Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Sediment quality of a Ramsar site assessed by chemical and ecotoxicological approaches

G.S. Araujo [a,b,*], A.C.F. Cruz [a,b], P.K. Gusso-Choueri [b], T.D. Saint'Pierre [c], R.A. Hauser-Davis [d], C.C. Martins [e], D.M.S. Abessa [b]

[a] *Instituto Oceanográfico, USP, São Paulo, SP, Brazil*
[b] *NEPEA, Campus do Litoral Paulista, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), São Vicente, SP, Brazil*
[c] *Laboratório de Espectrometria Atômica (Labspectro), Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Rio de Janeiro, Brazil*
[d] *Laboratório de Avaliação e Promoção da Saúde Ambiental, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*
[e] *Centro de Estudos do Mar, Universidade Federal do Paraná, PR, Brazil*

ARTICLE INFO

ABSTRACT

The sediment quality of the Protected Cananéia-Iguape-Peruíbe Area (APA-CIP), listed as one of the Wetlands of International Importance (Ramsar Site), was assessed through geochemical and ecotoxicological analyses, in order to determine if organic and inorganic contaminants retained in the sediments were bioavailable to benthic organisms and could trigger negative effects. Lethal (*Tiburonella viscana*) and sublethal (*Lytechinus variegatus* and *Nitokra* sp.) ecotoxicological endpoints were assessed, as well as sediment texture and organic and inorganic contaminants in sediments obtained at six stations distributed along the APA-CIP. Sites under the influence of the maximum turbidity zone and close to the main local urban center (Cananéia city) presented the worst environmental conditions, indicated by sediment toxicities and high contaminants levels. These conditions may be associated to fine-grained sediments. Metal (Pb, Co, Ni and Zn) concentrations exceeded the regional Sediment Quality Guidelines (SQGs) at stations PT and PM, while Pb also exceeded the Canadian Interim Marine Sediment Quality Guidelines (ISQGs). Total Aliphatic Hydrocarbons (AH) were close to threshold sediment contamination values at PT (maximum turbidity zone). These findings indicate that metals from former mining activities reach APA-CIP, in addition to contaminants from local sources (urban centers, docks, fishing wharfs). Contaminants accumulate in low energy regions, where fine particle and organic matter contents are high. Toxicities appeared to be associated to contaminants, suggesting that the multiple local anthropogenic sources can produce ecological risks, indicating the need for multiple efforts to control external and internal contamination sources in this protected estuarine area.

© 2020 Elsevier B.V. All rights reserved.

1. Introduction

Protected marine and estuarine areas have been established with different aims, including the protection of fish stocks and reduction of anthropogenic impacts (IUCN, 2013). The coastal zone of the state of São Paulo, Brazil, comprises a mosaic of marine, terrestrial and mixed protected areas (PAs), where the Cananéia-Iguape-Peruíbe Protected Area (APA-CIP, "Área de Proteção Ambiental de Cananéia-Iguape-Peruíbe") is inserted. The APA-CIP was established by Federal decrees 90.347/84, and 91.892/85 in 1984 and 1985, respectively, and is located in the area known as the Iguape and Cananéia Estuarine Complex (Lagamar). The area was recognized as a World Natural Heritage Site by UNESCO and is part of the UNESCO's Atlantic Rainforest Biosphere Reserve. Recently, the site was also included in the Ramsar's List of Wetlands of International Importance (https://rsis.ramsar.org/ris/2310).

Protected Areas have been frequently ineffective in accomplishing their conservation goals due to aquatic pollution (Gubbay, 2005; Jameson et al., 2002). However, few studies have focused on pollution as an important factor threatening biodiversity within protected areas (e.g. external contaminant sources). Previous studies indicate that metals are important contaminants in this estuarine complex (Abessa et al., 2014; Barcellos et al., 2005; Cruz et al., 2014; Guimarães and Sígolo, 2008; Gusso-Choueri et al., 2015, 2016, 2018; Mahiques et al., 2009, 2013), due to the disposal of mining residues in the upper areas of the Ribeira de Iguape River (RIR) basin, the main contributor to the estuarine complex surrounded by the APA-CIP. Sediments from the APA-CIP present comparable metal concentrations (especially

* Corresponding author at: NEPEA, Campus do Litoral Paulista, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), São Vicente, SP, Brazil.
E-mail addresses: giuliana_araujo@hotmail.com, giusaraujo@gmail.com (G.S. Araujo).

Pb) to those observed in polluted industrial areas, such as the Santos Estuarine System, also located in the state of São Paulo (Mahiques et al., 2009). Local intensive mining activities began in 1943 (Moraes, 1997), and included several Pb mines operating throughout the RIR basin. The mining and metallurgical process (ore and slag) residues were directly discharged into the river, leading to downstream contamination. However, the water disposal of these residues was prohibited, leading to depositing on the river banks, where storm water runoff, wind transport and other physical processes led to carry-over into the river. Mining activities ceased in 1995, but the residues remained deposited along the river banks (Guimarães and Sígolo, 2008; Kummer et al., 2011), representing river metal sources. The RIR mouth is located in the northern portion of the APA-CIP, consequently leading to mining contamination in this estuarine area (Amorim et al., 2011). The influence was intensified after the construction of the "Valo Grande" canal, causing approximately 60% of the RIR water flux to flow toward the estuary (Mahiques et al., 2013), converting this river into the major CIP estuarine complex nutrient and contaminant contributor (Barcellos et al., 2005; Mahiques et al., 2009).

After the construction of the Valo Grande canal, navigation along the estuary became easier. The number of fishing boats in the region experiences seasonal fluctuations, increasing from May to June and decreasing from August to September (Seckendorff and Azevedo, 2007). On the other hand, leisure navigation has expanded in the region, following ecotourism increases, especially during the austral summer (from December to February). Increased boat activity leads to increased anthropogenic hydrocarbon inputs (Egardt et al., 2018; Nasher et al., 2013; Yuan et al., 2001), and organic polycyclic aromatic and aliphatic hydrocarbons (PAHs and AHs, respectively) in water and fish from this region have been reported (Azevedo et al., 2012; Albergaria-Barbosa et al., 2016; Nishigima et al., 2001), although at relatively low concentrations. Hydrocarbons may not be the main contaminants at APA-CIP, but should not be disregarded, as they are known to be highly toxic and can combine with trace metals and other chemicals, producing complex contaminant mixtures that can affect the biota, even at low concentrations (Fleeger et al., 2007; Gauthier et al., 2015).

As mentioned previously, several studies have reported sediment toxicity of or adverse responses in organisms from the APA-CIP (Gusso-Choueri et al., 2015, 2016). However, these studies either presented a limited geographical scope (Cruz et al., 2014) or assessed biological effects by determining biomarkers in resident fish (Gusso-Choueri et al., 2015, 2016), a valuable tool for environmental quality assessments, but that may be limited for identifying ecological risks. As sediments may accumulate contaminants through time, sediment quality assessments constitute a reliable strategy to assess environmental quality and risks. Chemical analyses are useful to identify and quantify contaminants, whereas ecotoxicological approaches may evaluate potential effects on aquatic biota (Costa et al., 2009). Thus, studies integrating sediment toxicity and contamination relationships may provide more robust information concerning sediment quality at this Ramsar site.

Considering that the APA-CIP may be under the influence of both external and internal contamination, which may pose a threat to this protected area, this study aimed to evaluate sediment quality at the APA-CIP using geochemical and ecotoxicological approaches, in order to assess biota risks in this PA, due to historical and recent metal and hydrocarbons anthropogenic inputs.

2. Material and methods

2.1. Study area

The APA-CIP comprises 565,200 ha and is located between the southwestern coast of the state of São Paulo (Brazil) and the northeastern coast of the state of Paraná ($23°45'$–$25°15'$S, $46°45'$–$49°30'$W) (Fig. 1). The region consists in the lower course of the Ribeira de Iguape River and the Cananéia, Cardoso, Superagüi, Comprida and Iguape islands. Anthropogenic occupation is more intense in the northern portion of the system, especially near the urban centers at the Iguape and Ilha Comprida cities and in the lower RIR valley (Barcellos et al., 2005).

