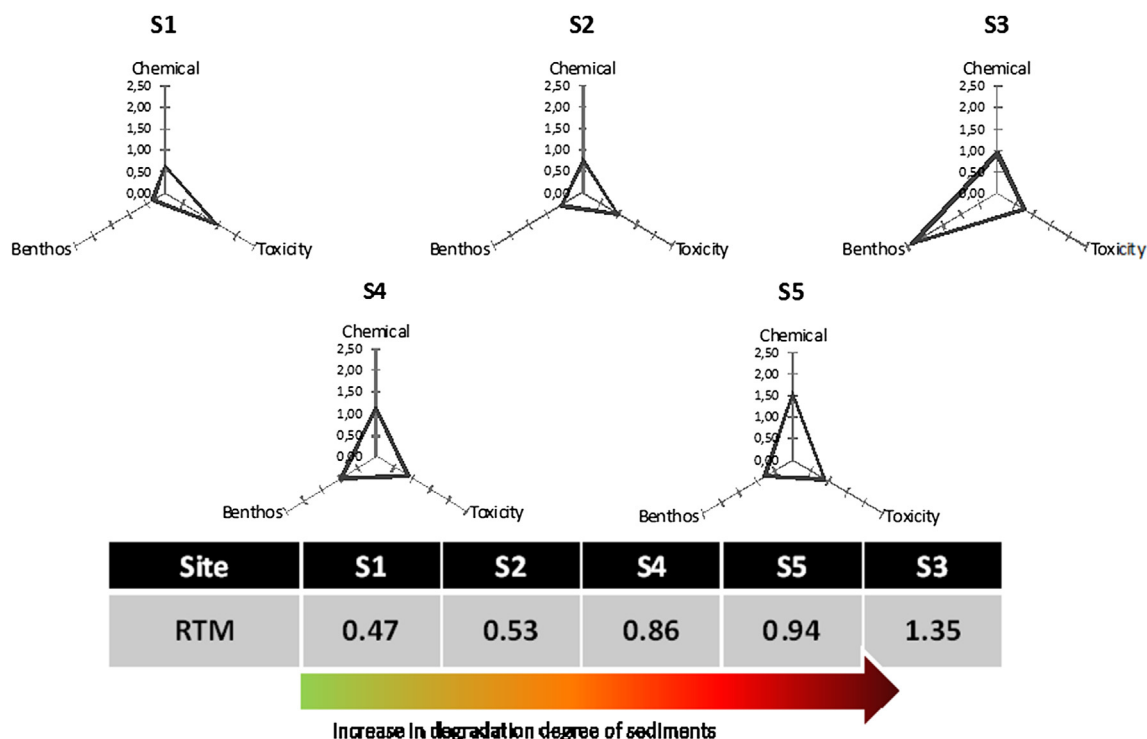


Fig. 2. Ratio-to-mean values estimated for sediment samples from Santos–São Vicente Estuarine System.

