

ANA CAROLINA FEITOSA CRUZ

Using chemical and ecotoxicological approaches to assess the ecological risk of
pollutants in the Cananéia-Iguape Estuarine Complex, SP, Brazil

Dissertation submitted to the
Oceanographic Institute of São Paulo
University, as part of the requirements to
obtain the title of Master of Science,
Oceanography Program, Biological
Oceanography area.

Supervisor: PhD. Denis Moledo de Sousa Abessa

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Oceanography Institute

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PhD. Denis Moledo de Souza Abessa

Concept

PhD. Rubens Lopes Figueira

Concept

PhD. Rodrigo Brasil Choueri

Concept

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*Vou viajar pra ver o mundo
Vim ver a linha do horizonte
Eu vou botar o pé na estrada
Em direção ao mar... O mar
Entre trilhas e cachoeiras
Eu quero ver o sol se por
E contemplar um céu de estrelas
Com meu amor... Só com o meu amor
Tudo que eu quero é te levar daqui
Poder te ver cantar
Poder te ver sorrir
Sentados na areia de frente pro mar
Ao som do violão, você dançando linda
E mesmo que o dia, não seja tão bonito assim
Seja do jeito que for, quero ter você perto de mim
Amor é tudo que eu tenho pra lhe dar
O mundo fica mais bonito
Quando vejo refletido em seu olhar!
(Paulo Amoedo)*

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Abstract

This study aimed to study the quality of sediments of the Cananéia-Iguape-Peruíbe estuarine complex (CIP) by using chemical and ecotoxicological analysis, in order to determine if metals from sediments would be bioavailable to benthic organisms. The different tools show that the estuary exhibits signs of alteration due to contamination by metals, which, in their turn, have their origin in mining activities situated upstream of Ribeira de Iguape River. Toxicities were attributed to metals and ammonia suggesting that the combination of natural and anthropic factors may be capable of producing ecological risks. Additionally, this study evidenced that depositional areas appear to move along the estuary, accompanying the displacement of the maximum turbidity zone, which in its turn depends on the freshwater inputs associate to seasonality. These results highlight that oceanographic processes should be considered as important factors that influence on the accumulation of contaminants in estuarine areas. The bioaccumulation test confirmed that these contaminants may be bioavailable to the organisms, especially in the more contaminated sites, and therefore, may be transferred to the food chain, with implications to ecological processes and human health.

Keywords: Contaminants, TIE, bioaccumulation, Cananeia-Iguape, estuary, sediment

Resumo

Esse estudo teve como objetivo avaliar os sedimentos do Complexo Estuarino Lagunar Cananéia-Iguape-Peruíbe (CIP) através da utilização de análises químicas e ecotóxicológicas, com a finalidade de determinar se os metais dos sedimentos estavam biodisponíveis para organismos bentônicos. As diferentes ferramentas mostraram que o estuário possui mudanças geradas pela contaminação por metais, provenientes de atividades de mineração situadas no alto do Rio Ribeira de Iguape. A toxicidade foi atribuída principalmente aos metais e a amônia, sugerindo que a combinação de fatores naturais e antrópicos são capazes de produzir risco ecológico. Esse estudo evidencia também que ao longo dos estuários, as áreas de deposição mudam acompanhando o deslocamento da zona máxima de turbidez, que está associada com entradas de água doce, regime de chuvas e conseqüentemente a sazonalidade. Dessa forma, os processos oceanográficos devem ser considerados importantes fatores que influenciam a acumulação de contaminantes em áreas estuarinas. O teste de bioacumulação mostra que esses contaminantes estão biodisponíveis aos organismos, especialmente em áreas mais contaminadas e, portanto, podem ser transferidos ao longo da cadeia alimentar, com implicações nos processos ecológicos e na saúde humana.

Palavras chave: Contaminantes, TIE, bioacumulação, Cananéia-Iguape, estuário, sedimento

Chapter 1 – Introduction

In Brazil, the specially protected areas are established by a wide legal framework, comprising the permanent preservation areas (PPA), such as riparian forests, mangroves, hillside areas and mountain tops; the legal reserves (a percent of the property which must be preserved, depending on the vegetal coverage); and the legally Protected Areas (PA) (Milano 2003). According to the Brazilian legislation, PAs consist in areas with natural characteristics and special attributes for conservation, which are under a special administration regime, and are legally instituted by the public power (IBAMA 2002). The designation of such areas aims to maintain the natural biodiversity and landscapes, to promote environmental education, and to preserve water resources, among other objectives (Cegana and Takahashi 2003).

The National System of Protected Areas (known as SNUC) was established by the federal law nº 9.985 on July 18th, 2000 (BRAZIL 2000), and concerns to the norms for creating and implementing the PAs in the country, as well as defining the different categories of PAs and providing some guidelines for their management. According to the SNUC, PAs can be divided into two main groups: the Strict-Use PAs and the Sustainable-Use PAs. The main purpose of the Strict-Use Protected Areas is the preservation of nature with the indirect use of natural resources, whereas the aim of the Sustainable-Use protected areas is the conservation of nature in harmony with the sustainable use of their natural resources.

The Environmental Protected Area (EPA) consists in a category of Sustainable-Use PA focused on the development and sustainable management of the natural resources, by means of planning, disciplining and ordering the economic activities (BRAZIL 2000). The EPAs are used to preserve the natural heritage and concomitantly to promote the improvement of the quality of life of local population, through a participative management (Maretti 1994; Moraes 2004).

The coastal zone of São Paulo State presents a wide system of terrestrial (coastal) and Marine Protected Areas (MPA) which altogether represent an almost continuous mosaic of PAs (Fig. 1). Among the PAs

comprised by this system, the Cananéia, Iguape and Peruíbe Protected Area (CIP-PA), and a set of Marine Protected Areas are of main concern, covering almost the entire coastal area of the state.

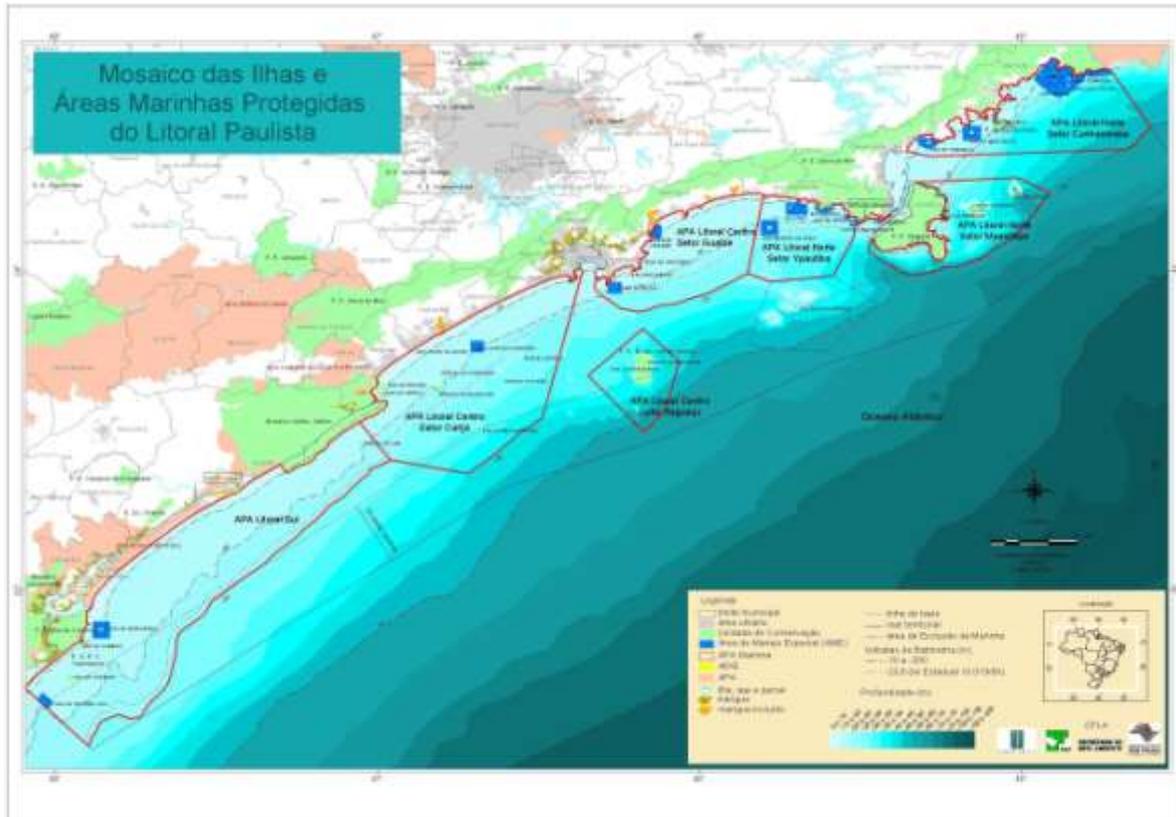


Fig. 1 Continuous mosaic of Protected Areas on the coast of São Paulo state. Source: Environment Secretary from São Paulo State.

The CIP-PA is located in the area known as Estuarine Complex of Iguape and Cananéia (or Lagamar), which is within the UNESCO Biosphere Reserve of Atlantic Rainforest and includes the cities of Cananéia, Iguape, Peruíbe, Itariri, Pedro de Toledo and Miracatu. This PA was created by the federal decree nº 90.347 in 1984 (Maretti 1994). This region is considered among the five more productive world's estuary and is of extreme priority for biodiversity conservation (Maretti 1994). Moreover, it has great relevance for the state Coastal Management Program (SMA 1997).

The Lagamar region presents its higher pluviometric rates from January to March (summer), with the monthly average of 266.9 mm and the lowest rate

from July to August, with a monthly average of 95.3 mm; and the mean annual pluviometric rate is about 2300 mm (Mishima 1985; Silva 1989). In summary, summer season is generally very hot and rainy, whereas winter season is cool and less rainy (Lima et al. 2007). The water circulation in its estuarine portion is predominantly generated by semidiurnal tides (Myao and Harari 1989), but it is also influenced by the contribution of a wide net of rivers, especially the Ribeira de Iguape River (RIR), and, in a smaller scale by the wind (Miranda et al. 1995). The estuarine connections to the sea (known as “*barras*”) as well as the Ilha Comprida (Comprida Island) are influenced by waves; swells from SW to NE may send waves that may penetrate into the Barra de Cananéia, Barra do Ararapira and Barra de Icapara, causing some influence inside the estuarine zone (Fig. 2).

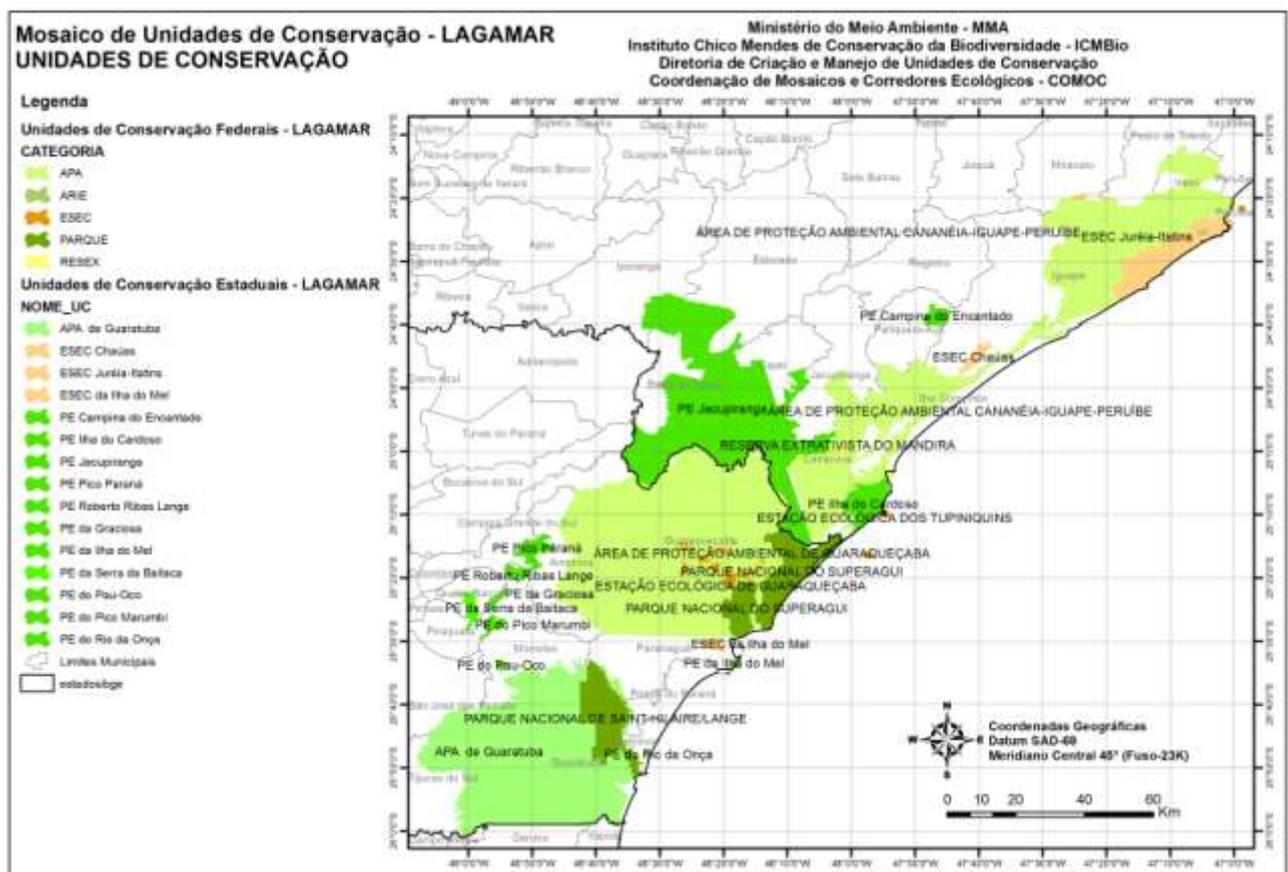


Fig. 2 Continuous mosaic of Protected Areas - Lagamar. Source: Ministry of Environment.

In terms of social and economic status, this region presents an economy based mainly on tourism, fishing, oyster farming and extraction, and subsistence agriculture. The indicators of education and family incomes are relatively low, as do the municipal gross domestic product (GDP); in addition, the public sanitation and health systems are not suitable and a relevant number of inhabitations are not attended by sewage collection systems (Morais and Abessa in press; Mendonça 2007; Chamy and Maldonato s/d).

According to CETESB (2009), the quality of waters and sediments in Cananeia was good, which was attributed to the low contaminants inputs, small resident population and little touristic activities. Azevedo et al (2009a; 2009b; 2011; 2012a; 2012b) conducted a set of analyses with fish, using chemical techniques and biomarkers, and suggested that the Cananéia region could be entirely considered as unpolluted, with low anthropogenic influence and thus its natural conditions would not cause risks or damage to the biota or to humans. According to these authors, CIP-PA could be considered as a reference clean area. Amorim et al. (2008) analyzed sediments from several stations in this region and observed low concentrations of metals in some estuarine portions, suggesting that the estuary presented natural conditions. On the other hand, other stations presented higher values for most of the analyzed elements and some metals such as As and Cr, were found in concentrations exceeding the TEL (Threshold effect level) values, when compared to the Canadian Sediment Quality Guidelines (Smith et al. 1996). The authors attributed these higher concentrations to the presence of fines and organic matter.

However, other studies showed that this system has passed through some significant changes during the last 150 years (Mahiques et al. 2009), the main of them related to the construction of a navigation channel (named Valo Grande). This channel aimed to reduce the navigation distance between the inner portion of RIR and the former port of Iguape, in order to facilitate the commerce. However, the channel was eroded to 300m width and the Valo Grande began to be the main watercourse, discharging approximately 70% of RIR waters within the estuarine complex (specifically, at Mar Pequeno region). Consequences to estuarine environment were dramatic, as the volume of discharged freshwater was very high, modifying the salinity, and because they

carried large amounts of suspended sediments, which started depositing in some estuarine areas.