The Iguape-Cananéia estuarine system is the major estuarine complex on the São Paulo coast, which, alongside the RIR forms the largest coastal plain in the state. As mentioned previously, this system currently receives a considerable part of the RIR drainage as, after the construction of the Valo Grande canal (Fig. 1, VG canal) in the mid-nineteenth century, at least 60% of the RIR flow was diverted toward the estuary, altering the estuarine hydrological regime (Mahiques et al., 2013). The RIR thus became the main freshwater, sediment and nutrient contributor to this system, especially in its central and north portions (Freitas et al., 2006). Additionally, rainstorms play a major role in removing surface soils and contaminants along the RIR basin (Costa et al., 2009), carrying and redistributing them across the estuarine complex.

2.2. Sediment sampling

Sediment sampling was performed along 6 stations distributed along the estuary, from the vicinities of the Valo Grande canal, where contaminant input influence is expected to be more intense, to Cardoso Island (reference area), as displayed in Fig. 1. The sampling campaign was carried out in September, 2013 (corresponding to the end of austral winter). Sediment samples were collected using a 0.036 m^2 stainless steel Van Veen grab sampler, transferred and conditioned in plastic vessels for metal and grain size analyses and in pre-cleaned aluminum foil for hydrocarbons assessments, and, placed on ice until arrival at the laboratory. Subsequently, aliquots for the toxicity tests were separated and maintained at 4 °C, and aliquots for the geochemistry and chemistry analyses were stored at −20 °C.

2.3. Sediment properties

Sediment grain size distribution was analyzed based on the protocol proposed by McCave and Syvitski (2007). About 30 g of previously dried sediment were wet-sieved 63 μm to separate fine particles (mud and silt). The material retained in the sieve was then dried and weighed. Initial and final weight differences indicated the mud fraction. Subsequently, the sandy material retained 0.063 μm was sieved through other different meshes (ϕ scale), in order to separate different sand classes, and the results were further classified based on the Wentworth scale. The calcium carbonate (CaCO$_3$) contents of each sample were determined using the method described by Hirota and Szyper (1975) which consists of separating 5 g fractions of each sediment sample and adding 10 mL of hydrochloric acid (5 mol L^{-1} HCl) for 24 h, in order to eliminate calcium carbonate. Next, samples were washed with distilled water and dried at 60 °C. The difference between the initial and final weights indicated the amount of CaCO$_3$ in each sample. Organic matter (OM) contents in the sediment samples were estimated using the ignition method (Luczak et al., 1997), in which 5 g aliquots of dry sediments were separated and calcinated at 500 °C for 4 h. Organic matter contents were obtained by calculating the difference between the initial and final weights.
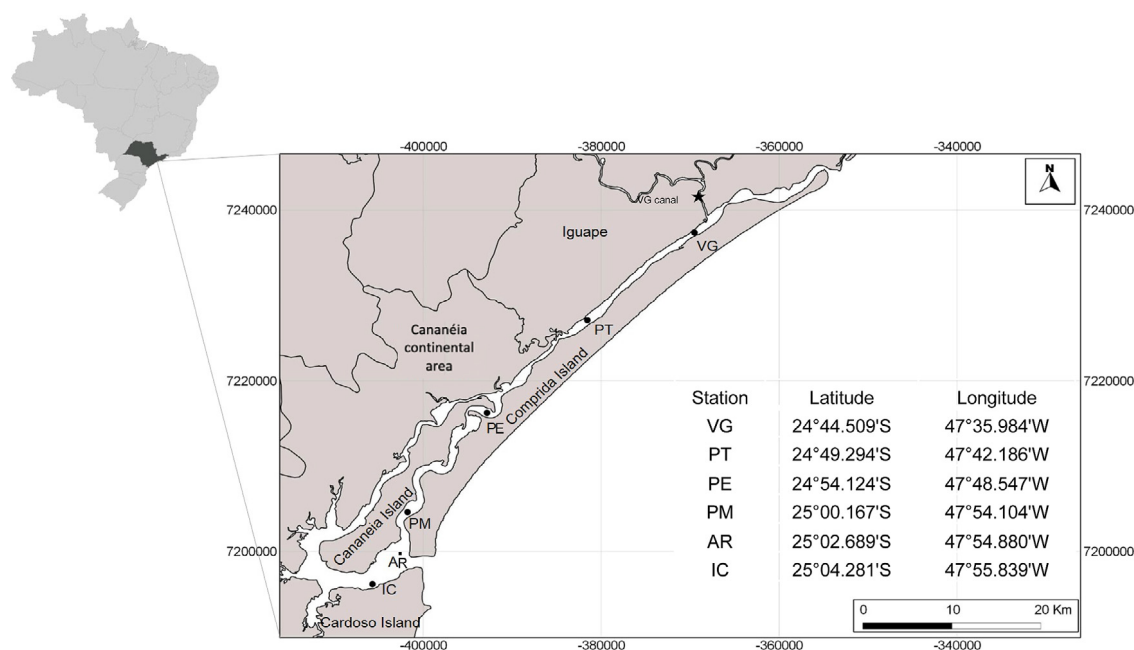
Fig. 1. Map and geographic coordinates of the sediment sampling sites within the Protected Cananéia-Iguape-Peruíbe Area.

2.4. Metals analyses

Elements were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) without the use of a reaction cell on a DRC II ELAN model (Perkin Elmer-Sciex, Norwalk, CT, USA), through HNO_3 extraction. ^{103}Rh was used as an internal standard at 20 $\mu g\ L^{-1}$ to monitor the nebulization process (standard Meinhard nebulizer type with twisted cyclonic chamber) and plasma stability. Al, Cr, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb were determined. Agreement between experimental and certified values was adequate (77.22–118.32%), and the precision of duplicate NIST 2711 (Montana Soil) and PACS-2 (Marine Sediment Reference Materials for Trace Metals) certified reference materials (CRM) were assessed, as recommended by the Environmental Protection Agency (US EPA, 1999) (Table 1).

2.5. Hydrocarbons analyses

Hydrocarbons were determined as described in detail by Wisnieski et al. (2016). Sediment samples were Soxhlet-extracted with a mixture of specific solvents (dichloromethane (DCM) and *n*-hexane, 1:1, v/v). The total organic extracts were then purified and fractionated by liquid chromatography on 5% deactivated silica and alumina columns. Aliphatic hydrocarbons (AHs, including individual *n*-alkanes, pristane and phytane) (Fraction 1) and PAHs (Fraction 2) were obtained by elution with 10 mL of *n*-hexane and 15 mL of a DCM and *n*-hexane mixture (3:7, v/v), respectively.

The instrumental analysis procedures used to quantify the AHs and PAHs were performed as described by Martins et al. (2015). AHs analyses were performed on a gas chromatograph (Model 7890A, Agilent) equipped with a flame ionization detector (FID), while PAHs were analyzed using a gas chromatograph (Model 7890A, Agilent) coupled to a mass spectrometer (Agilent 5973N inert MSD with Triple-Axis Detector). A fused silica capillary column coated with 5% diphenyl/dimethylsiloxane (30 m, 0.25 mm ID, 0.25 mm film thickness) was used in both systems. The oven temperature was programmed to ramp from 40 to 60 °C at 20 °C min^{-1}, then to 250 °C at 5 °C min^{-1}, and, finally, to 300 °C at 6 °C min^{-1}, with a final hold step for 20 min. Data acquisition during the PAHs analyses was performed in the selected ion

monitoring (SIM) mode. The individual *n*-alkanes, pristane and phytane were identified by matching their retention times with those of standard mixtures (n-C$_{10}$ to n-C$_{40}$), at concentrations ranging from 0.25 to 10.0 ng μL^{-1}. The PAHs were identified by matching the retention times and ion mass fragments with those of a standard mixture (Accustandard, Z-014G), at concentrations ranging from 0.10 to 1.50 ng μL^{-1}.