**Table 2**  
Principal component analysis results based on lines-of-evidence of SES sediments.

Variables	PC		
	1	2	3
Depth	<b>0.93</b>	0.36	0.04
CaCO <sub>3</sub>	0.07	<b>-0.76</b>	<b>0.64</b>
Sand	<b>0.45</b>	<b>-0.76</b>	-0.24
Mud	-0.44	<b>0.75</b>	0.28
OM	<b>-0.71</b>	-0.43	<b>0.55</b>
TOC	<b>-0.58</b>	<b>-0.74</b>	-0.04
N	<b>-0.71</b>	-0.49	-0.43
P	<b>-0.71</b>	<b>0.54</b>	-0.32
Al	<b>-0.98</b>	0.14	-0.11
Fe	<b>-0.79</b>	<b>0.50</b>	0.33
Hg	<b>-0.82</b>	<b>0.54</b>	-0.02
Cr	<b>-0.84</b>	0.46	0.26
Cu	<b>-0.73</b>	<b>0.59</b>	-0.07
Ni	<b>-0.75</b>	<b>0.60</b>	0.17
Pb	-0.06	0.13	-0.04
Zn	<b>-0.97</b>	0.15	-0.01
PAH	<b>-0.76</b>	<b>0.65</b>	0.04
LAB	-0.39	<b>-0.50</b>	<b>0.41</b>
TBT	<b>-0.71</b>	<b>0.69</b>	0.07
DBT	<b>-0.80</b>	-0.20	-0.23
MBT	<b>-0.71</b>	<b>-0.57</b>	-0.21
WS	<b>-0.67</b>	<b>-0.50</b>	<b>0.54</b>
PW	0.03	<b>0.47</b>	<b>0.82</b>
ELU	<b>1.00</b>	-0.01	0.03
SWI	<b>0.76</b>	<b>0.64</b>	-0.09
Species	<b>0.76</b>	0.33	<b>0.56</b>
Individuals	<b>0.78</b>	0.33	-0.45
Richness	<b>0.62</b>	0.24	<b>0.75</b>
Mollusca	-0.18	-0.33	<b>0.74</b>
Polychaeta	0.40	<b>0.83</b>	-0.34
Crustacea	-0.44	<b>-0.89</b>	-0.06
Nematoda	<b>0.64</b>	0.13	<b>0.64</b>
Eigenvalue	14.90	8.89	4.75
Variance (%)	46.57	27.79	14.84
Total of variance (%)	46.57	74.36	89.20

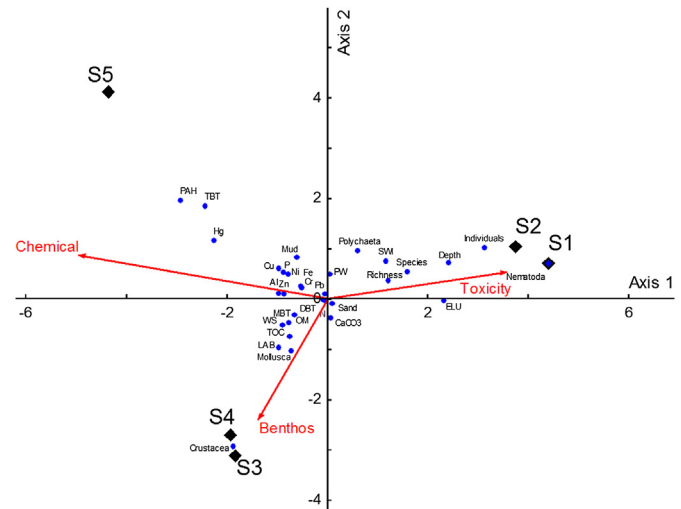
Bold indicates significant correlation.

## 4. Discussion

### 4.1. Time-trend of sediment quality: chemical contamination, toxicity and benthic community descriptors

Sediments from SES showed a predominance of sand in all sites, with increasing mud and organic matter contents towards inner estuary, where the presence of mangroves aids in retaining sediments (Fukumoto et al., 2006). Sediment transport in the medium estuary is influenced by tidal currents and by the interaction between fluvial and marine flows (Tessler et al., 2006) which resulting, thus, in a decrease of sediment transport and explains the OM contents in the inner estuary (S3–S5). Tessler et al. (2006) analyzed sediment cores SES by gamma spectrometry (<sup>210</sup>Pb and <sup>137</sup>Cs) and obtained a sedimentation rate of 4.0–5.6 mm y<sup>-1</sup> from the inner estuary, compared to 0.91 mm y<sup>-1</sup> from Santos Bay.

Considering the sampling site locations, it is also possible to observe the effect of the sedimentation described above in the distribution of nutrients and contaminants since the main industrial contamination sources to SES are located in this area (Lamparelli et al., 2001). Except for nitrogen and Cd (below the LD), metals and phosphorus were distributed along gradients, with increasing levels towards the internal portions of the estuary. Furthermore, for organic contaminants, the same gradient was observed to PAH and TBT, while, for LAB, the highest value was found in S4. This distribution pattern was also observed by other



**Fig. 3.** Ordination results of the principal component analysis based on lines-of-evidence data.

authors for TBT (Godoi et al., 2003), PAH (Bícego et al., 2006), metals (Hortellani et al., 2008) and LAB (Martins et al., 2010).