Thus, Ribeira Iguape River became the main contributor of suspended sediments, nutrients and metals to this system (Freitas et al. 2006). Some studies showed that the region of Iguape had a poor quality due to the introduction of metals from mining origins into the Ribeira river (CETESB 2009). Azevedo et al. (2012c) observed the presence of metals in the tissue of fishes, such as Pb, Cd, Hg and suggested that the higher concentrations were related to the proximity of estuary to an abandoned gold mine and the location of a fertilizer factory in the city of Iguape. At the highest portion of the Ribeira river a strong metal contamination in sediments was detected, due to the release of wastes from mining and metallurgy (Guimarães and Sígolo 2008). These metals are retained in suspended material and end up being deposited in estuarine area, producing environmental contamination (Guimarães and Sígolo 2008; Rodrigues et al. 2012; Melo et al. 2012). The concentrations of lead in sediments collected close to Iguape were comparable to those found in industrialized areas, as the Santos Estuary (SP) and the Aratu Bay, located in Bahia State (Aguiar et al. 2008; Mahiques et al. 2009; Cotrim 2006; Saito 2002). Guimarães and Sígolo (2008) observed high levels of lead and zinc in waters and suspended sediments from RIR, lead levels being 34 times higher than the permitted levels.

Furthermore, according to the study realized by Cruz et al. (submitted), sediments in this region showed systematically chronic toxicity, which was attributed to the influence of RIR along the most of the estuarine complex. This study also observed acute sediment toxicity close to city of Cananeia, indicating an additional influence of local sources of contaminants to the environmental degradation, possibly due to sewage, contaminated urban drainage, oil and antifouling paints, among others.

Urban centers can be installed near of Bays and estuaries, and as consequence they are more vulnerable to the impact caused by pollution (Pereira and Soares-Gomes 2002). Moreover, these water bodies have a restrict flow and are not capable of diluting and dispersing the contaminants,

which tend to settle into the bottom sediments, accumulating in this ecological compartment and consequently causing toxic effects to the aquatic organisms (Cruz 2010; Pozo 2011). Thus, sediments have been used as important indicators of the aquatic ecosystem quality (Power and Chapman 1995). Countless chemical, physic and biological processes can cause the release of contaminated substances to the water column or to the biota, producing ecological risks (Zamboni 1993).

The presence of uncontrolled contamination sources, and the evidences of environmental contamination and their potential effects to biota (e.g., through toxicity) suggest the CIP-PA is getting impacted by metals and display an alert that further studies must be conducted, especially because this area is preserved and considered a world heritage site. In this sense, chemical and ecotoxicological approaches have been widely applied aiming to evaluate the environmental contamination and its biological effects (Adams et al. 1992, Araujo et al. 2013). They provide information on the environmental quality considering the effect of the discharged contaminants and help to define critical and/or priority areas for protection or recovery (Abessa et al. 2008).

Chemical analyzes identify and quantify the concentrations of toxic substances, whereas toxicity test evaluate the potential effects that these substances can cause on biological systems (Báez et al. 2004). Thus, for environmental assessment and monitoring, chemical analyses and toxicity tests should be conducted together, in order to provide robust, reliable and complimentary information on environmental quality. Since the classic chemical and ecotoxicological approaches not always provide conclusive information for environmental management, some studies require the use of advanced ecotoxicological techniques. Among them, the toxicity identification evaluation (TIE) approach relies on the use of physical manipulation of the samples in order to remove, immobilize or modify the toxicity of some chemicals, in such way that it becomes possible to determine the main chemical groups that are responsible by the toxicity. The TIE can be divided into three phases: characterization (Phase 1); identification (Phase 2); confirmation (Phase 3) (USEPA 2007). During phase 1, the manipulation is made to remove toxic groups or raise the reactivity of any present chemical group, which change the

toxicity profile of the tested sample. Phase 2 consists in analyzing the chemicals from the groups which were identified in phase 1, and the third phase consists in returning the contaminants to sample in order to confirm if toxicity is back at the end of the manipulation. This approach has been used worldwide for liquid samples (mainly effluents) (Rachid 2002), but has not being intensively used for whole-sediment as well (Araujo et al. 2013).

CIP-PA has contamination by metals that comes from RIR, which would accumulate in Gradient from the VG, and would be affecting the environmental quality because the metals were bioavailable and could cause toxicity. Considering the well-known capacity that the estuaries have to retain and accumulate contaminants in their sediments (Chapman and Wang 2001), and due to the ecological and economic importance of CIP-PA, in a more detailed environmental assessment is required to this region, in order to estimate the ecological risk of pollutants.

Thus, this study was divided into two chapters: the first one consisted of a study on sediment quality that aimed to evaluate the contamination of sediments by metals, organic compounds and ammonia and the possible toxic effects, including the determination of which chemicals could be responsible for the observed effects. In the second chapter, the objective was to evaluate if metals from sediment would be bioavailable to benthic organisms, through the use of a new technique of laboratorial assays for evaluating bioaccumulation.

Capítulo 2 – Ecotoxicological approach

Ecotoxicological assessment of sediments from a Protected Coastal Area in Brazil (Cananeia and Iguape, SP)

Abstract

This study aims to evaluate the sediment quality in the CIP Protected Area. Chemical analysis, ecotoxicological tests, whole-sediment TIE, grain size analysis, and the analyses of organic matter and carbonate concentrations were performed. The sediments were predominantly composed of fine sand and mud. Higher concentrations of metals in the sediments from P1 and P2 were found, and in SEM/AVS analyses, the P1, P3, and P5 stations were found to have the potential to cause toxicity. Toxicity tests showed toxicity in the P1, P2, P3, and P4 stations. The PCA revealed associations between mud, metals and toxicity. TIE reduced the toxicity from metals and ammonia. Metals from upper the RIR are transported to CIP-PA and accumulate in the sediments at potentially toxic levels. Contamination was associated with muddy sediments, which is consistent with the fact that most of the suspended particulates in RIR come from mining residue. The location of extremely muddy and contaminated sediments appears to move along the estuary, accompanying the displacement of the maximum turbidity zone, which, in turn, depends on seasonal freshwater inputs. Thus, the most contaminated and toxic areas are not necessarily those close to the primary sources, but are actually those with finer sediments. Thus, actions are necessary to ensure control of the sources of contaminants in this area.

Keywords: contamination, TIE, Cananeia-Iguape, estuary, sediment, SEM/AVS

1. Introduction

A Protected Area (PA) is defined as “an area of land and/or sea especially dedicated to the protection and maintenance of biological diversity, and of natural and associated cultural resources, and managed through legal or other effective means” (McNeely et al. 1994). Marine and estuarine protected

areas have been established with varied objectives, which include protecting fish stocks, vulnerable habitats, endangered species and breeding areas, as well as reducing impacts from human populations (IUCN 1994). However, such Protected Areas (PAs) have frequently been ineffective in protecting aquatic organisms (Kelleher et al. 1995; Jameson et al. 2002) for several reasons, one of which is aquatic pollution (Gubbay 2005). Contamination generated from external sources may be transported into the marine protected areas, (MPAs) (Poza et al. 2009; Terlizzi et al. 2004) and may then have negative effects on both the biota (Pereira et al. 2012) and the sediment quality (Araujo et al. 2013; Nilin et al. 2013). However, few studies have focused on pollution as an important factor threatening biodiversity within marine and estuarine PAs.

The Cananéia Iguape Peruíbe Protected Area (CIP-PA) consists of a wide estuarine complex (CIP) that is located on the south coast of São Paulo state (southeastern Brazil), and which is comprised of a set of fragile ecosystems that require protection, especially mangroves, mudflats and other wetlands. This region has experienced some significant changes over the past 150 years. For example, it now receives part of the contribution of the Ribeira de Iguape River (RIR) after the construction of an artificial channel (named “Valo Grande”) that connects the river to the inner region of the estuary (Mahiques et al. 2009). This channel caused approximately 70% of RIR water flux to flow into the estuarine complex, a change which has modified the freshwater-saltwater balance within the estuary and discharged large amounts of suspended solids in the area. Because residues from former mining activities are deposited on the riverbanks from the upper portion of the RIR (Guimarães and Sígolo 2008; Kummer et al. 2011), unknown amounts of metals started to be continuously introduced into the river. The RIR thus represents a major contributor of both nutrients and contaminants to the CIP estuarine complex, particularly metals (Mahiques et al. 2009).

As a consequence, metals have accumulated in the sediments of the CIP-PA (Aguiar et al. 2008; Mahiques et al. 2009), and some elements have previously been found in concentrations that are comparable to those observed in polluted industrial areas, such as the Santos Estuarine System (Abessa et al. 2008) and Aratu Bay in Bahia state, Brazil (Onofre et al. 2007).

Sediment contamination represents a threat to aquatic organisms, because contaminants may accumulate in the biota, thus producing toxicity. They may also be transferred through the trophic chain, which causes risks for both ecosystems and public health. Thus, sediment quality has been used as an important indicator of the health of aquatic ecosystems (Power and Chapman 1995).

In this context, integrative studies involving different lines of evidence such as ecotoxicological and chemical approaches have been used to assess sediment quality in aquatic environments, with implications for ecosystem conservation (Araujo et al. 2013). Chemistry provides information on the degree and nature of the contamination, whereas ecotoxicological methods detect the occurrence of potential biological effects (Adams et al. 1992; Costa et al. 2008; Choueri et al. 2009; Petrovic and Barcelo 2004). Advanced techniques, such as the toxicity identification evaluation (TIE) approach, could also be used to determine the chemical compounds responsible for toxicity. Sediment TIEs involve some procedures that are designed to decrease, increase, or transform the bioavailable fractions of sediment contaminants in order to assess their contributions to the sample toxicity (Burgess et al. 2000). This technique has proven to be an effective tool for identifying chemicals responsible for sediment toxicity in environmental assessments (eg, Carr et al. 2001; Kwok et al. 2005; Araújo et al. 2006; Picone et al. 2009; Macken et al. 2009; Weston and Lidy 2010;. Anderson et al. 2010). However, its employment in tropical marine sediments is still incipient.

In light of the conservation purposes of PAs, this study aims to evaluate the sediment quality in the CIP-PA using geochemical and ecotoxicological analyses with benthic organisms, as well as whole-sediment TIE (phase I) in order to identify the main chemical groups responsible for toxicity.

2. Materials and methods

2.1. Study area

The study area was located in the CIP Estuarine Complex, on the south coast of São Paulo State, Brazil, which includes the cities of Iguape, Cananéia, and Ilha Comprida. This region is of international importance, and was included

within the Atlantic Rainforest Biosphere Reserve, according to the UNESCO. In Brazil, the national legislation establishes different categories of PAs (Brasil 2000). In the case of the CIP-PA, its PA category seeks to achieve a sustainable balance between the human need for natural resources and the protection of the natural ecosystems, landscapes, and resources from degradation (Moraes 2004).

The CIP region is mainly composed of barrier islands and estuarine channels, which form a complex net of water bodies, the banks of which are occupied by well-developed mangrove forests (Schaeffer-Novelli et al. 1990). The hydrodynamic circulation of the area is influenced by tidal waves and the contributions of several rivers (Miranda et al. 1995; Myao and Harari 1989), particularly the Ribeira de Iguape River (RIR).

Despite its high protection status, this region is the least developed region in the state of São Paulo in socio-economic terms, and it ranks low in health and sanitation infrastructure and services, education levels among residents, and household incomes. Official reports (CETESB 2009) indicated satisfactory environmental conditions in the CIP, including water and sediment quality, and they attributed the conditions to the small local population and a lack of tourist activity. However, as mentioned previously, studies have found evidence of contamination by metals in the sediments of this region (Aguiar et al. 2008; Guimarães and Sígolo 2008; Mahiques et al. 2009).

2.2. Sediment sampling

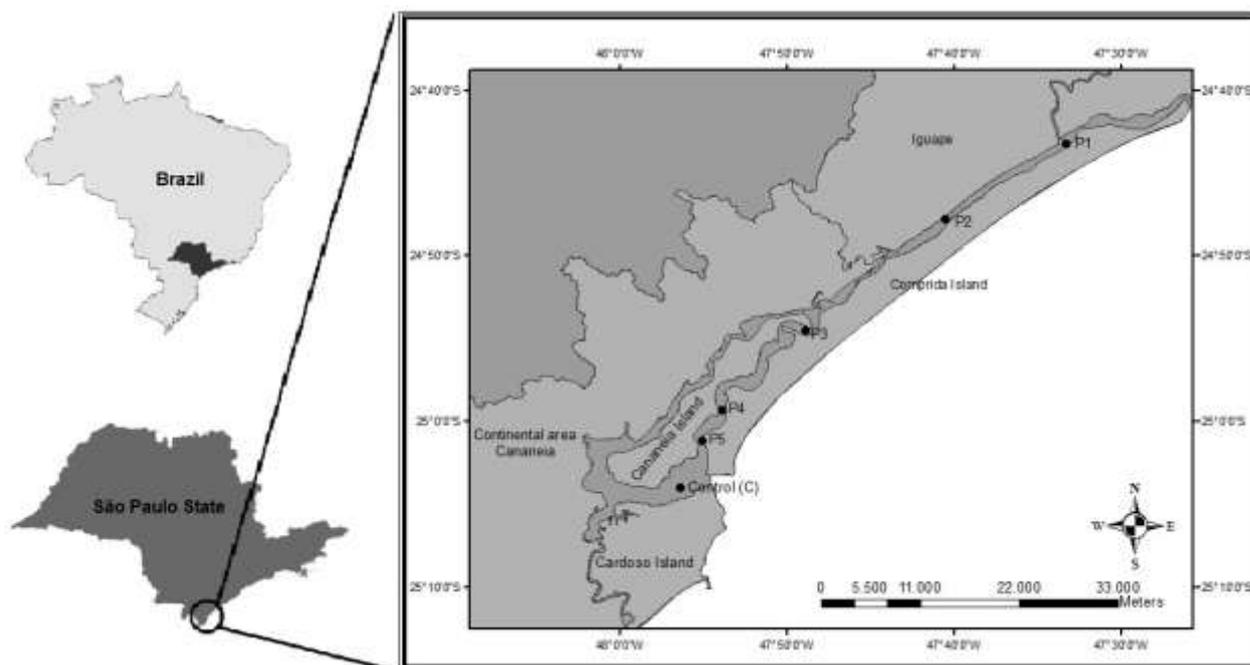


Fig. 3 Map showing the sediment collection stations around the study area within the CIP-PA, Brazil.

The sampling was conducted at 6 stations located from near the Valo Grande channel, where influence of contaminant sources is expected to be more intense, to Cardoso Island (considered the control area), as shown in fig. 3, table 1. The sampling surveys were conducted at three different times in order to observe temporal changes: May 2012, August 2012, and March 2013. In the May 2012 survey, the sample from P5 was not collected. Sediment samples were collected using a 0.036-m² stainless steel Van Veen grab sampler. In the laboratory, aliquots for toxicity tests were kept refrigerated at 4°C, while those for geochemical tests were stored frozen at -20°C.

Table 1 Geographic Coordinates of the collection stations

Station	Latitude	Longitude
P1	24°44.509'S	47°35.984'W
P2	24°49.294'S	47°42.186'W
P3	24°54.124'S	47°48.547'W
P4	25°00.167'S	47°54.104'W
P5	25°02.689'S	47°54.880'W
Control	25°04.281'S	47°55.839'W

2.3. Sediment properties

Sediment grain size distribution was analyzed based on the protocol proposed by Mudroch and MacKnight (1994). Approximately 30g of previously dried sediment were wet sieved through a 0.062- μm mesh to separate fine particles (mud and clay). The material retained on the sieve was then dried and weighed. Initial and final weight differences shows the percentage of mud and clay. Subsequently, the sandy material retained on the 0.062- μm mesh was sieved into different meshes (ϕ scale) in order to separate different classes of sands, and the results were further classified based on the Wentworth scale. The calcium carbonate (CaCO_3) contents in each sample were measured using the method described by Hirota and Szyper (1976), which consists of separating fractions of 5g of each sediment sample and then adding 10ml of hydrochloric acid (5N HCl) for 24 hours to eliminate calcium carbonates. Next, samples were washed with distilled water and dried at 60°C. The difference between initial and final weights showed the amount of CaCO_3 in the samples. Organic matter (OM) contents in the sediment samples were estimated using the ignition method (Luczak et al. 1997), which consists of separating 5g of dry sediment aliquots from each sample and incinerating them in a muffle (500°C) for 4 hours. Organic Matter contents were established by calculating the difference between initial and final weights.