A procedural blank analyses indicated that no peaks interfered with the target compound analyses. Mean recoveries of the spiked surrogate standards ranged from 67 to 78% for AHs and from 40 to 87% for PAHs. The instrumental limits of detection (LOD) were 0.001 $\mu g\ g^{-1}$ for *n*-alkanes and 0.50 ng g^{-1} for PAHs. These data are based on the lowest PAHs and *n*-alkanes concentrations sensitivities (0.02 and 0.04 ng μL^{-1}, respectively), multiplied by the final extracted volume (500 μL) and divided by the weight sediment (20 g) prior to the extraction. Precision expressed as the coefficient of variation between five replicates was lower than 15% for at least 85% of the analyzed hydrocarbons. The determined target hydrocarbon concentrations in the reference material (IAEA-408) were in adequate agreement (95%–105%) with the certified values.

2.6. Ecotoxicological assays

Quality control parameters were determined in all ecotoxicological assays (e.g. salinity, pH, dissolved oxygen (DO) and temperature) (Appendices A–C).

2.6.1. Sediment–Water interface embryonic Lytechinus variegatus toxicity

The embryonic toxicity test with the sediment–water interface (SWI) followed the method described by ABNT - Associação Brasileira de Normas Técnicas (2006), adopting the reduced volumes proposed by Cesar et al. (2004). Adult sea urchin *Lytechinus variegatus* individuals were obtained by snorkeling at Palmas Island, Guarujá, SP, and used as broodstock. About 2 g of sediment were transferred to glass test-tubes (15 mL), followed by 8 mL of filtered seawater (4 replicates/sample). To prevent direct contact between the embryos and the sediment, a mesh (45 μm) was introduced in each test-tube and placed on the sediment surface.

The same procedure was followed for the control tubes, which contained clean seawater (control water), or a 45 μm mesh and water (control mesh). The test system was allowed to stabilize for 24 h before beginning the exposure.

Gametes were obtained by osmotic induction (0.5 mol L^{-1} KCl), and ovules were fertilized by adding an aliquot of sperm solution containing activated sperm cells. Fertilization success was checked by the verification of control samples under a microscope, and >90% fertilization was achieved. For the toxicity test, approximately 500 eggs were added to each chamber (test tube) and incubated for 24 h, at constant temperature (25 ± 2 °C) and a 16 h light/8 h dark photoperiod. At the end of the test, the content of each chamber was transferred to other vessels containing buffered formaldehyde to preserve the larvae. Then, 100 embryos of each replicate were counted, and the percentage of normal embryos was calculated. Normal embryos (Pluteus stage) were identified based on typical larval development, considering branch symmetry, shape and skeleton size (Perina et al., 2011).

2.6.2. Chronic whole-sediment Nitokra sp. toxicity

The whole sediment chronic toxicity assay using laboratory cultured benthic copepod Nitokra sp., was based on the protocol developed by Lotufo and Abessa (2002). Four replicates were used for each sample, and 15 mL of high-density polyethylene flasks were used as test-chambers, filled with 2 mL of sediment and 8 mL of filtered seawater (salinity 17). Ten healthy ovigerous females were introduced into each chamber. The whole test system was incubated at 25 ± 2 °C for 7 days, under a 16:8 light/dark photoperiod. At the end of the test, the contents of each replicate were fixed with formalin (10%) and Rose-Bengal dye (1%). Finally, the numbers of adult females and their offspring (nauplii and copepodites) were counted using a stereomicroscope. Reproduction rates were calculated by dividing the number of offspring by the number of females of each replicate.

2.6.3. Acute whole-sediment Tiburonella viscana toxicity

The acute sediment toxicity assay using T. viscana (Thomas and Barnard, 1983) was performed following the protocol described by Melo and Abessa (2002) and ABNT - Associação Brasileira de Normas Técnicas (2006). The amphipods used in this assay were collected at Engenho d'água Beach, Ilhabela – São Paulo (23°48′–45°22′ W). Three replicates of each sediment sample were prepared in 1 L-polyethylene test chambers: a 2-cm layer of sediment and 750 mL of dilution seawater were gently added to each chamber. After 24 h, ten healthy adults and non ovigerous amphipods were introduced into each test chamber. The experiment lasted 10 days, and was kept under constant lighting and aeration, and 25 ± 2 °C. At the end of the test, the contents of each test chamber were sieved and the surviving organisms were counted. Missing organisms were considered dead.

2.7. Statistical analyses

The results of the ecotoxicological tests were first checked for homoscedasticity and normality by Bartlett's and Shapiro–Wilks tests, respectively. Then, all samples were compared to the reference sediment using One-Way Analysis of Variances (ANOVA), using the R software package. When significant differences in the toxicity endpoints were detected, a post-hoc test (Dunnett's t'-test) was performed to assess statistical differences between the references and the test-samples. If present, sediments were considered toxic ($p < 0.05$), while the absence of statistical differences indicated non-toxic sediments.

Geochemical and ecotoxicological data were transformed through logarithmic and Arcsin equations. Arcsin transformation is used to homogenize the residual variance of the data

$((Arcsin(\sqrt{x/100})) \cdot 180/\pi)$. Moreover, log transformation was applied to reduce the magnitude of the different variables, by the function $y = \log_{10}(x + 1)$. Then, data were integrated using principal component analysis (PCA), through variable correlations variables into a set of values (principal components), in order to reduce the number of variables. A cluster analysis was also performed, in order to confirm the PCA results for grouped stations.

3. Results

3.1. Sediment properties and metals

Sediment samples from VG, PE, PM, AR and IC were predominantly sandy (>75%) while the sediment from PT was predominantly muddy (>95%). Sediments presented low CaCO$_3$ contents (max. 6.35%), whereas OM amounts ranged from 0.28 to 14.4% (Table 1).

Metal concentrations in sediment samples are presented in Table 1. Higher concentrations of elements were observed in sediments from PT and PM. These results may be related to the sediment texture, as PT was predominantly muddy and PM presented fines >20%; these sediments presented also the higher amounts of OM. Sediments from AR and IC exhibited intermediate values, in comparison to the other stations. The recoveries (in percentage) obtained in the metal analysis indicate adequate recoveries (>70%) for most of analyzed metals, keeping in mind that the CRMs were analyzed through weak acid extraction (HNO$_3$) and that they are certified only for total metal extraction. Al and Cr (<70% recovery) are known as refractory elements, which may be strongly connected to silicates, interfering with digestion (see Bordon et al., 2011 for further details).

The concentrations of Pb in sediments exceeded the Interim marine sediment quality guidelines (ISQGs) at station PM (ISQGs = 30.2 mg kg^{-1}; CCME, 2001), and the regional sediment quality guidelines (SQGs) (Choueri et al., 2009) at stations PM (highly polluted >22.2 mg kg^{-1}) and PT (moderately polluted >10.3 mg kg^{-1}). The elements Co (moderately polluted, >4.1 mg kg^{-1}) and Ni (highly polluted, >6.02 mg kg^{-1}) exceeded SQGs values at station PT. Levels of Co (highly polluted, >10.3 mg kg^{-1}), Ni (highly polluted, >6.02 mg kg^{-1}), Zn (moderately polluted, >37.9 mg kg^{-1}) and Hg (moderately polluted, >0.08 mg kg^{-1}) exceeded the respective regional SQGs at station PM.

3.2. Hydrocarbons concentrations

The concentration of organic compounds in sediment samples are presented in Table 2. Total n-Alkanes, Total Aliphatics (resolved and unresolved) and PAHs were analyzed. Stations PT and PM presented, again, the highest values of contaminants. Pedra do Tombo indicated the highest value of Total Aliphatic Hydrocarbons (AH), reaching 96.3 μg g^{-1}. Volkman et al. (1992) suggested that unpolluted estuarine sediments should not present a total of AH higher than 100 μg g^{-1}. For PAHs, Baumard et al. (1998) classified sediments with less than 100 ng g^{-1} of total PAHs as low contamination, which include the sediments from all stations from APA-CIP. Therefore, low or no toxicity due to AHs and PAHs would be expected in sediments from stations PT and PM.