Regarding toxicity, in the whole sediment exposure only S3 was toxic, not repeating the results obtained in previous studies which reported high amphipod mortality (Cesar et al., 2007; Sousa et al., 2007; Abessa et al., 2008). More recently, Torres et al. (2009) observed the absence of whole sediment toxicity in the inner estuary related to dredging operations, although high levels of potentially bioavailable substances were found (metals, PAH and PCB).

All samples were toxic for pore water; in this situation, test-organism (from new fertilized eggs to pluteus embryos) are exposed to dissolved contaminants that can be absorbed by diffusion through their entire body surface, which may include confounding factors, such as unionized ammonia, that can contribute to the toxicity (Chapman et al., 2002). For SES, Abessa et al. (2008) observed toxicity in pore water samples even in low levels of ammonia, showing that the effects were due, possibly, to other contaminants. Regardless, in polluted environments, high concentrations of ammonia in sediments can be due to human activities and, thus, ammonia may be treated as a pollutant (Losso et al., 2007). Lamparelli et al. (2001) presented an inventory of contamination sources, including those related to nitrogen and ammonia, indicating several major industries, landfills, point and diffuse sewage inputs, ballast waters and atmospheric deposition as possible contributors of ammonia to SES.

The pattern of toxicity observed for sediment–water interface and elutriates suggest that SES may transfer contaminants to the water column through diffusion and re-suspension. Benthos distribution tended to follow the estuarine gradients already observed for SES, exemplified as particle size distribution, nutrients contents and salinity, which corroborates the condition of natural physiological stress of estuarine environments (Dauvin and Ruellet, 2009).

Abessa et al. (2008) and São Paulo (2010) also evidenced such gradient, with gradual decrease in richness, density and diversity towards the inner portions of the estuary, confirming, thus, the heterogeneity of SES. In these studies, benthic assemblages were predominantly composed by opportunistic polychaetes and mollusks, as seen in this investigation. In addition, the influences of different anthropogenic pressures (e.g., multiple contamination sources) increase the complexity of the system. Moreover, during

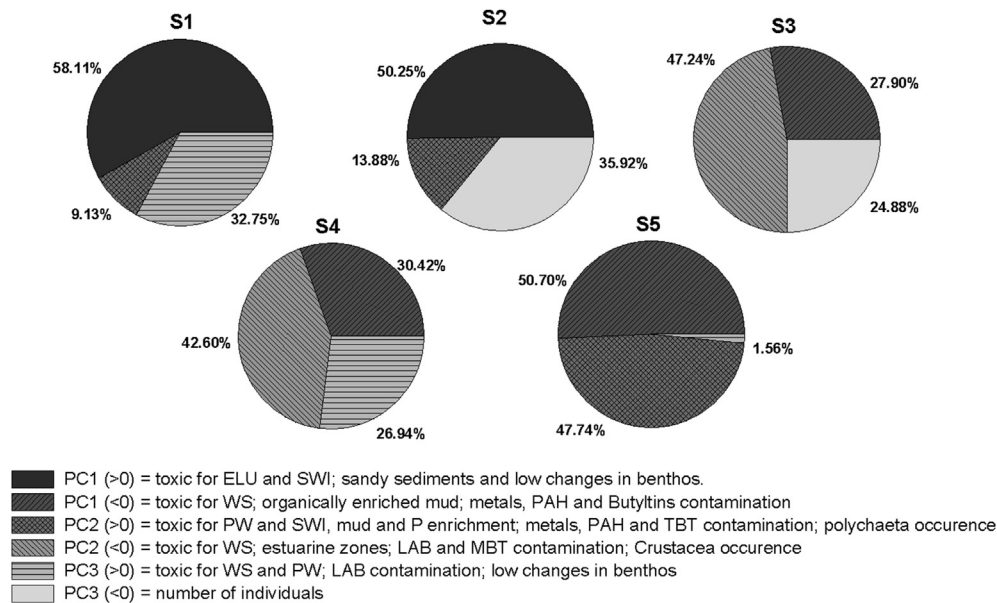


Fig. 4. Contribution in % of each axis to the total variance of the principal component analysis results for sediment samples of Santos-São Vicente Estuarine System.

the study, the harbour channel was being dredged, so it is possible that the low number of individuals in these areas can be attributed to the combined influence of natural factors, contamination and dredging activities, especially by the overflow plume.