2.4. SEM/AVS

This method consists of the procedures for the determination of acid volatile sulfides (AVS) and selected metals that are solubilized by a weak acidification (simultaneously extracted metals, or SEM). The AVS may control the bioavailability of metals in anoxic sediments (DiToro et al. 1990).

The acid extraction of metals from sediments in order to determine SEM/AVS was performed based on the technique adopted by the USEPA (Allen et al. 1991; 1993; Machado et al. 2004) for wet sediment samples. The procedures for AVS extraction include an attack with hydrochloric acid (HCl 6M). The sulfides in the sample are converted into hydrogen sulfide (H₂S) by a reaction with cold HCl. The H₂S produced reacts with DMPD (N,N-dimethylphenyl-p-diamine) in the presence of ferric chloride (FeCl₃) and is then quantified using UV–visible spectrophotometry. The remaining solution after the removal of sulfides is filtered, and the targeted dissolved metals (Cd, Cu, Ni, Pb, Zn, Cr, Fe and Mn) are determined using ICPOES (induced coupled plasma – optical emission spectrophotometry; Spectro, model Spectro Ultima 2). Finally, the SEM/AVS ratio is calculated using the molar concentrations of AVS and the target SEMs (Cd, Cu, Ni, Pb and Zn), following DiToro et al. (1992). When the ratio values are above 1, the sediment presents potential for causing toxicity, while sediment samples with values below 1 are unlikely to exhibit toxicity from SEMs.

2.5. Ecotoxicological tests

Three approaches were used to assess the sediment toxicity: the chronic toxicity test of whole-sediments using the benthic copepod *Nitocra* sp., the chronic test of sediment-water interface (SWI) with the sea urchin *Lytechinus variegatus*, and acute toxicity tests of whole-sediments with the burrowing amphipod *Tiburonella viscana*. For all tests, physical-chemical parameters (pH, salinity, dissolved oxygen, and temperature) of the overlying water within the test chambers were checked at the beginning and end of each experiment.

The whole sediment chronic toxicity assay with the benthic copepod *Nitocra* sp., which had been reared in the laboratory, was based on the protocol developed by Lotufo and Abessa (2002). Four replicates were set up for each sample, and 10-ml high-density polyethylene flasks were used as test-chambers; they were filled with 2ml of sediment and 8ml of filtered sea water. Five healthy ovigerous females were introduced into each replicate. The entire test system was incubated at $25\pm 2^{\circ}\text{C}$ for 7 days with a photoperiod of 16:8 light:dark and salinity 17. The content of each replicate was then fixed with formaldehyde (10%) and Rose-Bengal dye (0.1%). Finally, the numbers of adult females and their offspring (nauplii and copepodits) were counted using a stereomicroscope.

The acute sediment toxicity test with *T. viscana* (Thomas and Barnard 1983) was conducted following the protocol described by Melo and Abessa (2002) and ABNT (2008). The amphipods used in this test were collected at Engenho d'água Beach, in Ilhabela, São Paulo ($23^{\circ}48' \text{ S} - 45^{\circ}22' \text{ W}$). In this test, four replicates of each sediment sample were prepared in 1-L polyethylene test chambers that contained a 2-cm layer of sediment and 750ml of dilution seawater. After 24 hours, ten healthy adult and non ovigerous amphipods were introduced into each test chamber. The experiment lasted 10 days, and was kept under constant lightning, under constant aeration, and at $25\pm 2^{\circ}\text{C}$. 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.

The chronic test of the SWI followed the method described by ABNT (2006), and the reduced volumes proposed by Cesar et al. (2004) were adopted. Adult sea urchin specimens of the species *Lytechinus variegatus*, were obtained by snorkeling at Palmas Island, Guarujá, SP, and used as broodstock. Approximately 2 g of sediment were transferred to glass tubes (15 mL), and 8 mL of filtered seawater were then added (4 replicates/sample). To prevent direct contact between the embryos and the sediment, a 45- μm mesh was introduced in each tube and placed on the surface of the sediment. The tubes were allowed to stabilize for 24 h before exposure. The same procedure was made with control tubes, which contained clean seawater (control water) or a 45- μm mesh and water (control mesh).

The gametes were obtained using osmotic induction (0.5 M KCl), and the ovules were then fertilized by adding an aliquot of sperm solution containing activated sperm cells. Next, the fertilization success was checked through the observation of control samples under a microscope, and >90% fertilization was considered successful. For the toxicity test, approximately 500 eggs were added to each chamber and then incubated for 24 h at a constant temperature ($25 \pm 2^\circ\text{C}$) and under a 16h light/ 8h dark photoperiod. Next, the contents of each chamber were transferred to other vessels to preserve the larvae (500 mL formaldehyde). Then, 100 embryos were counted for each replicate, and the percentage of normal embryos was calculated. Normal plutei were defined based on typical larval development; the branch symmetry, shape and size of the skeleton were considered in the definition (Perina et al. 2011).

The results of the ecotoxicological tests were first checked for homocedasticity and normality by Bartlett's and Shapiro-Wilks tests, respectively. All samples were then compared to the reference sediment using unpaired Student-t'-test, with the help of Prism software. When significant differences in the toxicity endpoints were detected between the references and the test samples, the sediments were considered toxic; an absence of a statistical difference indicated non-toxic sediments.

2.6. Exploratory analyses

Geochemical and ecotoxicological data were integrated using exploratory techniques, including cluster analysis (Ward's method) and principal component analysis (PCA), with the help of the PAST software (Hammer et al. 2001) after log-transformation and/or arc-sen \sqrt{x} transformation of data prior to the analysis.

2.7. Whole-sediment TIE

The whole-sediment TIE – Phase I treatments were prepared based on the protocol developed by the USEPA (2007). The P2 sample was selected for

these techniques because this sediment exhibited the most frequent toxicity. To remove ammonia, treatment with *Ulva lactuca* was employed: the algae were collected in clean areas and kept under constant aeration and in a photoperiod of 16:8 h (light: dark) for four days. Then, 0.6 g of the algae were placed and kept in the test chambers for 24 h and were removed prior to the beginning of the test. Fenton's reagent was used to remove metals with the proportion of 1 mmol of Fe^{+2} to 50 mmol of H_2O_2 , i.e. by adding 0.018g of Fe^{+2} and 2.7 ml of H_2O_2 to each test chamber. The reaction was maintained for 24 h to achieve equilibrium, and then the reagent was removed. The sample was gently washed with seawater and the chamber was then filled with clean seawater. For organic toxicants, treatment consisted of the use of coconut charcoal. The charcoal was maintained in a vacuum system with deionized water for 18 h. Twenty-four hours before the addition of the organisms, 0.6 g of hydrated charcoal were added to each test chamber. Another treatment for organic toxicants was conducted with activated carbon following the same methodology used with the coconut charcoal. Then, sediments were tested for chronic toxicity to the copepod *Nitocra* sp, according to the protocol developed by Lotufo and Abessa (2002). Data was analyzed using a two-factor analysis of variance (ANOVA) followed by the Bonferroni test at 5% significance level (USEPA 2007).

3. Results

3.1. Sediment properties

In the first sampling survey, the sediment textural analyses revealed that the collected sediments were predominantly sandy (>80% sands) and were mainly composed of very fine sands, with exception of the sample from P1, which contained 55% mud. In the second survey (August 2012), most of the samples possessed a predominance of sand (>58%) except for the sample from P2, which was mainly composed of mud (>99%). Figure 4 shows that, in the last sampling survey (March 2013), samples from P1, P3, P5 and control stations were predominantly sandy (>80% sand), whereas sediments from P2 and P4 were muddy (>59%).

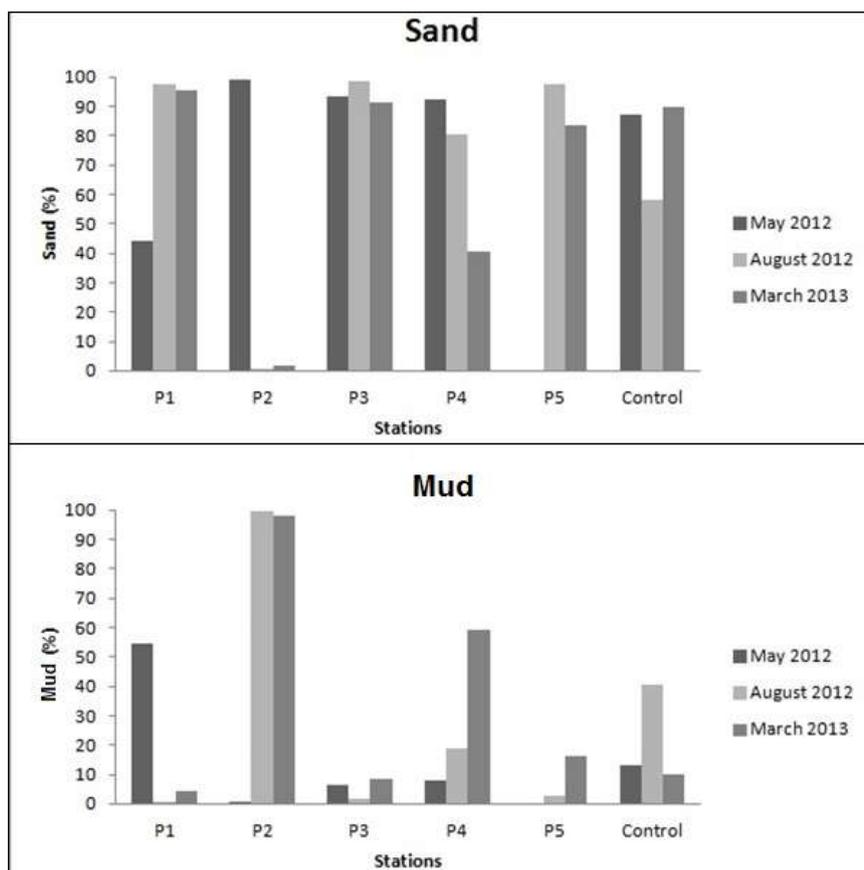


Fig. 4 Contents of Mud and Sand in sediments from the CIP-PA (São Paulo, Brazil).

The CaCO_3 contents were generally low (between $<QL$ and 16.60%), whereas OM amounts ranged from 0.20% to 16.80% (Table 2).

Table 2 Contents of Calcium Carbonate (%) and Organic Matter (%) in sediments from CIP-PA.

Station	May 2012		August 2012		March 2013	
	CaCO_3	OM	CaCO_3	OM	CaCO_3	OM
P1	5.83	7.16	4.50	0.47	0.00	0.20
P2	0.95	0.26	16.60	14.46	8.20	13.80
P3	2.99	3.83	2.64	0.60	1.60	0.60
P4	2.56	2.14	12.15	4.33	8.80	0.60
P5	NC	NC	9.17	1.22	5.00	16.80
Control	7.62	5.33	9.45	5.44	1.83	2.00

3.2. Sediment chemistry

The concentrations of metal in the sediment samples are presented in Fig. 5. Cd levels ranged from 0.008 to 0.283 mg/kg (Fig. 5a), and Cr concentrations ranged from 0.094 to 11.206 mg/kg (Fig. 5b). Cu levels ranged from 0.026 to 16.065 mg/kg (Fig. 5c), and iron levels ranged from 122.246 to 22308.769 mg/kg (Fig. 5d). Pb levels ranged from 0.857 to 51.628 mg/kg (Fig. 5e), and the Mn concentrations were between 0.200 and 387.408 mg/kg (Figure 5f). Levels of Ni ranged from 0.010 to 1.264 mg/kg (Fig. 5g), while Zn concentrations were between 0.040 and 37.014 mg/kg (Fig. 5h).

The results show that there was a trend of higher concentrations of metals occurring in the sediments from P1 and P2; this difference may be related to the proximity of these stations to the Valo Grande channel and, consequently, to the Ribeira River and its influence. Sediments from P4 exhibited intermediate values compared to the other stations. Figure 5 shows the temporal variation of metal concentrations for each station, and it reveals the occurrence of large variations over time. These variations were likely due to changes in the percentages of mud and organic matter, since metals were associated with fines and OM contents (see the results of the PCA analyses). For some elements (Cd, Cr, Cu, Fe, Pb, Mn and Zn), the highest concentrations were observed in the P1 sample in the first survey and in the P2 sample in the second and third surveys. In the case of Ni, the highest values were observed in the sediments from P4 (the first sampling survey). It is important to note that the concentrations of lead in the sediments exceeded the TEL at the P1 and P2 stations (TEL = 30.20 mg/kg; Smith et al, 1996), even considering the fact that this study used a weaker acid extraction than that which was used to derive the Canadian SQGs; therefore, TEL exceedance may indicate a more severe situation of contamination by Pb (Fig. 5).

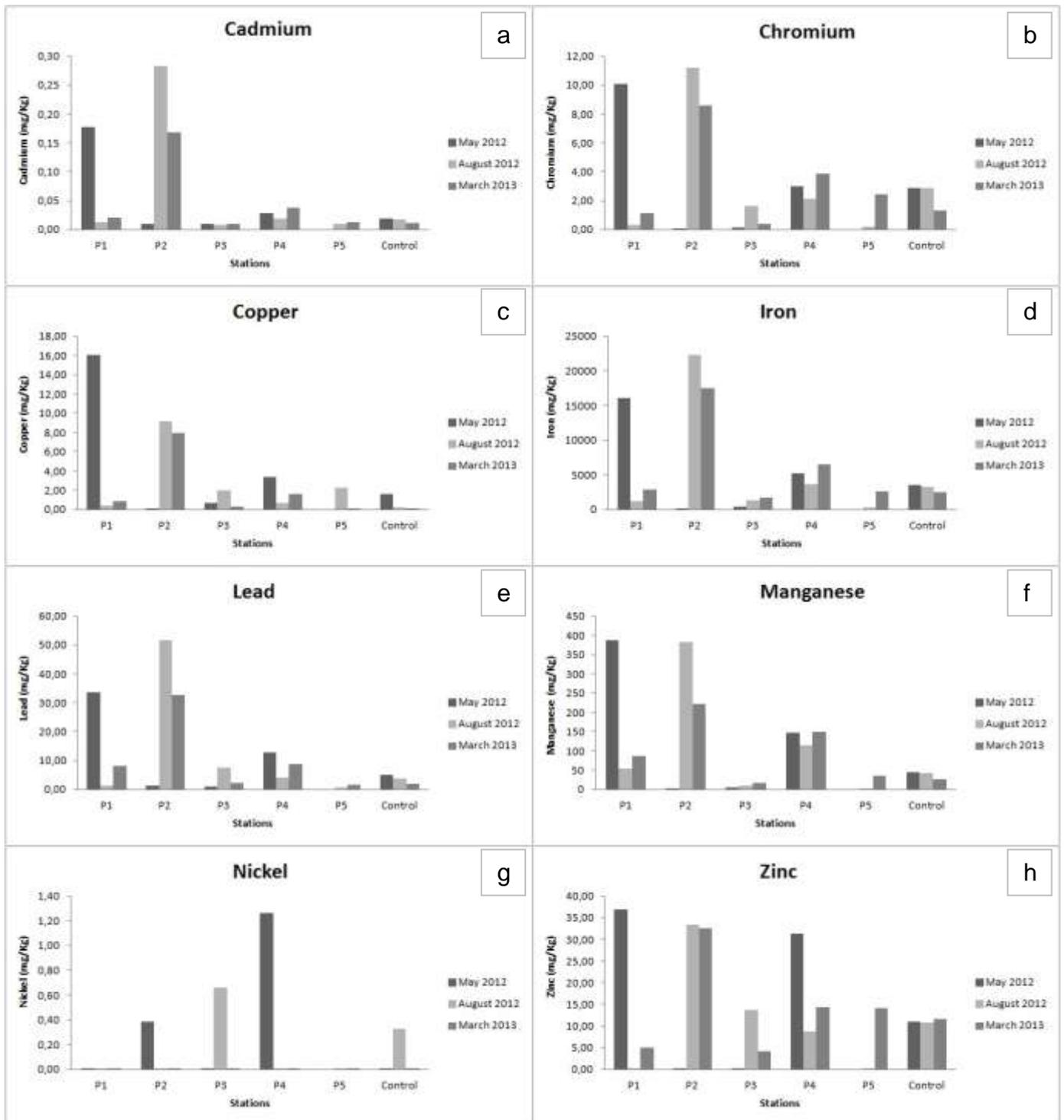


Fig. 5 Concentration of metals in sediment (mg/kg)

Table 3 SEM Contents (mg/kg), AVS Contents (mg/kg) and SEM/AVS Contents in sediments from CIP-PA (NC = not collected).