3.3. Ecotoxicological assays

3.3.1. Lytechinus variegatus

In the embryonic toxicity test of SWI, physical–chemical parameters of the overlying water in the test chambers remained within acceptable ranges (Cesar et al., 2004; ABNT - Associação Brasileira de Normas Técnicas, 2006) (Appendix A). Salinities

Table 1
Contents of calcium carbonate, organic matter, sand and mud, (in percentage) and concentrations of metals in sediments from Protected Cananéia-Iguape-Peruíbe Area (mg kg^{-1}). Bold values are above limits (CCME, 2001; Choueri et al., 2009). Certified Reference Materials (CRM) NIST 2711 (Montana Soil) and PACS-2 (Marine Sediment Reference Materials for Trace Metals) are indicated as * (NIST) and ** (PACS-2), respectively.

Sampling sites							
	VG	PT	PE	PM	AR	IC	Recovery (%)
CaCO$_3$	0.00	6.35	0.00	1.32	2.21	2.53	–
OM	0.28	14.4	0.82	6.06	1.00	2.05	–
Sand	98.9	3.84	96.7	77.2	92.5	89.6	–
Mud	1.12	96.2	3.25	22.8	7.54	10.4	–
Al	411.3	9642.4	622.8	13,630.7	1895.9	1401.8	17.9*
Cr	1.07	20.4	1.53	32.0	5.41	3.67	38.9**
Fe	907.4	1,5432.7	1722.8	26,661.3	3827.7	2813.9	75.6**
Co	0.43	**4.64**	0.82	**10.0**	1.63	0.97	77.2*
Ni	0.31	**7.20**	0.47	**11.7**	1.76	1.37	78.6*
Cu	0.28	5.47	0.46	16.3	0.80	0.68	104.1*
Zn	1.65	23.3	2.71	**45.2**	6.21	3.87	86.7*
Cd	0.01	0.07	0.01	0.14	0.02	0.01	118.3*
Hg	0.02	0.06	0.01	**0.12**	0.02	0.01	114.1**
Pb	0.83	**13.1**	2.93	**40.2**	3.08	2.04	81.9*

VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.

Table 2
Concentrations of total *n*-Alkanes, total Aliphatic and Polycyclic Aromatic Hydrocarbons in sediments from APA-CIP. Concentrations of *n*-Alkanes and Aliphatic hydrocarbons are expressed in $\mu g\ g^{-1}$ and those of PAHs are expressed in ng g^{-1}. LD is for Limit of Detection.

Variable	Sampling sites					
	VG	PT	PE	PM	AR	IC
n-Alkanes ($\mu g\ g^{-1}$)	0.2	17.4	0.17	3.40	2.97	0.04
Total Resolved. AHs ($\mu g\ g^{-1}$)	1.8	22.6	1.8	4.7	4.1	12.8
Total AHs ($\mu g\ g^{-1}$)	3.2	96.3	4.4	19.0	12.8	2.18
$\sum$PAHs* (ng g^{-1})	<LD	25.2	<LD	19.3	2.5	<LD
$\sum$PAHs EPA (ng g^{-1})	<LD	20.5	<LD	17.8	2.5	<LD

VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.

Fig. 3. Reproductive rate of *Nitokra* sp. exposed to sediments from Protected Cananéia-Iguape-Peruíbe Area. Asterisks (*) indicate significant differences relative to the control (IC) (p < 0.05). VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.

3.3.2. Nitokra sp.

In the test with copepods, the measured parameters of overlying waters in the test chambers were within acceptable levels in the most of samplings, except for PT (Appendix B), according to Lotufo and Abessa (2002). Salinities ranged from 17 to 19 PSU; pH levels were between 6.89 and 7.66, with exception of the test chamber with the sediments from PT in which the overlying water had low pH (4.65; however, reaching acceptable levels (7.43) by the end of the test); and DO levels ranged from 3.15 to 4.69 mg L^{-1}. Sediment from PT and PM were considered significantly toxic (Fig. 3).

3.3.3. Tiburonella viscana

During the acute toxicity test, physical and chemical parameters of the overlying water within the test chambers remained within acceptable ranges (Melo and Abessa, 2002; ABNT - Associação Brasileira de Normas Técnicas, 2006) (Appendix C). Salinities ranged from 30 to 34 PSU; DO levels ranged from 5.42 to 6.07 mg L^{-1}. The pH values ranged between 7.31 and 7.67. Sediment from station PT showed a significant statistical difference, producing 100% mortalities among the exposed amphipods (Fig. 4). Sediments from VG, PE and PM presented low survival rates (close to 50%); however, no statistical difference toward IC was indicated.
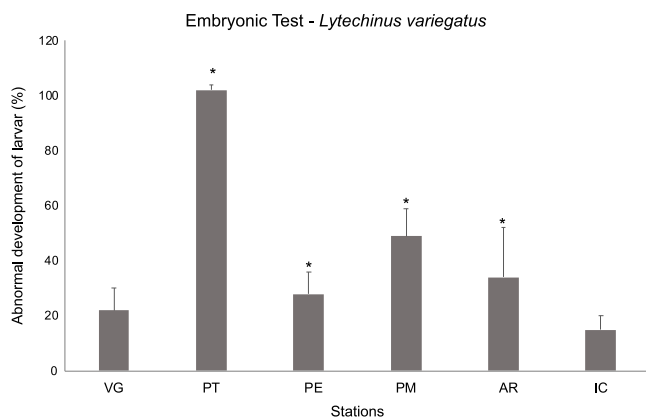

Fig. 2. Abnormal development of larvae of *L. variegatus* exposed to sediments from the Protected Cananéia-Iguape-Peruíbe Area. Asterisks (*) indicate significant differences relative to the control (IC) (p < 0.05). VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.

ranged from 31 to 35 Practical Salinity Unit (PSU); pH levels were between 5.84 and 6.57 and Dissolved Oxygen (DO) levels ranged from 2.77 (adjusted with aeration to 4 mg L^{-1}) to 4.16 mg L^{-1}. The normal embryonic development of organisms exposed to sediments from stations PT, PE, PM and AR were significantly altered, and sediments from PT were the most toxic (Fig. 2).
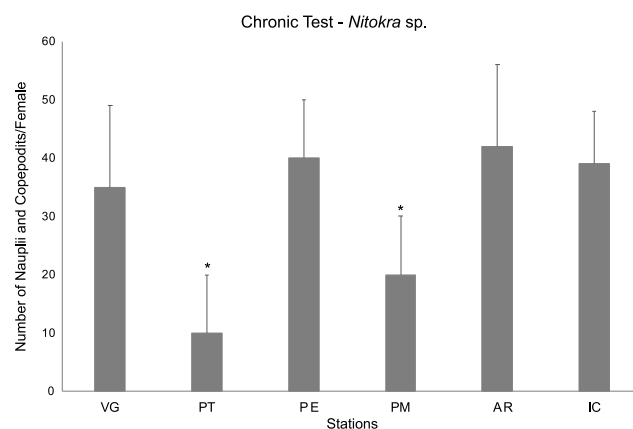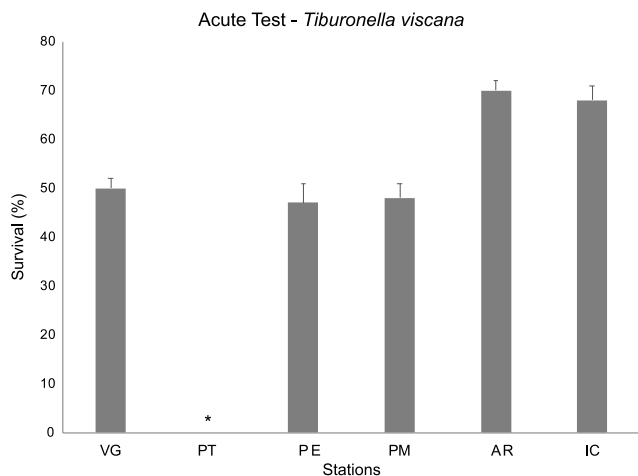
Fig. 4. Survival of *T. viscana* exposed to sediments from the Protected Cananéia-Iguape-Peruíbe Area. Asterisks (*) indicate significant differences relative to the control (IC) ($p < 0.05$). VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.