#### 4.2. Comparison of chemicals data with sediment quality guidelines

Chemistry data were compared to federal SQG (BRASIL, 2004) and local SQV (Choueri et al., 2009), and such comparison showed that site-specific values were more appropriate to predict toxicity than the values set in federal legislation. The later numbers were derived from international criteria and, consequently, do not reflect the realistic conditions of Brazilian environments. In the case of SES, the site-specific values have generated higher values for quotients which allowed better toxicity predictions (specifically for S1) than those proposed in federal legislation, which makes them more predictive compared to the Brazilian criteria. In Table 3 it is possible to observe the failure of SQG to predict impacts in S1, which was toxic for liquid phase tests.

#### 4.3. Integrated approach

Both RTM and PCA approaches allowed detecting the influence of contamination and environmental conditions to determine the recent state of sediment degradation in the SES. The application of RTM was useful to rank stations and to identify which line of evidence was more important to each site, even when considering the data matrix reduction to a single index. This was confirmed by the projection of RTM values jointed with PCA results showed in Fig. 3. Cesar et al. (2009) proved that RTM is a useful approach and provides an effective identification of contamination “hot spots” in environmental studies, including the complexity of chemical compounds measured.

By observing the results, metals, PAH and butyltins, were correlated with TOC and Organic Matter, geochemical carriers which are considered the important binders of organic contaminants in sediments (Langston and Pope, 1995; Burgess and Kester 2002; Mzoughi and Chouba, 2011). Pore water toxicity, mud and metals were also correlated in this case, the remobilization of

**Table 3**  
Comparisons of contamination levels in samples from Port of Santos with Brazilian sediment quality guidelines (SQG) and site-specific values (SQV) for Santos-São Vicente Estuarine System and their respective results for toxicity tests.

Stations	Brazilian SQGs		Site-specific SQV		Stations				
	Level 1	Level 2	Threshold	Probable	S1	S2	S3	S4	S5
Hg (mg kg <sup>-1</sup> )	0.15	0.71	0.08	0.32	0.04	0.15	0.18	0.28	0.64
Cd (mg kg <sup>-1</sup> )	1.2	9.6	–	0.75	<0.60	<0.60	<0.60	<0.60	<0.60
Cr (mg kg <sup>-1</sup> )	81	370	27.85	48.8	28.17	26.31	29.82	34.26	42.74
Cu (mg kg <sup>-1</sup> )	34	270	–	6.55	9.01	15.04	12.02	17.56	27.28
Ni (mg kg <sup>-1</sup> )	20.9	51.6	5.9	21.2	10.88	11.42	11.1	15.18	22.28
Pb (mg kg <sup>-1</sup> )	46.7	218	10.3	19.2	9.09	15.6	7.57	16.7	12.76
Zn (mg kg <sup>-1</sup> )	150	410	37.9	61.7	509.08	621.24	810.93	917.43	1077.33
PAHs (ng g <sup>-1</sup> )	–	3000	–	1660.00	193.02	501.61	748.37	1248.64	4803.62
Brazilian SQGs			SQGq		0.22	0.3	0.36	0.45	0.74
			Classification		Moderate	Strong	Strong	Strong	Strong
Site-specific SQV for SES			SQVq		1.35	1.7	2.06	2.46	3.24
			Classification		Strong	Strong	Strong	Strong	Strong
Toxicity					PW, ELU and SWI	PW, ELU and SWI	WS, PW, ELU and SWI	PW, ELU and SWI	PW and SWI

WS = whole sediment; PW = pore water; ELU = elutriates; SWI = sediment–water interface.



contaminants into the aqueous phase may have occurred. Wauhob et al. (2007) reported the increase of Cd, Cu, Al and organic contaminants in pore water and sediment–water interface chambers containing sediments from Corpus Christi Bay, Texas. Furthermore, natural processes (waves and tidal currents) or anthropogenic activities (dredging) can lead to resuspension of sediments and, once in the water column, metals may be remobilized to the dissolved phase (Cantwell and Burgess, 2004).