	May 2012			August 2012			March 2013		
	SEM	AVS	SEM/AVS	SEM	AVS	SEM/AVS	SEM	AVS	SEM/AVS
P1	86.81	8.70	3.62	1.92	-0.66	-0.69	14.24	191.43	0.02
P2	NC	NC	NC	94.48	2097.05	0.01	73.62	636.70	0.04
P3	1.97	-1.81	-0.17	23.77	2.48	5.91	6.69	-0.50	-5.10
P4	1.82	39.02	0.01	13.62	105.71	0.05	24.74	165.67	0.06
P5	48.79	109.70	0.13	3.21	0.75	1.76	15.72	18.41	0.39
Control	17.75	285.35	0.02	15.15	58.96	0.10	13.64	33.03	0.18

The SEM, AVS, and SEM/AVS values are shown in Table 3. SEM values ranged from 0.01 to 94.98 mg/kg. The AVS values ranged from 0.75 to 2097.05 mg/kg. Some negative AVS values were obtained, and they indicate that the AVS had lower values than the blank sample; thus, this method could not be used to determine the true sulfide concentration. In these cases, it was assumed that all of the SEMs would be in the solubilizable form. According to the SEM/AVS values, only the samples from P1 in May 2012, and the samples from P3 and P5 in August 2012 presented high concentrations of metals when compared to sulfides.

3.3. Ecotoxicological tests

3.3.1. *Nitocra* sp.

In the test with copepods, the measured parameters of overlying waters were within acceptable levels in most of the samplings (Appendix A), according to Lotufo and Abessa (2002). Salinities ranged from 14 to 22; pH levels were between 5.89 and 8.01 except for P2 in August 2012 and March 2013, which had lower final pH (4.25 and 4.11 respectively). This acidification in the sediment (pH<6) probably was due to the high levels of Fe and sulfides which, when oxidized, produce H⁺ ions. Also, DO levels ranged from 1.56 to 6.5 mg.L⁻¹,

and low values were observed in all test samples from March 2013 in the beginning of the test ($2.93 \text{ mg.L}^{-1} < \text{values} < 1.56 \text{ mg.L}^{-1}$). However, at the end of the test, values were greater than 3.63 mg/L . In May 2012, significantly low copepod fecundity was observed in P1 and P4 sediments (Fig. 6a). In August 2012, sediment from P2 was considered chronically toxic to the copepods (Fig. 6b). In March 2013, sediments from P2 and P3 were considered significantly toxic when compared to the control (Fig. 6c).

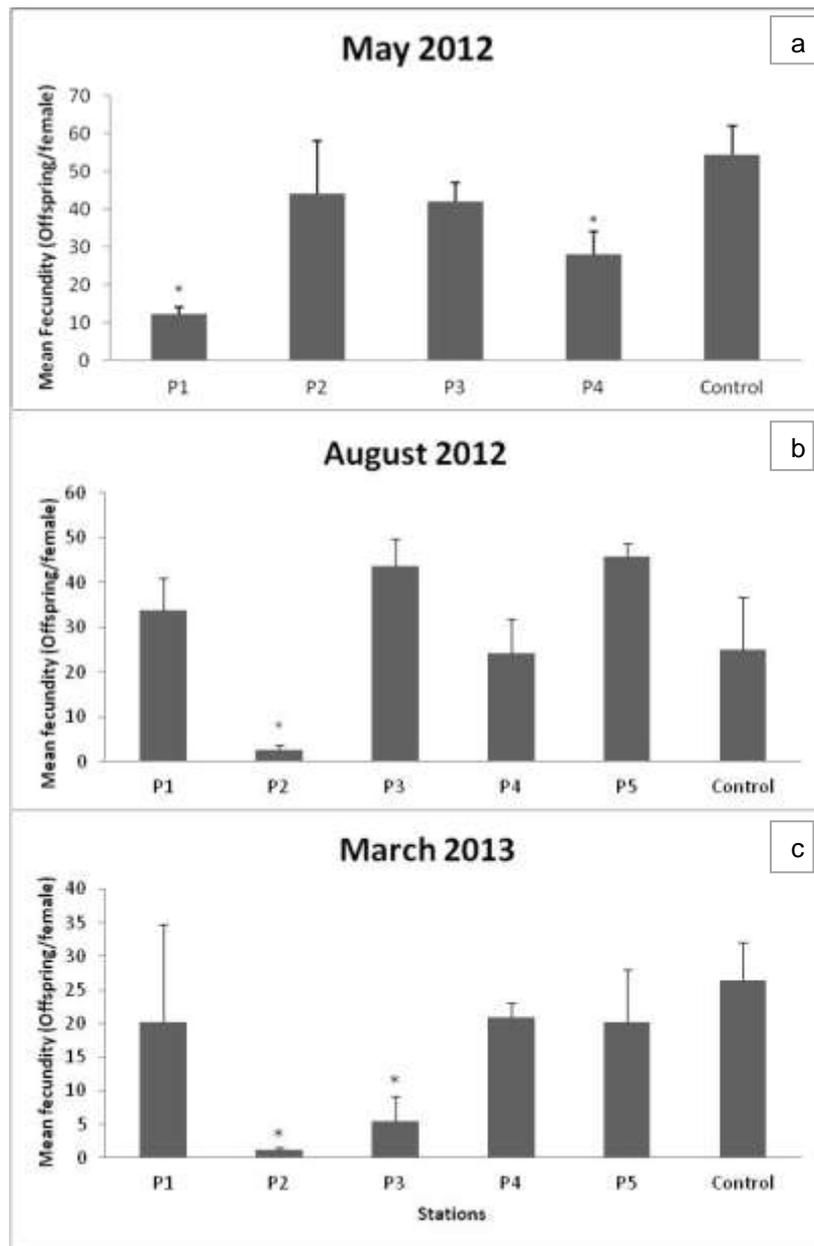


Fig. 6 Mean Fecundity rates of *Nitocra* sp. exposed to sediments from the CIP-PA in three different sampling surveys. Asterisks (*) indicate significant differences relative to the control ($p < 0.05$)

3.3.2. *Tiburonella viscana*

Appendix B shows that, 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 2008). Salinities ranged from 31 to 37; DO levels ranged from 4.65 to 6.85 mg.L⁻¹. The pH values ranged from 7.23 to 8.37, except for P1 in the first sampling (May 2012) and P2 in the third sampling (March 2013), which presented lower final pH values (5.3 and 4.09, respectively). Survival of organisms exposed to the sediments from the P1 station in the first sampling survey and the P2 station in the third sampling were significantly low, and these sediments were considered toxic (Fig. 7).

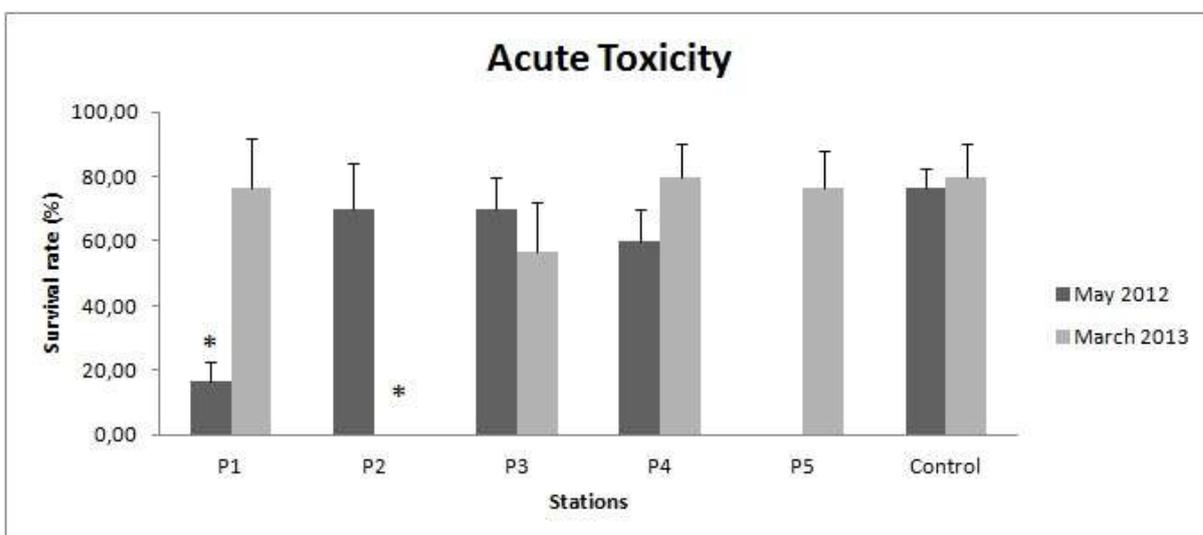


Fig. 7 Mean survival rates of the amphipod *Tiburonella viscana* exposed to sediments from the CIP-PA. Asterisks (*) indicate significant differences relative to the respective control ($p < 0.05$). P5 was not collected in the first sampling.

3.3.3. *Lytechinus variegatus*

In the chronic toxicity test with sea-urchin embryos and SWI, physical-chemical parameters of the overlying water within the test chambers remained within acceptable ranges (ABNT 2006; Cesar et al. 2004), as shown in Appendix C. Salinities ranged from 33 to 36; pH levels were between 6.4 and

7.76, and DO levels ranged from 3.08 to 6.38 mg.L⁻¹. Normal embryonic development was significantly altered in the organisms exposed to the sediments from the P1, P2, P3, and P4 stations during the first and third sampling surveys), and from the P2 station in the second survey. Thus, these sediments were considered toxic (Fig. 8).

Results also showed that, during rainier months (May 2012 and March 2013), sediment toxicity tended to be widespread, since samples from P1, P2, P3 and P4 were considered toxic. On the other hand, in the dry season (August 2012), only the sediment from the P2 station was toxic to the larvae of *L. variegatus*.

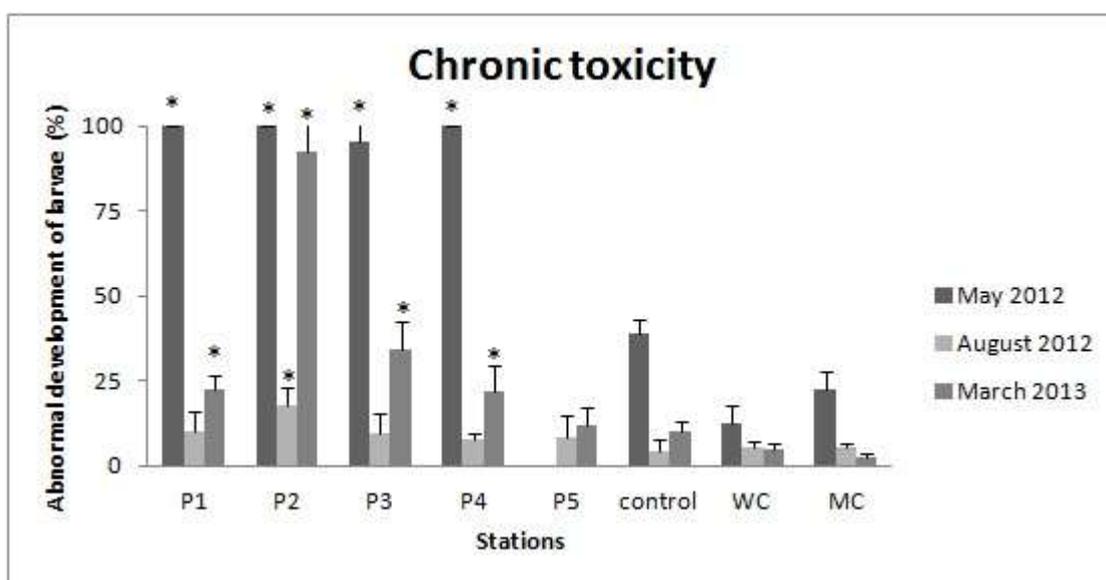


Fig. 8 Abnormal development of larvae of *Lytechinus variegatus* exposed to sediments from the CIP-PA. WC: Water Control; MC: Mesh Control. Asterisks (*) indicate significant differences relative to the respective control ($p < 0.05$). P5 was not collected in the first sampling.

3.4. Integrative approach

3.4.1. May 2012

In the survey conducted in May 2012, there were significant correlations ($p \leq 0.05$) between SEM/AVS values and sediment toxicity. The SEM/AVS

values were negatively correlated with amphipod survival ($r = -0.97$) and copepod fecundity ($r = -0.86$). The SEM values were also negatively correlated to amphipod survival ($r = -0.93$) and copepod fecundity ($r = -0.89$).

Results of the PCA showed that the two first axes explained more than 90% of variances (Table. 4, Fig. 9). The first axis accounted for 74.47% of variances and revealed negative correlations with Cd, Cr, Cu, Fe, Mn, Pb, Zn, OM, CaCO₃ and mud; this axis presented positive correlations with sand, copepod fecundity and amphipod survival. Thus, this axis tended to associate toxicities, contaminants and geochemical carriers, including mud, OM and CaCO₃. In the second axis, negative correlations were observed with abnormal development of sea urchin, amphipod survival, copepod fecundity, Zn, OM, and CaCO₃, whereas some positive correlations were observed with Ni.

Table 4 Results from PCA integrating sediment properties, chemistry and toxicities, in samples from the CIP-PA, São Paulo, Brazil. (values in italics indicate significant correlations with the respective axis)

Axis	Eigenvalue	% Variance	Cumulative Variance
PC1	8.38	74.47	74.47
PC2	1.85	16.44	90.91

	PC1	PC2
Cd	<i>-0.89</i>	0.30
Cr	<i>-0.97</i>	-0.07
Cu	<i>-0.99</i>	0.13
Fe	<i>-0.95</i>	-0.21
Mn	<i>-0.94</i>	-0.15
Ni	0.11	<i>0.40</i>
Pb	<i>-0.96</i>	0.13
Zn	-0.03	<i>-0.89</i>
OM	<i>-0.74</i>	<i>-0.54</i>
CaCO ₃	<i>-0.68</i>	<i>-0.71</i>
Amp Surv	<i>0.86</i>	<i>-0.40</i>
Cop Fec	<i>0.86</i>	<i>-0.44</i>
Abn Dev Urc	0.15	<i>-0.97</i>
Sand	<i>0.89</i>	-0.19
Mud	<i>-0.94</i>	-0.24

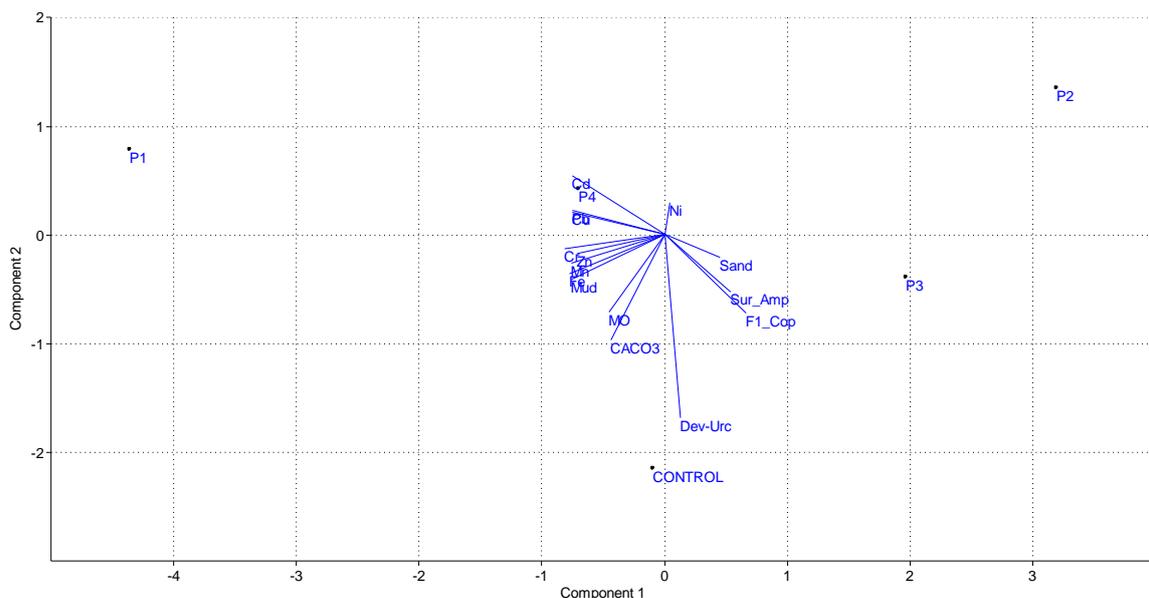


Fig. 9 . Bi-dimensional distribution of PCA results using geochemical and ecotoxicological data obtained using sediments from the CIP-PA (PC1 x PC2).