3.4. Integrative approach

The first two PCA components explained >98% of variances (Table 3, Appendix D). The 1st PC explained 75.4% of the total variance, indicating separation of the most contaminated sites (stations PT and PM, with negative scores) from the rest of the investigated sites (VG, AR, IC, PE), which presented positive scores. All the variables presented high correlations to PC1, indicating an association between toxicities (abnormal *L. variegatus* larvae, low *Nitokra* sp. fecundity rates and *T. viscana* mortality), geochemical factors (mud, OM and CaCO$_3$), organic compounds (*n*-Alkanes, UCM, total AHs and PAHs) and all the analyzed metals. The 2nd PC explained 23.6% of the total variance, e mainly associated to stations PM (positive values for Pb) and PT (negative values for UCM, total AHs, n-alkanes and whole sediment toxicity toward amphipods). Therefore, PM was highly associated to Pb, the main contaminant from the mining activities along the RIR in PC2, while PT indicates a higher association with hydrocarbons (UCM, total AHs and *n*-alkanes). At this site, n-alkanes indicate terrigenous inputs, while total AHs suggest a certain degree of anthropic influence, as unresolved complex mixture (UCM) amounts were higher.

A cluster analysis was also performed in order to verify the PCA results. This analysis grouped stations PT and PM together and all the other stations in another cluster, with VG more similar to PE and AR to IC.

4. Discussion

Our results indicate that high contaminant concentrations were observed in sediments from stations PT (maximum turbidity zone in regular conditions; Mahiques et al., 2013; Tessler and Souza, 1998; Tramonte et al., 2018) and PM (close to the city of Cananéia). In these sediments, levels of Pb, Co, Ni, and Zn exceeded SQGs limits (Choueri et al., 2009), and in sediments from PT indicated the total AHs concentrations were close to the threshold of contamination (Volkman et al., 1992). The total volume of freshwater flowing into the APA-CIP involves the contribution of various small rivers, which contribute with hydrocarbons from terrestrial plants and little anthropic activity (Nishigima et al., 2001). Cananéia has been historically considered low impacted; however, PAHs metabolites were found in fish

Table 3
PCA eigenvalues integrating sediment properties, chemistry and toxicity for samples from Protected Cananéia-Iguape-Peruíbe Area. Bold indicate relevant associations.

PC	Eigenvalue	%Variance
PC1	15.07	75.36
PC2	4.72	23.59
	PC1	PC2
Sea Urchin_abn.	**−0.815**	−0.559
Copepod	**−0.915**	−0.384
Amph._mort.	**−0.716**	**−0.662**
Mud	**−0.828**	−0.546
OM	**−0.932**	−0.274
CaCO$_3$	**−0.907**	−0.408
Al	**−0.960**	0.280
Cr	**−0.938**	0.344
Fe	**−0.918**	0.396
Co	**−0.862**	0.506
Ni	**−0.931**	0.361
Cu	**−0.810**	0.586
Zn	**−0.893**	0.450
Cd	**−0.882**	0.469
Hg	**−0.872**	0.482
Pb	**−0.793**	**0.607**
UCM	**−0.781**	**−0.623**
n-alkane	**−0.779**	**−0.617**
Tot-AH	**−0.777**	**−0.628**
PAHs	**−0.993**	−0.109
PC Scores		
Stations	PC1	PC2
VG	2.945	−0.083
PT	−5.333	−3.270
PE	2.663	−0.169
PM	−4.638	3.585
AR	1.928	−0.068
IC	2.434	0.005

VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.

bile of individuals collected in the estuarine system (Albergaria-Barbosa et al., 2016; Gusso-Choueri et al., 2015; Azevedo et al., 2012; Nishigima et al., 2001). Volkman et al. (1992) stated that organic-rich estuarine sediments may reach up to 100 μg g^{-1} of total AHs, however, concentrations above this limit usually are due to petroleum inputs. The highest AHs concentration was found in station PT (96.3 μg g^{-1}), however, a high percentage (77%) of such hydrocarbons was composed of the unresolved complex mixture (UCM), suggesting that some anthropic influence is occurring in this estuarine system. Despite being of anthropic or biogenic source, organic compounds are present in the estuarine system and may combine with other contaminants (e.g. metals), resulting in negative effects to the biota. Mixture of contaminants can be toxic even at low concentration levels (Monosson, 2005). A recent study showed that Pb is still a problem for this estuarine region, because it occurs in a bioavailable form and presents the highest levels in comparison to other elements (Tramonte et al., 2018). This element was also considered of main concern for the region, due to its high concentrations and potential bioavailability (Guimarães and Sígolo, 2008; Mahiques et al., 2009; Bonnail et al., 2017); besides, fish used as food source to local inhabitants' present contamination by Pb (Gusso-Choueri et al., 2018). In this study, concentrations of Pb exceeded the ISQGs (CCME, 2001) in samples from PT and PM. In addition to fine sediments (silt and clay), deposition areas tend to accumulate organic matter as well (Gordon and Goñi, 2004). In this sense, contamination (metals and AHs) and toxicity (embryonic, chronic and acute) tended to associate with the occurrence of mud and OM.

The worse conditions tended to occur in the muddy sediments (stations PT and PM), where chemical levels and toxicity were

strongly associated to muds and OM, corroborating with other studies which show this type of association (Burton and Landrum, 2003; Cruz et al., 2014). These results also show that depositional areas of APA-CIP possibly tend to accumulate contaminants (e.g. metals, PAHs, AHs) from different sources. Interestingly, the higher concentrations of hydrocarbons occurred in sediments from PT, while sediments from PM presented the higher concentrations of metals. These results suggest that, in addition to the mining activities, APA-CIP contamination sources include docks, ferry boats, sewage and urban drainage (Cruz et al., 2014). As previously mentioned, demersal fish from APA-CIP associated PAHs in its bile content to genotoxicity and DNA damage (Gusso-Choueri et al., 2016), indicating the role of hydrocarbons as concerning contaminants to this area. Thus, although concentrations of AHs in sediments from PT were not high, their presence should not be disregarded, because they seem to be accumulating together with metals in this site, and thus it may combine with metals to cause or increase toxicity (D. et al., 2014). Synergistic effect was found after mixing metals and PAHs, increasing toxicity and mortality (Fleeger et al., 2007). An additive effect (enhancing toxicity) was also found for mixtures of metals and PAHs (Gauthier et al., 2015).

Regarding metals, the past mining activities situated on the upper RIR represent the main sources to the RIR and to the APA-CIP (Mahiques et al., 2009; Moraes, 1997), since metals are mainly carried downstream associated with suspended particles (Abessa et al., 2014; Guimarães and Sígolo, 2008) and tend to precipitate close to the Valo Grande canal, within the estuarine system (Mahiques et al., 2009). Coarser grained sediments tend to be deposited close to areas presenting higher energy (e.g., mainly along the upper and mid RIR portions), whereas finer fractions tend to move longer distances and reach the estuarine canal (Mahiques et al., 2013). However, Tramonte et al. (2018) observed a different contaminant distribution along the estuary, suggesting that contaminated sediment is transported independently of the tide and is also influenced by RIR floods.