#### 4.4. Sediment quality in the Port of Santos

Sediments from port zones in Brazil (Abessa et al., 2008; Choueri et al., 2009) and in other urbanized areas, such as United States (Iannuzzi et al., 2008; McGee et al., 2009), Canadá (Belan, 2003), Europe (Riba et al., 2004; Silva et al., 2006) and China (Cheung et al., 2003), have the same levels of contamination reported here. Nevertheless, for some of those studies in estuaries, associations of cause and effect of such contamination were not well defined. The pattern revealed for the quality of sediments from Port of Santos evidences a gradient of contamination from the inner estuary towards the sea, where toxicity was expressed in different forms of exposure and impaired benthic community.

Iannuzzi et al. (2008) could not evidence any single driver (chemical or physical) of sediment toxicity or of alterations to the benthic community from the Passaic River (New Jersey, US), and this observation was, therefore, related to the effects of mixtures and chemical interactions (e.g. chemical synergism or antagonism). In the present study, contaminants were associated with TOC and mud, features that McGee et al. (2009) considered as contaminant binders in sediments from Anacostia River (Washington, US). Following the estuarine gradient, the levels of contamination in Port of Santos clearly decreases alongside with these binder contents, while the toxicity of the liquid phases increased (from S5 to S1). Changes in equilibrium partitioning can explain that, since the decrease of organic matter contents can cause substantial changes in interstitial water chemistry (USEPA, 2005) and, thus, some compounds can be remobilized into liquid phase, as there are evidences of such mobilization of contaminants to the liquid phase in literature (Burgess and Kester, 2002; McDonald, 2005; Wauhob et al., 2007).

Concerning to benthos, the pattern found suggests the combination effects of natural and anthropogenic factors. A high number of species and individuals occurred in zones of marine waters influence (S1 and S2) whereas the chemicals contamination occurred in the inner portion of estuary. This suggests that the conditions in the estuarine environment control the structure of the benthic community, alongside with the contamination levels.

According to the “estuarine quality paradox” concept (Elliott and Quitino, 2007; Dauvin and Ruellet, 2009), “the dominant faunal and floral community of estuaries is adapted to and reflects the spatial and temporal variability of highly naturally-stressed areas” and, thus, communities may have features very similar to those found in anthropogenically-stressed areas, making it difficult to detect effects of anthropogenic activities in estuaries. However, due to high levels of contamination, this factor cannot be ignored because, in contaminated sites, opportunistic and tolerant species have the ability to reproduce at high rates, which makes them able to proliferate in contaminated habitats (Hartwell and Hameedi, 2006).

Besides the complexity described above, dredging activities also appear to contribute to the structure of communities in Port of Santos, since it is commonly associated with a reduction of diversity and density in benthic fauna from dredged areas and in their vicinity (Newell et al., 1998). Moreover, looking for a better interpretation on benthic data, Abessa et al. (2008) suggested an interpretation based on exploratory analyzes, such as cluster

analysis, multidimensional scaling and correlation coefficients between the species and the variables concerned, since the reduction of data into a single index limits the perception of the effects on the benthos in complex environments as SES.

## 5. Conclusions

Sediments from the harbour areas of SES are contaminated by metals (mainly Hg and Zn), PAH, LAB and butyltins (TBT), and such contaminants were associated to biological effects. This fact is important because it demonstrates that the policies for contamination control were ineffective to deal with the population expansion and economic activities in the region (harbor, industries, tourism), since contaminants continue to be released into the environment and cause toxicity. Moreover, the comparison with sediment quality guidelines revealed that Brazilian standards present low sensitivity to predict impacts, when compared to site-specific values.

For benthos, the estuarine influence was a primary factor to control the structure of community, but anthropogenic stressors such as contamination and dredging events represents a secondary, but still relevant factor, to control benthic fauna in SES. The integrated analysis of these three lines of evidence allows noticing a gradient of contamination, which increased towards the inner estuary; moreover, as some binders decrease, the toxicity for liquid phases gets more significant.

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