The PCA analysis showed 4 groups based on sediment quality. The first group included only the P1 station, which is located near the Valo Grande channel (in the north) and which was negatively associated with axis 1. Another group was made up of P2 and P3. These areas are located between Cananéia and Iguape and were positively associated with axis 1. The third group was formed only by the P4 station, which is located close to Cananéia and was negatively associated with the axis 2. The last group included only the control, which was situated near Cardoso Island and which had a weak positive association with axis 2 and a weak negative association with axis 1.

3.4.2. August 2012

In the sampling survey conducted in August 2012, the SEM/AVS was found not to be significantly correlated with the sediment toxicities. The SEM presented significant negative correlation with abnormal development of sea urchin ($r = -0.78$) and copepod fecundity ($r = -0.66$).

Results of the PCA showed that the two first axes explained 87.61% of variances (Table. 5, Fig. 10). The first axis accounted for 77.62% of variances

and revealed associations between the metals (Cd, Cr, Cu, Fe, Mn, Pb, Zn), geochemical carriers (OM, CaCO₃, sand and mud), and toxicity (both copepod fecundity and abnormal development of sea urchin). As for the second axis, associations were observed with abnormal development of sea urchin, CaCO₃ contents, and Ni and Zn levels in the sediments.

Table 5 Results from PCA integrating sediment properties, chemistry and toxicities, in samples from the CIP-PA, São Paulo, Brazil. (values in italics indicate significant correlations with the respective axis)

Axis	Eigenvalue	% Variance	Cumulative Variance
PC1	9.23	77.62	77.62
PC2	1.19	9.99	87.61

	PC1	PC2
Cd	<i>-0.95</i>	0.29
Cr	<i>-0.92</i>	-0.38
Cu	<i>-0.50</i>	0.33
Fe	<i>-0.95</i>	-0.15
Mn	<i>-0.81</i>	-0.04
Ni	0.13	<i>-0.93</i>
Pb	<i>-0.91</i>	0.22
Zn	<i>-0.75</i>	<i>-0.62</i>
OM	<i>-0.89</i>	0.06
CaCO ₃	<i>-0.66</i>	<i>0.48</i>
Cop Fec	<i>0.96</i>	-0.20
Abn Dev Urc	<i>0.66</i>	<i>-0.41</i>
Sand	<i>0.91</i>	-0.26
Mud	<i>-0.88</i>	-0.05

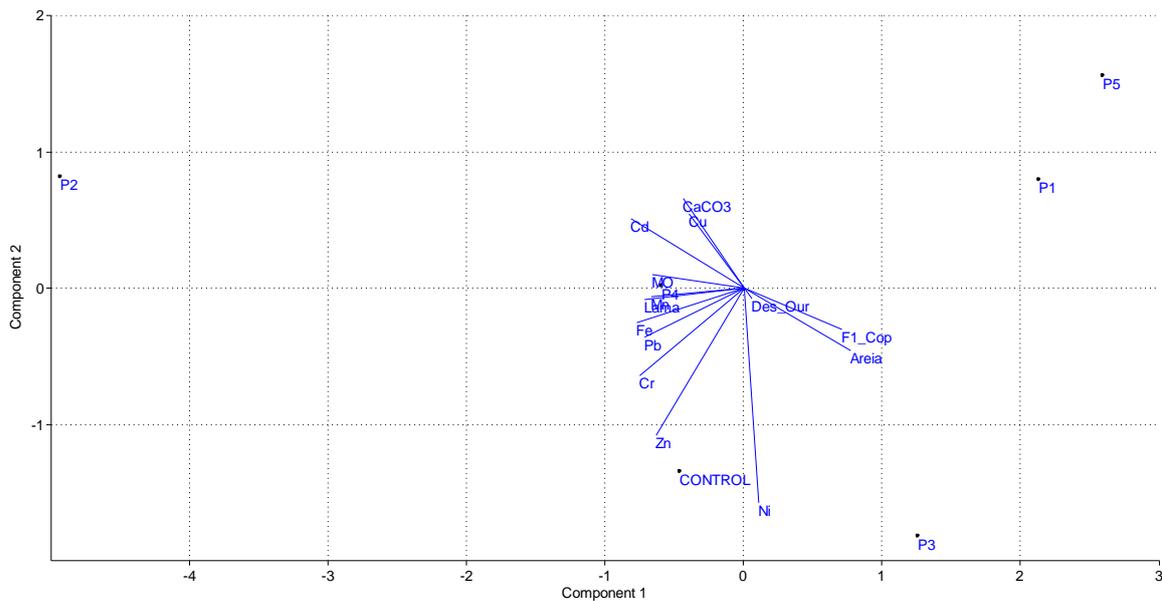


Fig. 10 . Bi-dimensional distribution of PCA results using geochemical and ecotoxicological data obtained using sediments from the CIP-PA (PC1 x PC2).

The PCA suggested the existence of three groups based on sediment quality. The first group included only P2, which was located in the estuarine zone (at the maximum turbidity zone), relatively far from the Valo Grande channel, and it presented high concentrations of contaminants and a predominance of mud. The second group included P4 and the control area, with high values of natural elements and a predominance of fine sand. The third group included the sediments from P1, P3 and P5, which presented low concentrations of contaminants and a predominance of sand (Fig. 10).

3.4.3. March 2013

In this sampling survey, there were no significant correlations between SEM/AVS and the sediment toxicities. The SEM was found to be negatively correlated with amphipod survival ($r = -0.86$) and abnormal development of sea urchin ($r = -0.87$), and also to have a low negative correlation with copepod fecundity ($r = -0.56$).

Results of the PCA showed that the first two axes explained 86.58% of variances (Table. 6, Fig.11). The first axis accounted for 74.27% of variances and revealed associations between the metals (Cd, Cr, Cu, Fe, Mn, Pb, Zn), geochemical carriers (OM, CaCO₃ and mud), and toxicity (amphipod survival, copepod fecundity and abnormal development of sea urchin). In the second axis, associations were observed with OM and CaCO₃, which were found to be negatively associated with Pb and Zn concentrations.

Table 6 Results from PCA integrating sediment properties, chemistry and toxicities, in samples from the CIP-PA, São Paulo, Brazil. (values in italics indicate significant correlations with the respective axis)

Axis	Eigenvalue	% Variance	Cumulative Variance
PC1	8.10	74.27	74.27
PC2	1.34	12.31	86.58

	PC1	PC2
Cd	<i>-0.97</i>	0.17
Cr	<i>-0.94</i>	-0.25
Cu	<i>-0.95</i>	<i>0.31</i>
Fe	<i>-0.98</i>	0.02
Mn	<i>-0.81</i>	0.17
Pb	<i>-0.87</i>	0.43
Zn	<i>-0.85</i>	-0.47
OM	<i>-0.55</i>	-0.60
CaCO ₃	<i>-0.68</i>	-0.62
Amp Surv	<i>0.92</i>	-0.19
Cop Fec	<i>0.73</i>	-0.33
Abn Dev Urc	<i>0.92</i>	-0.24
Mud	<i>-0.89</i>	-0.30

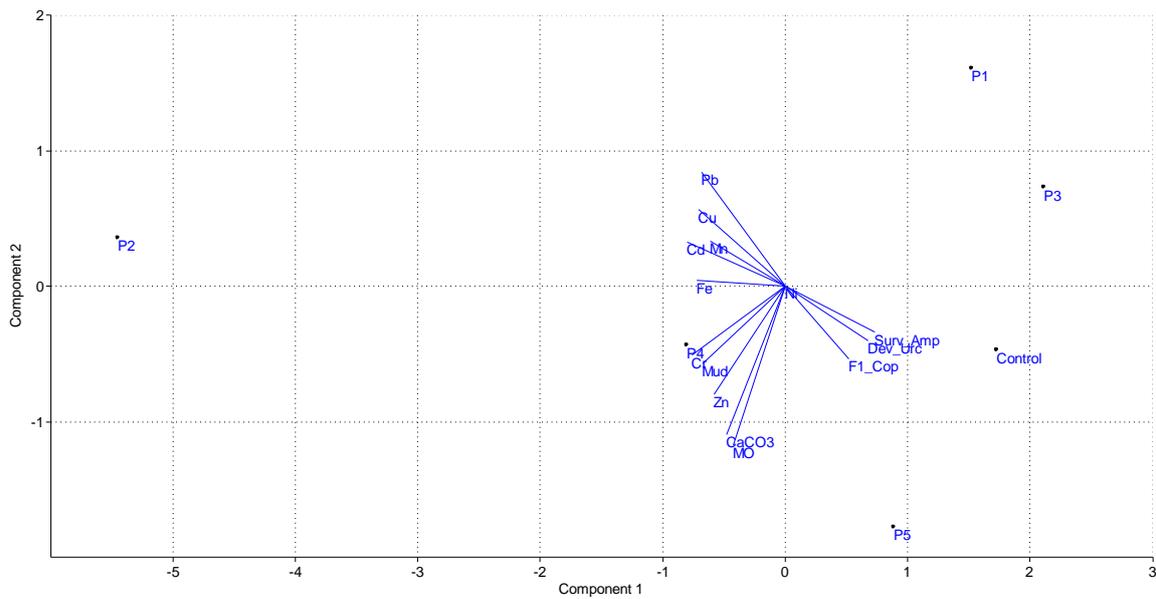


Fig. 11 . Bi-dimensional distribution of PCA results using geochemical and ecotoxicological data obtained using sediments from the CIP-PA (PC1 x PC2).

The PCAs of the three surveys showed associations between geochemical carriers, especially for mud, metals and toxicity. This finding suggests that the adverse effects may be related to the metals discharged into the estuary by the Ribeira River. The PCA groups tended to isolate the most degraded stations (P1 in the first survey and P2 in the second and third surveys). This data suggests that depositional areas accumulate metals and become toxic to benthic organisms.

3.5. Whole-sediment TIE

In the whole-sediment TIE experiment, the treatments were not effective in removing toxicity at the lower sediment dilutions. At the higher dilutions (P2_25 and P2_12.5), however, toxicity reduction was possible, and the chemical groups responsible for the toxicity were able to be identified (Fig. 12). In these dilutions, effective treatments for toxicity removal were Fenton's reagent, Activated Carbon and *Ulva lactuca*, with Fenton's reagent being the most effective in toxicity reduction. Treatment with coconut charcoal did not

reduce toxicity. The results suggest that metals and ammonia were the main causes of the sediment toxicity.

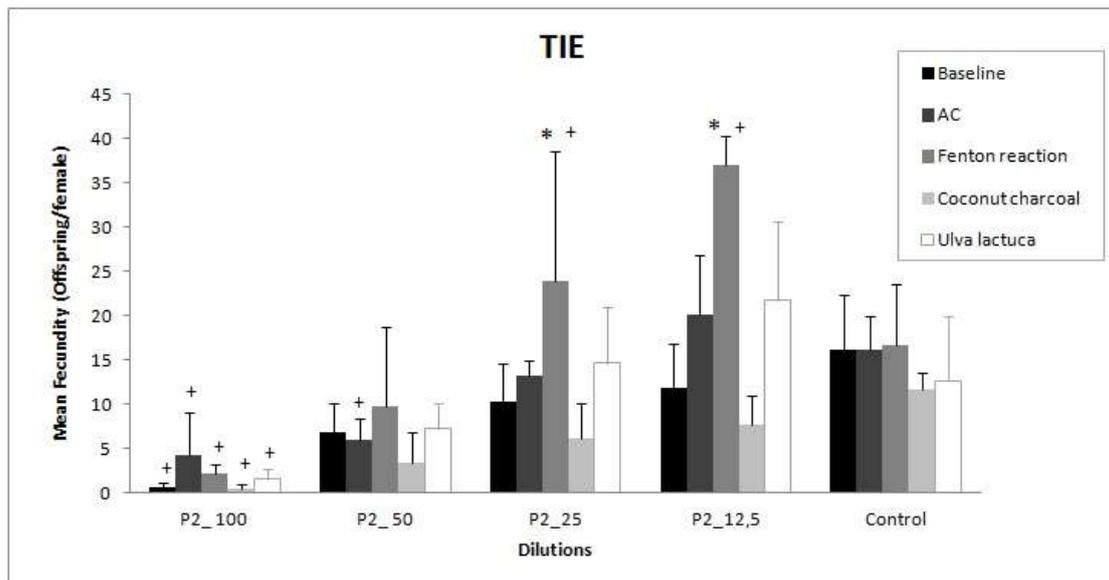


Fig. 12 Mean fecundity of *Nitocra* sp. + show statistical difference with the control treatment and * show statistical difference from baseline in the same treatment. ($p < 0.05$)

4. Discussion

Results obtained on the CIP-PA showed that there are associations between geochemical carriers (mainly fines), sediment contamination by metals, and toxicity. Worse conditions tended to occur in muddy sediments, a finding which indicates that contaminants are strongly associated with fines. Guimarães and Sígolo (2008) showed that the suspended sediments that are transported from the RIR toward the CIP-PA are mainly composed of metal-rich mining residues or muddy particles with adsorbed metals, which may spread along the estuarine system toward Cananéia and cause sediment contamination. A previous study conducted in the CIP-PA region (Amorim et al. 2008) reported that organic matter and fines were important for retaining trace elements, and thus contributed to the understanding of the areas at risk due to contamination by metals.

However, even though the Valo Grande channel is the secondary source of metals to the estuary, there was no clear gradient of pollution. This

lack occurred because sediments from P2 (and eventually from P4) in the second and third sampling surveys were those that were richer in metals and more toxic. In this sense, contamination and toxicity tended to be associated with the occurrence of fines. Even so, sites exhibiting worse sediment quality were located closer to Valo Grande (P1 and P2), which indicates a relationship with the source of contamination. Thus, the proximity to the contamination sources and the presence of depositional areas seem to be the main factors that contribute to sediment quality in CIP-PA.

However, results suggest that depositional areas in the CIP-PA change over time, since they are influenced by waves, currents, and freshwater inputs. In the dry season, fines tend to concentrate closest to the Valo Grande channel (P1), while during the rainy season, the zone of maximum turbidity appears to move southward (towards P2). This difference may be associated with differences in rainfall. This fact is extremely important because it shows that, in a given coastal system, the ecological risk may have connections to oceanographic and geomorphological aspects, which become as relevant as the distance from the contaminating sources. Therefore, low energy areas tend to facilitate the deposition of mud and contaminants. Moreover, because there is a seasonal phenomenon of a larger freshwater inflow in the summer (the rainy season), the areas with highest rates of deposition may change throughout the year; therefore, the areas at higher risk change throughout the year, and this may have consequences for biota. This fact corroborates the findings reported by Amorim (2008), who observed that the volume of water is more important in the summer and that the transport of particulate matter increases in this period. Thus, seasonality may influence the transport of sediments and contaminants, since the maximum depositional areas tend to migrate southward during the rainier months. This phenomenon may increase pollution in sediments, because it spreads contaminated particles southward. This phenomenon has implications for remediation projects.