Extreme rainfall episodes can contribute significantly to the supply of terrestrial OM and metals to the APA-CIP (Abessa et al., 2014; Gusso-Choueri et al., 2015, 2018). Thus, the areas with highest deposition may change throughout the year, which can promote a displacement of the areas under higher risk with possible consequences for the biota (Cruz et al., 2014). In our study, sediments presented higher levels of contaminants, increased toxicity and higher OM and mud contents were found at stations PT and PM (midway distance between the Valo Grande canal and the city of Cananéia) and not at VG, which was initially expected. Tessler and Souza (1998) indicated that the predominant flux in this estuarine system occurs from VG to PT, where a low tide inversion point concerning flow direction is noted. Thus, this point may vary toward the southeast, near PM, highlighting both depositional areas.

The integration of chemical and ecotoxicological data clearly shows that some portions of the APA-CIP are negatively impacted by the input of metals and hydrocarbons, which cause risks to the biota, despite the protection status of the entire region. Sites PT and PM presented worse conditions, but PT presented the higher concentrations of hydrocarbons, while PM exhibited the higher levels of metals. Our results suggest that the APA-CIP is being affected by more than one source of contamination (e.g. mining, sewage, recreational docks, fishing boats and terminals), even though the mining activity seems to be the most important. The contaminants introduced by these multiple sources tend to be carried to and sink on depositional areas, creating sites with poor environmental quality.

Recent studies, such as that carried out by Tramonte et al. (2018), indicate that Pb remains a concern for this estuarine

Table A.1

Data of salinity, pH, dissolved oxygen (DO) and temperature in the *L. variegatus* test. DO was measured at mg/L.

	Salinity (PSU)	pH	DO (mg L^{-1})	Temperature (°C)
VG	35	5.84	4.16	23 ± 2
PT	35	6.17	3.60	23 ± 2
PE	31	6.31	2.77[a]	23 ± 2
PM	35	6.33	3.34	23 ± 2
AR	35	6.43	3.51	23 ± 2
IC	35	6.57	3.32	23 ± 2

VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.
[a]Values were adequately adjusted (4 mg L^{-1}).

system and that RIR floods may increase Pb contamination in this area. Gusso-Choueri et al. (2018) stated that fish from APA-CIP presented Cd, Pb, and As concentrations higher than the permissible levels for human consumption, while Albergaria-Barbosa et al. (2016) suggested that nautical activity may have increased PAH bioavailability at the Cananéia estuary. Thus, the results reported herein are valuable in furthering knowledge in this regard.

Based on the results obtained in the present study and the literature (Cruz et al., 2014; Mahiques et al., 2013; Gusso-Choueri et al., 2018, 2016, 2015; Tramonte et al., 2018), we can conclude that the APA-CIP present contamination in depositional areas and cannot be considered homogeneous (in terms of environmental quality), since some of its portions present moderate environmental degradation. Jameson et al. (2002) stated that Marine Protected Areas could be negatively influenced by external sources, since the fluid nature of their environment would not be efficient to retain pollutants outside the MPAs. Some examples indicated that MPAs could be affected by pollution (Araujo et al., 2013; Pozo et al., 2009; Terlizzi et al., 2004). The levels of contamination in the depositional areas are enough to produce potential risk to the local biota, especially due to metals. The results also indicate that the APA-CIP is not being totally effective to protect this area from external impacts, and that policies are required to control the contamination sources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Acknowledgments

Table B.1

Data of salinity, pH, dissolved oxygen (DO) and temperature in the *Nitokra* sp. test. DO was measured at mg/L.

	Initial				Final			
	Salinity (PSU)	pH	DO (mg L^{-1})	Temperature (°C)	Salinity (PSU)	pH	DO	Temperature (°C)
VG	17	7.66	4.69	23 ± 2	18	7.36	6.45	23 ± 2
PT	17	4.65	4.47	23 ± 2	16	7.43	4.9	23 ± 2
PE	18	6.89	4.34	23 ± 2	17	7.52	5.65	23 ± 2
PM	19	7.04	3.15	23 ± 2	19	7.30	4.10	23 ± 2
AR	19	7.09	3.84	23 ± 2	19	7.26	5.20	23 ± 2
IC	19	7.01	3.15	23 ± 2	20	7.21	4.71	23 ± 2

VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.

Table C.1

Data of salinity, pH, dissolved oxygen (DO) and temperature (T °C), in the *T. viscana* test. DO was measured at mg/L.

	Initial				Final			
	Salinity	pH	DO	T °C	Salinity	pH	DO	T °C
VG	31	7.67	6.07	25° ± 1	34	8.0	6.23	25° ± 1
PT	30	7.36	5.42	25° ± 1	30	6.6	5.75	25° ± 1
PE	32	7.61	5.85	25° ± 1	32	8.0	6.28	25° ± 1
PM	32	7.54	5.61	25° ± 1	34	8.2	6.21	25° ± 1
AR	33	7.31	5.85	25° ± 1	34	8.00	6.09	25° ± 1
IC	34	7.62	5.78	25° ± 1	34	8.00	5.94	25° ± 1

VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.

Appendix A. Physical–chemical parameters of *L. variegatus*

See Table A.1.

Appendix B. Physical–chemical parameters of *Nitokra sp*

See Table B.1.

## Appendix C. Physical–chemical parameters of T. viscana

See Table C.1.

Appendix D. Bi-dimensional distribution of PCA results using geochemical and ecotoxicological data from sediments from Protected Cananéia-Iguape-Peruíbe Area (PC1 × PC2)

See Fig. D.1.

Appendix E. Cluster analyses using geochemical and ecotoxicological data from sediments from Protected Cananéia-Iguape-Peruíbe Area

See Fig. E.1.
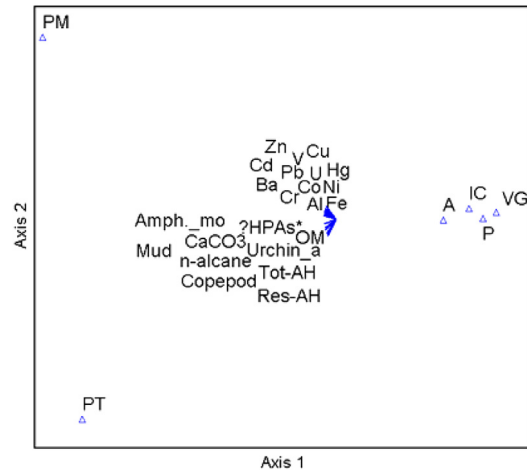
Fig. D.1. PCA integrating sediment properties, chemistry and toxicity for samples from Protected Cananéia-Iguape-Peruíbe Area.
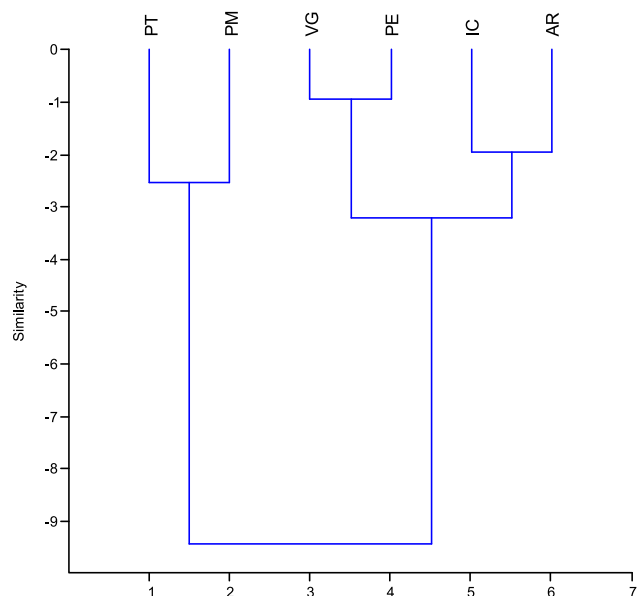
Fig. E.1. VG = Valo Grande. PT = Pedra do Tombo. PE = Pedrinhas. PM = Pai Mato. AR = Arrozal. IC = Ilha do Cardoso.

References

Abessa, D.M.S., Gonçalves, L., Perina, F., Davanso, M.B., Rodrigues, V.G., Martins, L., Sígolo, J.B., 2014. Sediment geochemistry and climatic influences in a river influenced by former mining activities : the case of ribeira de. Open J. Water Pollut. Treat. 1, 43–54.

ABNT - Associação Brasileira de Normas Técnicas, 2006. NBR_15350 - Toxicidade Crônica de Curta Duração L. variegatus.

Albergaria-Barbosa, A., Patire, V., Taniguchi, S., Fernandez, W., Dias, J., Bícego, M., 2016. Mugil curema as a PAH bioavailability monitor for Atlantic west sub-tropical estuaries. Mar. Pollut. Bull. 114, 609–614. http://dx.doi.org/10.1016/j.marpolbul.2016.09.039.

Amorim, E.P., Favaro, D.I.T., Braga, E.S., 2011. Toxic metal assessment in estuarine sediments from Cananeia, São Paulo, Brazil, determined by Atomic Absorption Spectrometry, in: Congresso Brasileiro de Geoquímica. pp. 83–86.

Araujo, G.S., Moreira, L.B., Morais, R.D., Davanso, M.B., Garcia, T.F., Cruz, A.C.F., Abessa, D.M.S., 2013. Ecotoxicological assessment of sediments from an urban marine protected area (Xixová-Japuí State Park, SP, Brazil). Mar. Pollut. Bull. 75, 62–68. http://dx.doi.org/10.1016/j.marpolbul.2013.08.005.

Azevedo, J.S., Braga, E.S., Roche, H., Alberto, C., Ribeiro, O., Bebianno, M.J., 2012. Evidence of contamination by oil and oil products in the Santos-São Vicente Estuary, São Paulo, Brazil. Braz. J. Oceanogr. 60, 117–126.

Barcellos, R.L., Berbel, G.B.B., Braga, E., Furtado, V.V., 2005. Distribuição e características do fósforo sedimentar no Sistema Estuarino Lagunar de Cananéia-Iguape. Geochim. Bras. 19, 22–36.

Baumard, P., Budzinski, H., Garrigues, P., 1998. Polycyclic aromatic hydrocarbons in sediments and mussels of the Western Mediterranean Sea. Environ. Toxicol. Chem. 17, 765–776. http://dx.doi.org/10.1897/1551-5028(1998)017<0765:PAHISA>2.3.CO;2.

Bordon, I.C.A.C., Sarkis, J.E.S., Gobbato, G.M., Hortellani, M.A., Peixoto, C.M., 2011. Metal concentration in sediments from the Santos Estuarine system: a recent assessment. J. Braz. Chem. Soc. 22, 1858–1865.

Burton, G.A., Landrum, P.F., 2003. Toxicity of sediments. In: Encycl. Sediments Sediment. Rocks, Vol. 751. pp. 748–751. http://dx.doi.org/10.1007/3-540-31079-7_241.

CCME, C.C. of M. of the E., 2001. Canadian sediment quality guidelines for the protection of aquatic life. Can. Counc. Min. Environ. 5.

Cesar, A., Marín, A., Marín-Guirao, L., Vita, R., 2004. Amphipod and sea urchin tests to assess the toxicity of Mediterranean sediments: the case of Portman Bay. Sci. Mar. 68, 205–213. http://dx.doi.org/10.3989/scimar.2004.68s1205.

Choueri, R.B., Cesar, A., Abessa, D.M.S., Torres, R.J., Morais, R.D., Riba, I., Pereira, C.D.S., Nascimento, M.R.L., Mozeto, A.A., DelValls, T.A., 2009. Development of site-specific sediment quality guidelines for North and South Atlantic littoral zones: Comparison against national and international sediment quality benchmarks. J. Hazard. Mater. 170, 320–331. http://dx.doi.org/10.1016/j.jhazmat.2009.04.093.

Costa, Filho, S., Risso, 2009. Modelagem espaço-temporal da erosão e potencial contaminação de Arsênio e Chumbo na bacia hidrográfica do rio Ribeira de Iguape (SP). 39, 338–349.

Cruz, A.C.F., Davanso, M.B., Araujo, G.S., Buruaem, L.M., Santaella, S.T., de Morais, R.D., Abessa, D.M.S., 2014. Cumulative influences of a small city and former mining activities on the sediment quality of a subtropical estuarine protected area. Environ. Monit. Assess. 186, 7035–7046. http://dx.doi.org/10.1007/s10661-014-3908-1.

D., Salem, F., Morsy, A., El-Nemr, A., El-Sikaily, A., Khaled, 2014. The monitoring and risk assessment of aliphatic and aromatic hydrocarbons in sediments of the red sea. Egypt. Egypt J Aquat Res 40, 333–348. http://dx.doi.org/10.1016/j.ejar.2014.11.003.

Egardt, J., Mørk Larsen, M., Lassen, P., Dahllöf, I., 2018. Release of PAHs and heavy metals in coastal environments linked to leisure boats. Mar. Pollut. Bull. 127, 664–671. http://dx.doi.org/10.1016/j.marpolbul.2017.12.060.

Environmental Protection Agency, U.E., 1999. Determination of metals in ambient particulate matter using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS). EPA/625/R-96/010a.

Fleeger, J., Gust, K., Marlborough, S., Tita, G., 2007. Mixtures of Metals and Polynuclear Aromatic Hydrocarbons Elicit complex, nonadditive, toxicological interactions in meiobenthic copepods. Environ. Toxicol. Chem. 26, 1677–1685.

Freitas, R.C., Barcellos, R.L., Pisetta, M., Rodrigues, M., Furtado, V.V., 2006. O canal do valo grande e o assoreamento no sistema estuarino-lagunar de Cananéia-Iguape. III Simpósio Brasileiro de Oceanografia 14p.

Gauthier, P.T., Norwood, W.P., Prepas, E.E., Pyle, G.G., 2015. Metal-polycyclic aromatic hydrocarbon mixture toxicity in *Hyalella azteca*. 1. Response surfaces and isoboles to measure non-additive mixture toxicity and ecological risk. Environ. Sci. Technol. 49, 11772–11779. http://dx.doi.org/10.1021/acs.est.5b03231.

Gordon, E.S., Goñi, M.A., 2004. Controls on the distribution and accumulation of terrigenous organic matter in sediments from the Mississippi and Atchafalaya river margin. Mar. Chem. 92, 331–352. http://dx.doi.org/10.1016/j.marchem.2004.06.035.

Gubbay, S., 2005. Evaluating the Management Effectiveness of Marine Protected Areas. WWF.

Guimarães, V., Sígolo, J.B., 2008. Associação de Resíduos da Metalurgia com Sedimentos em Suspensão - Rio Ribeira de Iguape. Rev. do Inst. Geociências - USP, pp. 1–10.

Gusso-Choueri, P.K., Araújo, G.S.D., Cruz, A.C.F., Stremel, T.R.D.O., Campos, S.X.D., Abessa, D.M.D.S., Oliveira Ribeiro, C.A.D., Choueri, R.B., 2018. Metals and arsenic in fish from a ramsar site under past and present human pressures: Consumption risk factors to the local population. Sci. Total Environ. 628–629. http://dx.doi.org/10.1016/j.scitotenv.2018.02.005.

Gusso-Choueri, P.K., Choueri, R.B., de Araújo, G.S., Cruz, A.C.F., Stremel, T., Campos, S., Abessa, D.M., Ribeiro, C.A.O., 2015. Assessing pollution in marine protected areas: the role of a multi-biomarker and multi-organ approach. Environ. Sci. Pollut. Res. 22. http://dx.doi.org/10.1007/s11356-015-4911-y.

Gusso-Choueri, P.K., Choueri, R.B., Santos, G.S., de Araújo, G.S., Cruz, A.C.F., Stremel, T., de Campos, S.X., Cestari, M.M., Ribeiro, C.A.O., de Sousa Abessa, D.M., 2016. Assessing genotoxic effects in fish from a marine protected area influenced by former mining activities and other stressors. Mar. Pollut. Bull. 104. http://dx.doi.org/10.1016/j.marpolbul.2016.01.025.