The data clearly shows a close relationship between the estuary and the RIR basin, because metals represented one of the main factors responsible for the sediment toxicity, and particularly because mining was and still is conducted along the RIR basin. Thus, sources located at the upper area of the

river should be controlled. Previous studies found that sediments were enriched by some metals, such as Pb and Cr, and that the observed contamination in the sediments was directly related to the former mining activities in the upper RIR basin (Moraes et al. 2004, Mahiques et al. 2009; Morais et al. 2013).

There is a very high consistency between the results of the acute and chronic toxicity tests, and there are also associations of this toxicity with metals and fines. Sediments from P1 and P2 tended to exhibit more frequent toxicity, as well as higher SEM values.

A factor that may have influenced the results of the toxicity test, particularly in the case of P1 and P2, was the trend of decreasing pH levels over the course of the experiments. In the experiments, under oxidizing conditions, metals and sulfides are oxidized. This releases H^+ ions and causes the pH to decrease in a phenomenon similar to acid drainage. Such conditions may turn metals into more reactive forms, thus increasing sediment toxicity. This phenomenon of acidification is not expected to occur in the RIR or in the estuary, due to both the high pH of the RIR waters and the buffering system actions in the estuarine complex. However, in the shallow minor channels spread throughout the estuary, where water circulation is restricted and deposition is intense, this possibility should be taken into account, as should the possibility that these small water bodies are more susceptible to pollution. In any case, contamination by metals was strongly associated with toxicity, and this association suggests that metals were involved in the effects that were observed.

In TIE (only for P2 sediments), the treatment for metal removal was the most effective in reducing toxicity, which again points to the presence of metals in this region as the main cause of toxicity (Moraes 1997; Saito 2002; Guimarães and Sígolo 2008; Araujo et al. 2013; Moraes et al. 2013). The whole-sediment TIE results reflect the contribution of ammonia to the sediment toxicity. In this case, ammonia is likely of natural origin (Ankley et al. 1992; Ward et al. 2011). However, when in low concentrations, ammonia may interact with other contaminants, such as metals, which results in additive or synergistic effects and, consequently, in increases in toxicity (Ankley et al. 1990;

Environment Canada 2001). Further studies should be done in order to evaluate the toxicity that results from the combination of ammonia and metals.

5. Conclusion

Results show that metals from the upper region of the RIR are transported to the CIP-PA and accumulate in the sediments at potentially toxic levels. Contamination was associated with the muddiness of the sediments, a finding which is consistent with the fact that most of the suspended particulates in the RIR consist of mining residues. The location of extremely muddy and contaminated sediments (e.g. depositional areas) appears to move along the estuary, and to accompany the displacement of the maximum turbidity zone, which, in turn, depends on the seasonal freshwater inputs. Thus, the most contaminated and toxic areas are not necessarily those closest to the primary sources, but are actually those with finer sediments. These results highlight the fact that oceanographic processes should be considered as important factors in the accumulation of contaminants in estuarine areas. In addition, metals and, to a lesser extent, ammonia, were recognized as the main causes of sediment toxicity. Thus, actions must be taken to ensure control of the sources of contaminants for this area, especially those located upstream.

6. Acknowledgements

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Chapter 3 – Bioaccumulation

Using bioaccumulation tests to estimate the quality of estuarine sediments contaminated by metals

Abstract

This investigation aimed to propose and use the bioaccumulation test with sediments using a tropical estuarine bivalve. Chemical analyses, bioaccumulation tests and grain size analysis were performed, as well as organic matter and carbonate concentrations analyses. The sediments were composed predominantly by fine sand and mud, with variable levels of organic matter and carbonates. Higher concentrations of metals in the sediments from P2 are showed and in the SEM/AVS the stations P3 and P5 showed potential to cause toxicity. BAF showed high ratios for Cd, Ni and Zn and the PCA gave associations between mud, OM, CaCO₃, metals in sediment and in tissues of *A. brasiliiana*. These analyzes show that contaminants are bioavailable to the organisms. The use of bioaccumulation test proved to be an efficient technique in the evaluation of contaminants present in sediments of estuarine regions and clams from the specie *Anomalocardia brasiliiana* proved been good bioindicators.

Keywords: Contaminants, bioaccumulation, Cananeia-Iguape, estuary, sediment, *Anomalocardia brasiliiana*,

1. Introduction

The assessment of environmental pollution and its effects have employed several lines of evidence (LOE), which include chemical analyses of water, sediment and organisms collected in field, toxicity testing, analysis of community structure, evaluation of biomarkers, among others (Choueri et al. 2010; Chapman 2007; Chapman and Anderson 2005). The use of LOEs relies on the principle that each method has its own advantages and limitations, and that the combined use of different techniques provides a holistic-level interpretation (USEPA 2000; Newman and Unger 2003).

Sediment consists in an important indicator of the health of aquatic ecosystems (Power and Chapman 1995), because most of contaminants tend to sink to the bottom and accumulate in the sediments. Recent studies concerning to assess the quality of sediments have used a set of approaches (Chapman and Hollert 2006; Chapman 2007; Torres et al. 2009; Choueri et al. 2009; Buruaem et al. 2013; Poleza et al. in press); however the most of them do not use laboratory tests for bioaccumulation as an additional LOE, despite the recommendations to include them in sediment quality assessment (Chapman et al. 1997).

Bioaccumulation tests provide information on the bioavailability of contaminants and may be designed to assess the accumulation of chemicals in the tissue of organisms through single and combined exposure routes (respiration, ingestion, or dermic contact) (USEPA 2000).

There are few protocols available for conducting such experiments with sediments (e.g. USEPA 1993; 2000), and most of them concern for organisms from temperate regions. Thus it is important to develop and apply protocols for use in different types of environments, such as tropical and subtropical estuaries.

In this sense, fish and invertebrates are potentially good biological models for bioaccumulation tests with sediments, and among them, mollusks, polychaetes and crustaceans have been used as main biological models in bioaccumulation studies (Mac and Schmitt 1992; USEPA 1993; USEPA and USACE 1991). Mollusks constitute important ecological functional elements in estuarine ecosystems and were the first marine resource to be used worldwide for bioaccumulation researches (USEPA 1993; Silva-Cavalcanti and Costa 2011). Their sessile habit ensures the exposure to contaminants, whereas the easy collection allows the use of a large number of individuals in laboratorial experiments. Thus, they consist in suitable indicators of the environmental quality and pollution (Martins et al. 2005; Kehrig et al. 2006; Silva et al. 2006). In special, bivalves have an additional advantage to be used as biomonitors of pollution due to their importance in the food chain of estuarine ecosystems and commercial importance for local populations.

Thus, this investigation aimed to propose and use the bioaccumulation test with sediments using a tropical estuarine bivalve. To achieve that, clams from the species *Anomalocardia brasiliiana* were exposed to field collected sediments with different concentrations of metals, and afterwards the concentrations of metals in the soft tissues from the animals were analyzed to determine if contaminants were available to the organisms.

2. Materials and Methods

2.1. Study area

The study area consists in the Cananeia-Iguape estuarine complex (named CIP), which is located on the south coast of São Paulo state (Brazil), between the latitudes 24° 40'S and 25° 05'S and longitudes 47 ° 25'W and 48 ° 10'W (Fig. 13). CIP is one of the main estuarine complexes of the São Paulo coast, comprising the cities of Iguape, Cananéia and Ilha Comprida. The CIP connects to the valley of the Ribeira de Iguape River (RIR), forming the largest coastal plain of São Paulo (Freitas et al. 2006).

The CIP region is formed by estuarine channels and barrier islands and forms a complex net of water bodies, which banks are occupied by broad mangrove forests (Schaeffer-Novelli et al. 1990). The hydrodynamic circulation of the area is influenced by tidal waves and the contribution of several rivers, especially the Ribeira de Iguape River (Miranda et al. 1995; Myao and Harari 1989), which is the largest river of São Paulo that runs towards the coast.

Since the XIX century, after the construction of the Valo Grande Channel, about 70% of RIR flow started to be deflected into the internal portion of CIP, thus altering the pattern of the freshwater discharges of RIR and introducing large amounts of nutrients and metals into this estuarine system. Since then, RIR became the main contributor of freshwater, sediments and nutrients inputs to the system, especially in its central and northern portion (Freitas et al. 2006).

The CIP region is considered of international concern, and was recognized as a Biosphere Reserve by UNESCO (the Atlantic Rainforest Biosphere Reserve) (Moraes 2004). Besides, the CIP is also protected by

federal law, as the entire region was designated as a legally protected area (the Cananéia-Iguape-Peruíbe protected area, also known as Lagamar).

The CIP region is characterized by the relative low socio-economic development, and by precariousness in the health and sanitation infrastructure and services, education levels among residents. On the other hand, official reports (CETESB 2009) indicated satisfactory environmental conditions, including water and sediment quality. However, previous studies have found evidence of contamination by metals in sediments of this region (Aguiar et al. 2008; Guimarães and Sígolo 2008; Mahiques et al. 2009), especially close to the RIR mouth.

2.2. Sediment sampling

The study area includes the estuarine portion of Iguape and Cananeia, with 6 sampling stations located since the proximity of Valo Grande channel (theoretically under higher influence of contaminant sources) until the Cardoso Island (control area, theoretically out of RIR influence). The stations are shown in fig. 13 and the geographic coordinates were shown in the table 1 (see the chapter 2 of this study). Sediments and organisms were collected in August 2012. Sediment samples were collected with a 0.036 m² stainless steel Van Veen grab sampler and in the laboratory, aliquots for bioaccumulation test were kept refrigerated at 4°C, while those for geochemistry were stored frozen at -20°C.

Bivalve individuals of the species *Anomalocardia brasiliiana* were collected in September 3, 2012 from some sand bars located in Cananeia, close to the Cardoso Island State Park by turning the sediment over with a shovel and picking out larger animals. The animals were conditioned in plastic boxes containing humid paper, and transported, at ambient temperature to the laboratory, where they were transferred to tanks containing dilution water, which were maintained at 25±2°C with constant aeration.

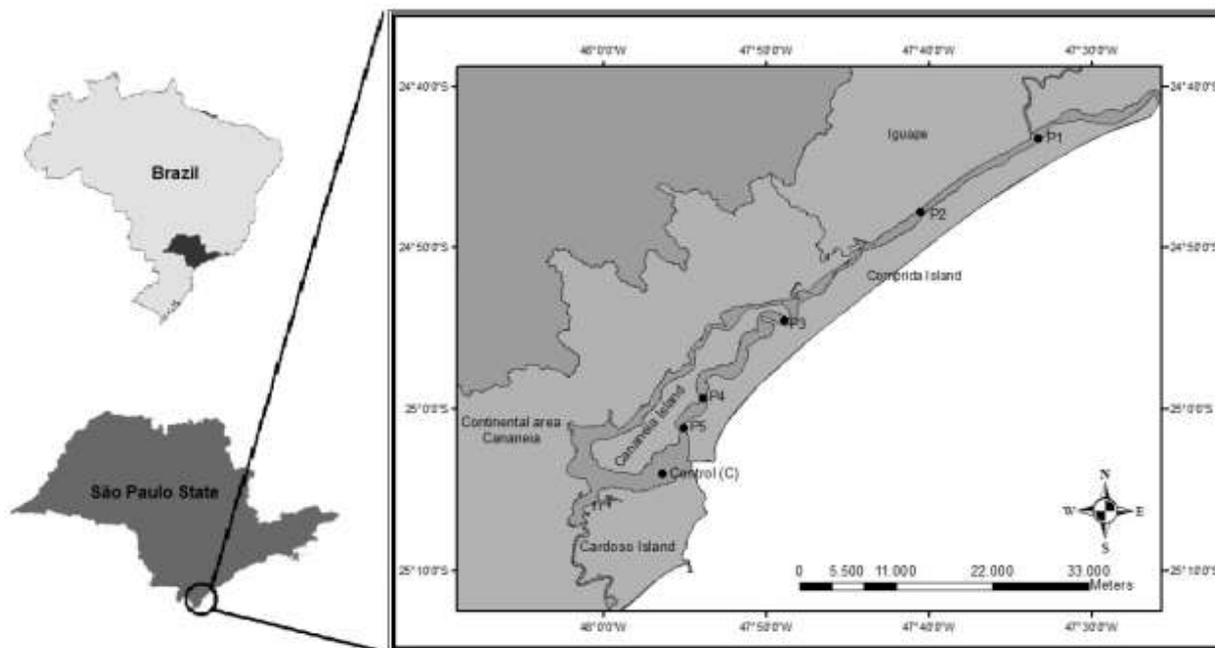


Fig. 13 Map showing the sediment collection stations around the study area, within CIP-PA, Brazil.

2.3. Sediment properties

Sediment grain size distribution was analyzed based on the protocol proposed by Mudroch and MacKnight (1994). About 30g of previously dried sediment were wet sieved through a 0.062 μ m mesh to separate fine particles (mud and clay). The material retained on the sieve was then dried and weighed. Initial and final weight differences demonstrated the mud fraction (mud and clay). Subsequently, the sandy material retained on the 0.062 μ m mesh was sieved into different meshes (ϕ scale) in order to separate different classes of sands, and the results were further classified based on the Wentworth scale. The calcium carbonate (CaCO_3) contents in each sample were measured using the method described by Hirota and Szyper (1976), which consists of separating fractions of 5g of each sediment sample and then adding 10ml of hydrochloric acid (5N HCl) for 24 hours to eliminate calcium carbonates. Afterwards, samples were washed with distilled water and dried at 60°C. The difference between initial and final weights showed the amount of CaCO_3 in the samples. Organic matter (OM) contents in the sediment samples were estimated using the ignition method (Luczak et al. 1997), which consists of the

separation 5g of dry sediment aliquots from each sample, and their incineration in a muffle (500°C) for 4 hours. Organic Matter contents were established by calculating the difference between initial and final weights.

2.4. SEM/AVS

This method describes the procedures for the determination of acid volatile sulfides (AVS) and selected metals that are solubilized by a weak acidification (simultaneously extracted metals, SEM). The AVS may control the bioavailability of metals in anoxic sediments (DiToro et al. 1990).

The acid extraction of metals from sediments to determine SEM/AVS was performed based on the technique adopted by the USEPA (Allen et al. 1991; 1993; Machado et al. 2004), for wet sediment samples. The procedures for AVS extraction involves the attack with hydrochloric acid (HCl 6M). The sulfides in the sample are converted into hydrogen sulfide (H₂S) by reaction with cold HCl. The produced H₂S reacts with DMPD (N,N-dimethylphenyl-p-diamine) in the presence of ferric chloride (FeCl₃) and is then quantified by UV-visible spectrophotometry. The remaining solution after the removal of sulfides is filtered and the targeted dissolved metals (Cd, Cu, Ni, Pb, Zn, Cr, Fe and Mn) are determined by ICPOES (induced coupled plasma – optical emission spectrophotometry) (Spectro, model Spectro Ultima 2). Ultimately, the ratio SEM/AVS is calculated by using the molar concentrations of AVS and the target SEMs (Cd, Cu, Ni, Pb and Zn), (Di Toro et al. 1992). When the ratio values are above 1, the sediment presents potential for causing toxicity, while for values below 1, the sediment probably does not exhibit toxicity due to SEMs.

2.5. Bioaccumulation test

The bioaccumulation test was adapted from the protocols developed by the USEPA (1993; 2000). For the experiments, animals with an average size of 3 cm were selected. The dilution water for use in the bioaccumulation test was collected in the same station of the animals (near P5).