Hirota, J., Szyper, J.P., 1975. Separation of Total Particulate Carbon Into Inorganic and Organic Components and organic components ' carbon into inorganic of total particulate Separation. Limnol. Oceanogr. 20, 896–900.

IUCN, I.U. for C. of N., 2013. Guidelines for applying protected area management categories including IUCN WCPA best practice guidance on recognising protected areas and assigning management categories and governance types.

Jameson, S.C., Tupper, M.H., Ridley, J.M., 2002. The three screen doors: can marine "protected" areas be effective? Mar. Pollut. Bull. 44, 1177–1183.

Kummer, L., Melo, V.F., Barros, Y., Azevedo, J.C., 2011. Sequential extraction of lead and zinc from soils of heavy metal mining and processing area. Rev. Bras. Ciênc. Solo 35, 2005–2018. http://dx.doi.org/10.1590/S0100-06832011000600017.

Lotufo, G.R., Abessa, D.M.S., 2002. Testes de toxicidade com sedimentos total e água intersticial estuarinos utilizando copépodos bentônicos. In: Nascimento, I.A., Sousa, E.C.P.M., Nipper, M.G. (Eds.), Métodos Em Ecotoxicologia Marinha: Aplicações No Brasil. Artes Gráficas e Indústria Ltda, São Paulo, pp. 151–152.

Luczak, C., Janquin, M.A., Kupka, A., 1997. Simple standard procedure for the routine determination of organic matter in marine sediment. Hydrobiologia 345, 87–94. http://dx.doi.org/10.1023/A:1002902626798.

Mahiques, M., Burone, L., Figueira, R., Lavenére-Wanderley, A., Capellari, B., Rogacheski, C., Barroso, C., Santos, L., Cordero, L., Cussioli, M., 2009. Anthropogenic influences in a lagoonal environment : A multiproxy approach at the valo grande mouth, Cananéia-Iguape system (SE Brazil). Braz. J. Oceanogr. 57, 325–337.

Mahiques, M.M., Figueira, R.C.L., Salaroli, A.B., Alves, D.P.V., Gonçalves, C., 2013. 150 years of anthropogenic metal input in a Biosphere Reserve: the case study of the Cananéia–Iguape coastal system, Southeastern Brazil. Environ. Earth Sci. 68, 1073–1087. http://dx.doi.org/10.1007/s12665-012-1809-6.

Martins, C.C., Doumer, M.E., Gallice, W.C., Dauner, A.L.L., Cabral, A.C., Cardoso, F.D., Dolci, N.N., Camargo, L.M., Ferreira, P.A.L., Figueira, R.C.L., Mangrich, A.S., 2015. Coupling spectroscopic and chromatographic techniques for evaluation of the depositional history of hydrocarbons in a subtropical estuary. Environ. Pollut. 205, 403–414. http://dx.doi.org/10.1016/j.envpol.2015.07.016.

McCave, I.N., Syvitski, J.P.M., 2007. Principles, Methods, and Application of Particle Size Analysis. Cambridge Univ. Press, http://dx.doi.org/10.1017/CBO9780511626142.

Melo, S.L.R., Abessa, D.M.S., 2002. Testes de toxicidade com sedimentos marinhos utilizando anfípodos. In: Nascimento, I., Sousa, E.C.P.M., Nipper, M.G. (Eds.), Ecotoxicologia Marinha: Aplicaçõesno brasil (Cap. XIV). Editora Artes Gráficas, Salvador, pp. 163–178.

Monosson, E., 2005. Chemical mixtures: Considering the evolution of toxicology and chemical assessment. Environ. Health Perspect. 113, 383–390. http://dx.doi.org/10.1289/ehp.6987.

Moraes, R.P., 1997. Transporte de chumbo e metais associados no Rio Ribeira de IguapeTransporte de chumbo e metais associados no Rio Ribeira de Iguape, São Paulo, Brasil.. In: 94f. Dissertação (mestrado) - Universidade Estadual de Campinas, Instituto de Geociencias, Campinas, SP. São Paulo, Brasil, Available in: <http://www.repositorio.unicamp.br/handle/REPOSIP/287199>.

Nasher, E., Heng, L.Y., Zakaria, Z., Surif, S., 2013. Concentrations and sources of polycyclic aromatic hydrocarbons in the seawater around Langkawi Island. Malaysia. J. Chem. 2013, http://dx.doi.org/10.1155/2013/975781.

Nishigima, F.N., Weber, R.R., Bícego, M.C., 2001. Aliphatic and aromatic hydrocarbons in sediments of Santos and Cananéia, SP, Brazil. Mar. Pollut. Bull. 42, 1064–1072.

Perina, F.C., De Souza Abessa, D.M., Pinho, G.L.L., Fillmann, G., 2011. Comparative toxicity of antifouling compounds on the development of sea urchin. Ecotoxicology 20, 1870–1880. http://dx.doi.org/10.1007/s10646-011-0725.

Pozo, K., Lazzerini, D., Perra, G., Volpi, V., Corsolini, S., Focardi, S., 2009. Levels and spatial distribution of polychlorinated biphenyls (PCBs) in superficial sediment from 15 Italian Marine Protected Areas (MPA). Mar. Pollut. Bull. 58, 773–776. http://dx.doi.org/10.1016/j.marpolbul.2009.03.003.

Seckendorff, R.W., Azevedo, V.G., 2007. Abordagem histórica da pesca da tainha Mugil platanus e do parati *Mugil curema* (Perciformes: Mugilidae) no litoral Norte de São Paulo, No. 28. Série Relatórios Técnicos, pp. 1–8.

Terlizzi, A., Delos, A.L., Garaventa, F., Faimali, M., Geraci, S., 2004. Limited effectiveness of marine protected areas: Imposex in *Hexaplex trunculus* (Gastropoda, Muricidae) populations from Italian marine reserves. Mar. Pollut. Bull. 48, 188–192. http://dx.doi.org/10.1016/j.marpolbul.2003.10.019.

Tessler, M., Souza, L., 1998. Dinâmica sedimentar e feições sedimentares identificadas na superfície de fundo do sistema Cananéia-Iguape, SP. Rev. Bras. Ocean. 46, 69–83. http://dx.doi.org/10.1590/S1679-87591998000100006.

Thomas, J.D., Barnard, J.L., 1983. The Platyischnopidae of America (Crustacea: Amphipoda). Smithson. Contrib. Zool. 375, 33.

Tramonte, K.M., Figueira, R.C.L., Majer, A.P., de Lima Ferreira, P.A., Batista, M.F., Ribeiro, A.P., de Mahiques, M.M., 2018. Geochemical behavior, environmental availability, and reconstruction of historical trends of Cu, Pb, and Zn in sediment cores of the Cananéia-Iguape coastal system, Southeastern Brazil. Mar. Pollut. Bull. 127, 1–9. http://dx.doi.org/10.1016/j.marpolbul.2017.11.016.

Volkman, J.K., Holdsworth, D.G., Neill, G.P., Bavor, Jr., H.J., 1992. Identification of natural, anthropogenic and petroleum hydrocarbons in aquatic sediments. Sci. Total Environ. 112, 203–219. http://dx.doi.org/10.1016/0048-9697(92)90188-X.

Wisnieski, E., Ceschim, L.M.M., Martins, C.C., 2016. Validação de um método analítico para determinação de marcadores orgânicos geoquímicos em amostras de sedimentos marinhos. Quim. Nova 39, 1007–1014. http://dx.doi.org/10.5935/0100-4042.20160103.

Yuan, D., Yang, D., Wade, T.L., Qian, Y., 2001. Status of persistent organic pollutants in the sediment from several estuaries in China. Environ. Pollut. 114, 101–111. http://dx.doi.org/10.1016/S0269-7491(00)00200-1.