For each sample, including the control sediment, three replicates were set up in chambers containing a layer of approximately 2 cm of sediment and 3L of dilution water (at salinity 30). The system was kept for 24h with aeration and temperature of $25 \pm 2^\circ\text{C}$, in order to reach equilibrium. Afterwards 6 healthy organisms were placed in each replicate; 3 replicates were used per sample. The tests were kept in photoperiod (12 h:12 h light/dark) under constant aeration and temperature. Water renewals were performed every seven days or if any test-chamber was exhibiting water with high turbidity. After 14 days, the test was finished, and then the surviving organisms were counted and sacrificed. The soft tissues of each individual were removed and frozen at -20°C until their use in the chemical analyzes. The tissues of the survivors in each replicate were grouped into pools, thus for each tested sediment 3 pools were prepared. Physical-chemical parameters (pH, dissolved oxygen, ammonia and salinity) were measured at the beginning and end of the tests, and also at each water renewal.

Afterwards all the bivalve soft tissues were lyophilized and used in chemical analyses. Prior to the chemical analysis, approximately 1.0 g of lyophilized samples *A. brasiliiana* were digested in pressurized microwave system with 5.0 mL of concentrated HNO_3 for 10 minutes in a fluorocarbon polymer chamber, according to USEPA 3051A method (5.5 min until the temperature reaches 175°C followed by an additional period of 4.5 min at 175°C). After cooling, the solution volume was completed up to 10 mL with ultra-pure water and the resulting mixture was then centrifuged. Before carrying out the digestion procedure, the samples were kept immersed in HNO_3 for approximately 1 hour in order to remove part of the organic matter and thus minimize the risks of loss and explosion.

The metals contained in the supernatant were determined by ICP-MS (induced coupled plasma – mass spectrophotometry). In order to assure the reliability of the chemical analysis, a certified reference material was used (SRM NIST 1566b oyster tissue) for bivalve analysis, and the recoveries are presented in the table 7. For the sediment, as there are not certified materials for SEM/AVS, we calculate the precision of the analysis, by comparing the

difference (in %) between the mean concentration and that obtained by one of the replicates (Table 7).

Table 7 Contents of certified reference material of *Anomalocardia brasiliiana* (mg/kg) and in sediment (%).

Compared with	Certified	Cr	Mn	Fe	Ni
	NIST 1566b	-	18.8	209.4	1.08
<i>A. brasiliiana</i>	Measured	-	18.5±0.2	205.8±6.8	1.04±0.09
	Recovery (%)	-	98.40	98.28	96.30
Sediment	Precision (%)	100	90.28	88.46	91.66
	Certified	Cu	Zn	Cd	Pb
	NIST 1566b	71.0	1440	2.50	0.301
<i>A. brasiliiana</i>	Measured	71.6±1.6	1424±46	2.48±0.08	0.308±0.009
	Recovery (%)	100.85	98.89	99.2	102.33
Sediment	Precision (%)	99.21	100	99.84	90.30

Finally, the bioaccumulation factor (BAF) was calculated and consisted of the ratio of a chemical concentration in an organism to the concentration in sediment (Lee 1992; Boese and Lee 1992; USEPA 2000).

2.6. Exploratory analyses

The concentrations of each metal in the soft tissues of *A. Brasiliiana* were compared by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. The geochemistry and bioaccumulation data were integrated by the use of a two-matrix ordination, based on PCA. In this case a matrix containing the geochemistry data was used as the main matrix, while the second matrix consisted in the set of information regarding to the concentration of metals in the tissues of *A. brasiliiana*. Prior to the analysis, data were log-transformed. This analysis was conducted using the PC-ORD software.

3. Results

3.1. Sediment properties

The samples presented predominance of sand (>58%) with exception of P2 which was composed mainly by mud (>99%).(Fig. 14). Amounts of CaCO_3 were generally low (between 2.64 and 16.60%), whereas OM amounts ranged from 0.47% to 14.46% (Table 8).

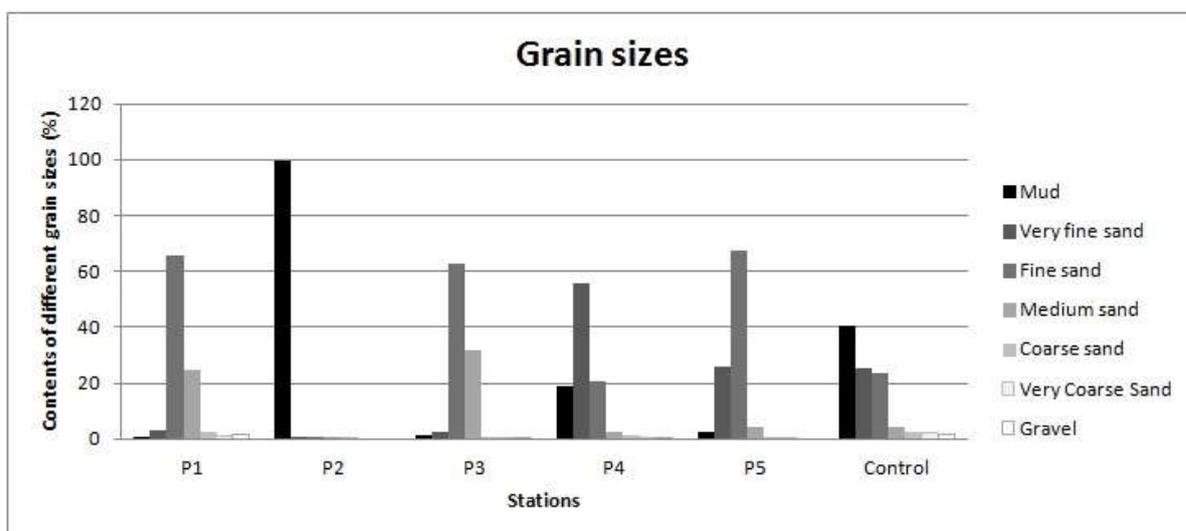


Fig. 14 Contents of different grain sizes in sediments from the CIP-PA (SP, Brazil).

Table 8 Contents of Calcium Carbonate (%) and Organic Matter (%) in sediments from CIP-PA.

Stations	CaCO_3	OM
P1	4.50	0.47
P2	16.60	14.46
P3	2.64	0.60
P4	12.15	4.33
P5	9.17	1.22
Control	9.45	5.44

3.2. Sediment chemistry

The concentrations of metal in the sediment samples and in soft tissues of *A. brasiliiana* are presented in the Table 9. For the most of elements (Cd, Cr, Cu, Fe, Pb, Mn and Zn), the highest concentrations were observed in the sediments from P2. The highest Ni concentrations occurred in the sediments from P3. It is noteworthy that the concentrations of lead in the sediments from P2 exceeded the TEL (TEL = 30.20 mg/kg; Smith et al, 1996), even considering that this study used a weaker acid extraction than that used to derive the Canadian SQGs.

Table 9 Values of metals in sediment and in the tissues of *A. brasiliiana* (mg/kg).

Sediment								
	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
P1	0.01	0.34	0.39	1211.39	53.66	0.01	1.46	0.04
P2	0.28	11.21	9.13	22308.77	381.95	0.01	51.63	33.43
P3	0.01	1.61	1.97	1366.77	10.94	0.66	7.43	13.71
P4	0.02	2.12	0.65	3610.82	115.87	0.01	4.21	8.73
P5	0.01	0.18	2.30	271.66	1.78	0.01	0.86	0.04
Control	0.02	2.88	0.26	3225.71	43.32	0.32	3.72	10.83
<i>A. brasiliiana</i>								
	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
P1	0.82	0.01	0.16	8.64	2.44	0.12	0.01	1.74
P2	1.46	0.02	0.20	15.94	14.77	0.14	0.02	2.69
P3	0.88	0.01	0.19	11.66	2.28	0.15	0.02	2.42
P4	0.91	0.02	0.24	11.85	1.23	0.17	0.01	2.60
P5	1.05	0.01	0.19	11.73	1.55	0.14	0.02	2.38
Control	0.96	0.01	0.22	12.33	1.00	0.16	0.01	2.57

The values of SEM, AVS and SEM/AVS are shown in Table 10. SEM ranged from 1.92 to 94.98 mg/kg. AVS values ranged from 0.75 to 2097.05 mg/kg. A negative value of AVS was obtained and these indicate that the AVS had lower values than white, and it is not possible to determine the sulfide concentration by this method; in this case, we can assume that all SEM would be in a solubilizable form. According to this relationship, the metals were

supposed to be bioavailable in the sediments from stations P3 and P5, and possibly in sediments from P1 station.

Table 10 Contents of SEM (mg/kg), AVS(mg/kg) and SEM/AVS for the different stations.

Stations	SEM	AVS	SEM/AVS
P1	1.92	-0.66	-0.69
P2	94.48	2097.05	0.01
P3	23.77	2.48	5.91
P4	13.62	105.71	0.05
P5	3.21	0.75	1.76
Control	15.15	58.96	0.10

In the bioaccumulation test, salinities ranged from 23 to 32; pH levels were between 6.78 and 8.30 and DO levels ranged from 1.58 to 7.8 mg.L⁻¹ and low values were observed in the beginning of the test. However at the end of the test, values were greater than 2.84 mg/L. Unionized ammonia ranged from 0.002 to 2.83 mg/L and total ammonia ranged between 2.12 and 3632,46 µg/L (Appendix D). For the tissues of *A. brasiliiana* the highest concentrations were observed for P2 (Cd, Fe, Mn and Zn) being Mn significantly different from the other stations ($p < 0.05$). There were significant differences between P1 and P2 for Fe and Zn ($p < 0.05$).

The BAF values are presented in table 11. The highest ratios were found for Cd, Ni and Zn. Animals from P1 and P5 presented higher BAF values for Zn and those from P1, P2, P4 and P5 showed high values for Ni. For all stations BAF values for Cd were high, including the control, which suggests that these high values were related the normal metabolism of *A. brasiliiana*.

Table 11 Bioaccumulation factor (BAF) in different stations of CIP-PA.

	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
P1	65.903	0.041	0.404	0.007	0.045	11.593	0.008	43.455
P2	5.158	0.002	0.022	0.001	0.039	13.975	0.000	0.081
P3	109.515	0.008	0.096	0.009	0.208	0.231	0.002	0.176
P4	48.511	0.010	0.376	0.003	0.011	17.040	0.003	0.298
P5	105.148	0.081	0.081	0.043	0.867	14.426	0.028	59.566
Control	53.028	0.005	0.851	0.004	0.023	0.505	0.004	0.237

3.4. Integrative approach

There were positive correlations ($p \leq 0.05$) between concentrations in sediment and in soft tissues of *A. brasiliensis* for Cd ($r = 0.94$), Fe ($r = 0.85$), Mn ($r = 0.94$) and Zn ($r = 0.64$). Apparently these metals were more related to natural concentrations. No correlations were observed for Pb or for Cu. The figure 15 showed the determination coefficient which confirms the correlation data.

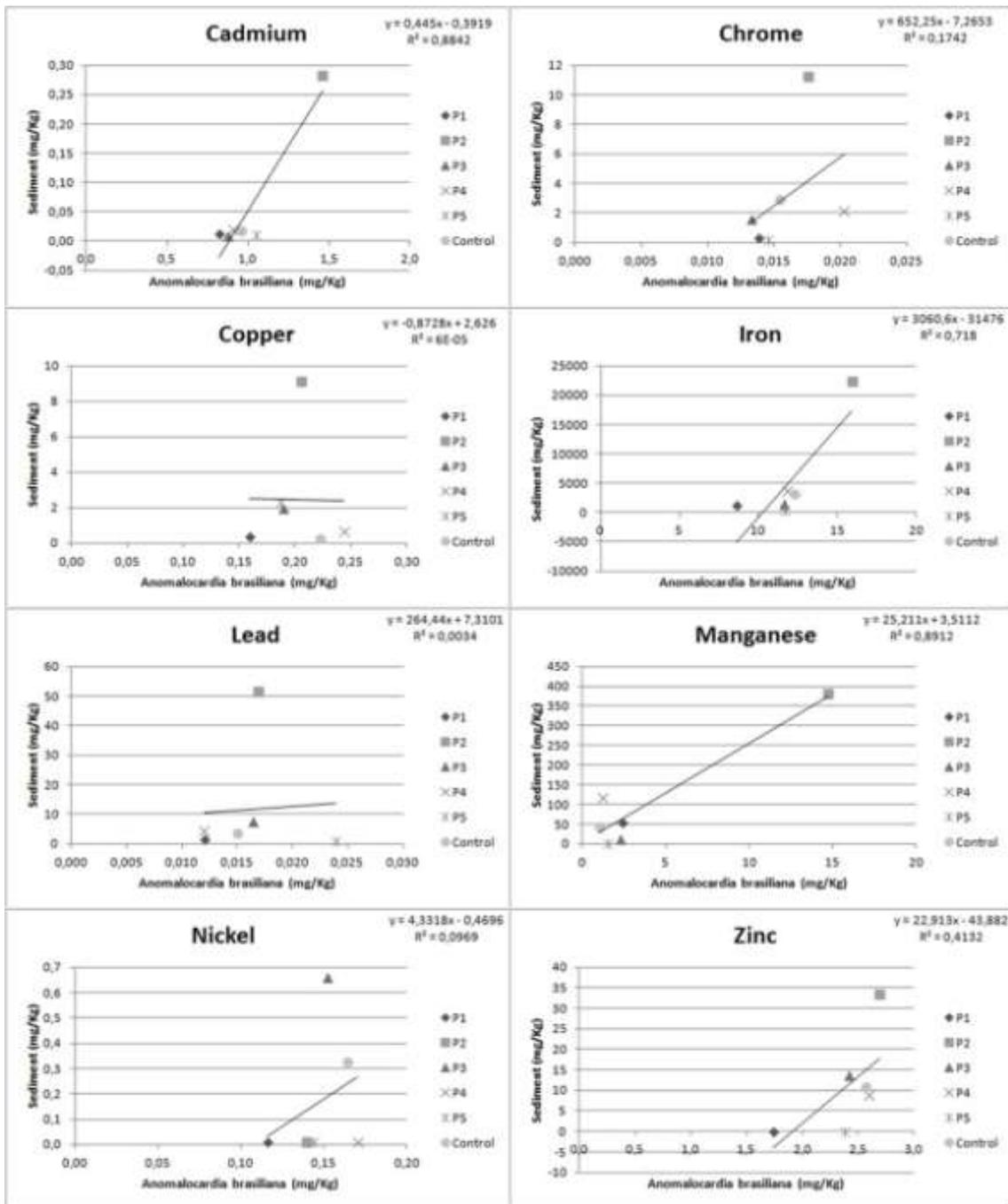


Fig. 15 Correlations between the concentrations of metals in the sediment and in the tissues of *A. brasiliana* (mg/kg).

Results of the matrix showed that the two first axes explained 94.54% of variances (Table. 12, Fig. 16). The first axis accounted for 77.43% of variances and revealed associations between the metals in sediments (Cd, Cr, Cu, Fe, Mn, Pb, Zn and SEM) and tissues of *A. brasiliana* (Cd, Cr, Fe and Mn) as well as the geochemical carriers (OM, CaCO_3 , mud and sand). For the

second axis, associations were observed for SEM and SEM/AVS contents, CaCO₃, Cr in the tissues of *A. brasiliiana* and Ni in the sediments.

Table 12 Results from two matrix ordination, based on PCA, with sediment properties, sediment chemistry and chemistry of tissues *A. brasiliiana* (-B), for samples from the CIP-PA, São Paulo, Brazil. (values in italics indicate significant correlations with the respective axis)

Axis	Eigenvalue	% Variance	Cumulative Variance	Broken-Stick Eigenvalue
1	10.066	77.434	77.434	3.18
2	2.224	17.108	94.542	2.18

	PC1	PC2
Cd	<i>-0.99</i>	0.01
Cr	<i>-0.99</i>	0.08
Cu	<i>-0.92</i>	0.15
Fe	<i>-0.99</i>	0.006
Mn	<i>-0.97</i>	-0.11
Ni	0.28	<i>0.90</i>
Pb	<i>-0.98</i>	0.13
Zn	<i>-0.92</i>	0.35
Cd-B	<i>-0.93</i>	-0.02
Cr-B	<i>-0.52</i>	<i>-0.49</i>
Cu-B	-0.25	-0.13
Fe-B	<i>-0.88</i>	0.20
Mn-B	<i>-0.96</i>	0.07
Ni-B	0.03	0.17
Pb-B	-0.02	0.15
Zn-B	<i>-0.55</i>	0.22
OM	<i>-0.98</i>	-0.09
CaCO ₃	<i>-0.81</i>	<i>-0.42</i>
Sand	<i>0.96</i>	-0.23
Mud	<i>-0.96</i>	-0.05
SEM/AVS	0.29	<i>0.90</i>
SEM	<i>-0.88</i>	<i>0.46</i>

Enter text and then drag to position

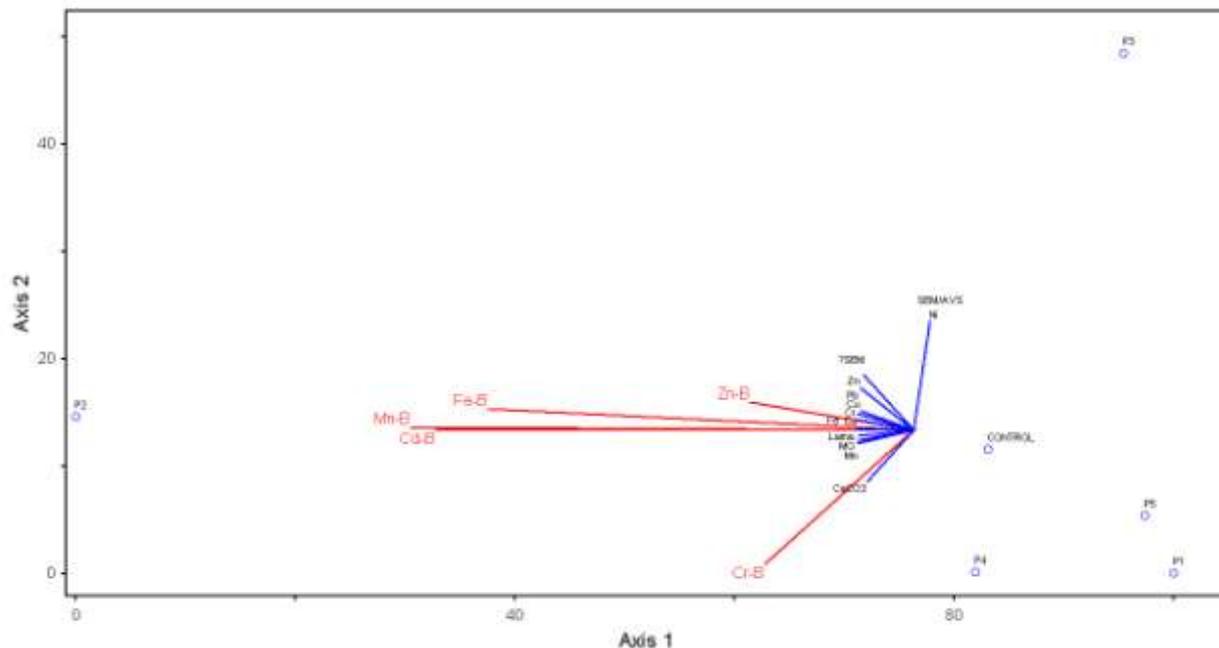


Fig. 16 Bi-dimensional distribution from two matrix ordination, based on PCA results using geochemical and bioaccumulation data obtained using sediments from the CIP-PA.

The ordination analysis suggested the existence of 3 groups based on sediment quality. The first group included only P2, which was located in the estuarine zone (at the maximum turbidity zone), and presented high concentrations of contaminants and predominance of mud. The second group included P4 and the control sediment with high values of natural elements and predominance of fine sands. The third group included the sediments from P1, P3 and P5, which presented low concentrations of contaminants and predominance of sand (Fig. 16).

4. Discussion

It can be seen that most of the sediments presented low to moderate levels of contaminants, as indicated in the model SEM/AVS. These data corroborate with Amorim et al. (2008), who observed low levels of contaminants for most of the sample sediments. Moreover the P2 station showed higher concentrations of metals and in the previous chapter it was noted that the contamination by metals was strongly associated to the toxicity, suggesting that

metals were related to the observed effects, at least in laboratorial experiments with organisms that feed on sediment particles, and that worse conditions tended to occur in muddy sediments, indicating that contaminants are strongly associated to fines. For *A. brasiliiana* can observe a greater accumulation of metals in P2 sediments than in those from the other stations, but the rates are not very high and this can be related to the fact that metals were not available to this species, due to exposure route and the test conditions. *Anomalocardia brasiliiana* presents a filter feeding habit and live in shallow waters ranging from 0.5 m to 1.5 m depth (Moueza et al. 1999). Thus, since it feeds on suspended particles at the sediment-water interface zone; and considering that along the tests (under laboratory conditions), the sediments tend to precipitate and remain on the bottom surface, then the exposure tends to be low, for both dietary route and dermal contact (dissolved metals).

Although the SEM/AVS has not been effective in predicting the toxicity through dissolved metals, not only because it predicted bioavailability for sediment in which no bioaccumulation significant bioaccumulation was observed (P1, P3, P5) but also because this approach did not predict that sediments from P2 present bioavailable elements. This occurs because SEM/AVS does not evaluate efficiently the bioavailability through the dietary route. A similar result was observed by Torres et al (submitted), in a study using different LOEs in which the AVS/SEM approach was not unable to predict sediment toxicity as well as bioaccumulation in bivalves.

It is evident that the sediments from P2 have contamination and the organisms present there accumulated some elements. Fernandez (2011) conducted experiments on fish in the region and noted high concentrations of several metals (Cu, Cr, Fe and Zn) which generated severe responses to animals. Kuniyoshi (2011) in a study with fish observed high concentrations only for the As above stipulated for human consumption and stated that the amounts of metals are higher in areas closer Iguape which corroborates the present study receiving higher amounts of metals in P2 stations. These results evidence that bioaccumulation depend on several factors, including the biology of the studied species, the route of exposure and how the contaminant is absorbed. In this sense, further studies with different species (i.e., concerning to different

exposure forms) are required for a good comprehension of the bioaccumulation phenomenon.

For the other stations it is possible that a higher accumulation has not occurred due to the low time of exposure of animals to sediment, which may not have allowed the achievement of a steady-state. USEPA (1993) recommend a 28-day exposure and this will result in tissue residues within 80% of the steady-state tissue residues. The test was finished after 14 days due to high mortality of organisms. But even with short test period is possible to observe a trend in the results, in special the higher concentrations in metals among the organisms of the most contaminated site.

The biomonitor species appears to be appropriate to assess the bioavailability of trace elements. Pereira (2003) and Garcia (1999) indicate the *Anomalocardia brasiliiana* as an excellent bioindicator species, which corroborates with the current study. However, studies with longer exposure time as well as with other species are needed in Brazil.

5. Conclusion

The use of bioaccumulation test proved to be a suitable technique in the evaluation of contaminants present in sediments of estuarine regions and clams from the specie *Anomalocardia brasiliiana* proved to be an appropriate bioindicators, since the animals exposed to the more contaminated site tended to present the higher levels of metals in their tissues. This technique may be used alone or together with other approaches, composing an additional LOE in studies concerned to the environmental monitoring and assessment.

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Chapter 4 – Final Conclusion

The CIP-PA region has been considered as a reference for many years by different authors and institutions. However, using different tools, it can be seen that the region has undergone changes in their physical characteristics, and now exhibits signs of alteration due to contamination by metals. The combination of toxicity and chemical analyses showed that the region is being affected by the input of metals by the Ribeira de Iguape River, and sediments present a toxic potential. The whole-sediment TIE approach indicated that sediment toxicity was due to metals and ammonia, suggesting that the combination of natural and anthropic factors may be capable of producing ecological risks. As the contamination was associated to the muddy sediments, there is evidence that the contaminated suspended particulates coming from the RIR are the geochemical carriers for metals. Additionally, this study evidenced that muddy sediments (i.e., depositional areas) appear to move along the estuary, accompanying the displacement of the maximum turbidity zone, which in its turn depends on the freshwater inputs associated to seasonality. In other words, there is a migration on the deposition of fine particles and consequently the estuarine degradation tends to migrate according to the changes in depositional areas. These results highlight that oceanographic processes should be considered as important factors that influence on the accumulation of contaminants in estuarine areas. The bioaccumulation test confirmed that these contaminants may be bioavailable to the organisms and therefore, may be transferred to the food chain, with implications to ecological processes and human health.

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Appendix

Appendix A: Physical-chemical parameters of *Nitocra* sp

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

May 2012								
	Initial				Final			
	Salinity	pH	DO	T°C	Salinity	pH	DO	T°C
P1	15	6.58	5.00	23±2	15	4.38	5.88	23±2
P2	17	5.89	6.50	23±2	22	4.40	6.07	23±2
P3	16	6.49	6.25	23±2	22	5.88	4.90	23±2
P4	14	6.32	6.19	23±2	17	5.87	5.72	23±2
Control	20	6.48	6.26	23±2	22	7.83	5.30	23±2

August 2012								
	Initial				Final			
	Salinity	pH	DO	T°C	Salinity	pH	DO	T°C
P1	16	7.66	4.41	23±2	17	7.82	3.84	23±2
P2	16	6.6	3.92	23±2	17	4.25	3.82	23±2
P3	17	7.4	4.39	23±2	21	7.39	3.97	23±2
P4	18	7.3	2.17	23±2	22	7.77	3.49	23±2
P5	18	7.5	3.92	23±2	22	7.71	4.24	23±2
Control	16	7.4	2.96	23±2	22	7.36	3.39	23±2

March 2013								
	Initial				Final			
	Salinity	pH	DO	T°C	Salinity	pH	DO	T°C
P1	16	7.10	2.46	23±2	16	7.21	5.05	23±2
P2	15	6.24	1.97	23±2	15	4.14	3.65	23±2
P3	17	6.98	2.45	23±2	16	7.47	4.30	23±2
P4	19	7.31	1.66	23±2	19	7.81	3.62	23±2
P5	18	7.22	1.64	23±2	19	7.71	4.92	23±2
Control	18	7.29	1.56	23±2	19	8.01	4.42	23±2

Appendix B: Physical-chemical parameters of *Tiburonella viscana*

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

May 2012								
	Initial				Final			
	Salinity	pH	DO	T°C	Sal.	pH	DO	T°C
P1	34	7.73	6.76	23±2	35	5.30	6.25	23±2
P2	35	7.78	6.85	23±2	34	7.62	6.81	23±2
P3	34	7.79	6.65	23±2	34	7.48	6.41	23±2
P4	35	8.03	6.65	23±2	34	7.23	6.14	23±2
Control	35	8.09	7.00	23±2	34	8.37	6.45	23±2

March 2013								
	Initial				Final			
	Salinity	pH	DO	T°C	Salinity	pH	DO	T°C
P1	31	7.82	4.61	25°±1	31	7.82	5.75	25°±1
P2	31	7.80	5.38	25°±1	32	4.09	6.21	25°±1
P3	32	7.60	6.08	25°±1	35	8.02	6.28	25°±1
P4	35	7.77	5.31	25°±1	37	7.89	6.34	25°±1
P5	35	7.69	5.13	25°±1	35	8.05	6.09	25°±1
Control	35	7.56	4.86	25°±1	36	8.25	5.84	25°±1

Appendix C: Physical-chemical parameters of *Lytechinus variegatus*

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

May 2012								
	Initial				Final			
	Salinity	pH	DO	T°C	Salinity	pH	DO	T°C
P1	34	6.85	4.92	23±2	33	6.60	3.86	23±2
P2	34	6.93	6.23	23±2	35	6.59	5.88	23±2
P3	35	7.00	5.33	23±2	34	6.60	4.04	23±2
P4	35	7.06	5.10	23±2	34	6.50	3.69	23±2
Control	35	7.66	5.22	23±2	36	7.30	3.91	23±2

August 2012								
	Initial				Final			
	Salinity	pH	DO	T°C	Salinity	pH	DO	T°C
P1	34	7.65	6.38	23±2	34	7.40	5.10	23±2
P2	34	7.30	6.34	23±2	34	7.00	5.20	23±2
P3	34	7.45	6.26	23±2	34	7.20	5.80	23±2
P4	34	7.50	5.78	23±2	34	7.20	5.50	23±2
P5	34	7.50	6.34	23±2	34	7.10	6.20	23±2
Control	34	7.60	5.90	23±2	34	7.40	5.80	23±2

March 2013								
	Initial				Final			
	Salinity	pH	DO	T°C	Salinity	pH	DO	T°C
P1	34	7.32	4.17	23±2	34	7.42	4.52	23±2
P2	34	6.40	3.50	23±2	35	6.65	3.08	23±2
P3	35	7.40	3.39	23±2	35	7.52	3.88	23±2
P4	35	7.30	4.22	23±2	35	7.54	4.06	23±2
P5	35	7.65	3.63	23±2	35	7.60	3.94	23±2
Control	35	7.66	3.92	23±2	34	7.63	3.81	23±2

Appendix D: Physical-chemical parameters of *Anomalocardia brasiliiana* test

Appendix D Data of salinity, pH, dissolved oxygen (DO), temperature, total ammonia and unionized ammonia in the bioaccumulation test. DO and total ammonia were measured at mg/L and unionized ammonia at µg/L.

05/09/2012 – 1 st Day						
	Salinity	pH	DO	Unionized ammonia	Total Ammonia	Temperature
P1 A	32	7.22	4.11	0.13	129.62	25 ± 1
P1 B	30	7.59	7.08	0.20	205.01	25 ± 1
P1 C	30	7.59	7.04	0.12	123.13	25 ± 1
P2 A	28	7.08	7.39	0.84	842.10	25 ± 1
P2 B	30	7.14	7.58	0.69	686.37	25 ± 1
P2 C	30	6.97	1.58	0.25	245.74	25 ± 1
P3 A	30	7.05	5.02	0.16	155.99	25 ± 1
P3 B	30	7.52	6.92	0.33	331.23	25 ± 1
P3 C	30	7.51	5.93	0.51	506.04	25 ± 1
P4 A	31	7.64	5.29	2.76	2762.52	25 ± 1
P4 B	30	7.62	3.43	3.63	3632.46	25 ± 1
P4 C	31	7.38	5.36	0.81	809.62	25 ± 1
P5 A	31	7.46	6.51	0.13	134.46	25 ± 1
P5 B	30	7.56	7.48	0.18	179.80	25 ± 1
P5 C	30	7.61	7.27	0.31	314.62	25 ± 1
Control A	30	7.55	5.62	0.15	154.72	25 ± 1
Control B	30	7.53	5.82	0.13	130.16	25 ± 1
Control C	30	7.56	5.09	0.12	122.63	25 ± 1
13/09/2012 – 9 th Day						
	Salinity	pH	DO	Unionized ammonia	Total Ammonia	Temperature
P1 A	30	7.96	5.93	0.69	688.23	25 ± 1
P1 B	31	7.89	5.77	0.59	589.41	25 ± 1
P1 C	30	7.90	6.26	1.30	1295.57	25 ± 1
P2 A	27	7.31	6.15	0.55	547.16	25 ± 1
P2 B	23	7.26	5.91	0.80	798.54	25 ± 1
P2 C	30	7.31	6.02	0.50	501.77	25 ± 1
P3 A	31	7.95	6.06	2.41	2410.71	25 ± 1

P3 B	30	7.73	6.20	0.89	886.31	25 ± 1
P3 C	30	7.97	6.45	0.75	749.92	25 ± 1
P4 A	30	8.14	6.30	2.83	2830.12	25 ± 1
P4 B	31	8.08	6.33	2.33	2331.07	25 ± 1
P4 C	30	8.08	5.69	1.08	1084.31	25 ± 1
P5 A	31	8.04	6.20	1.13	1128.80	25 ± 1
P5 B	29	8.07	6.18	0.89	895.48	25 ± 1
P5 C	31	8.30	6.23	0.55	551.32	25 ± 1
Control A	31	7.89	5.95	0.46	456.69	25 ± 1
Control B	30	7.81	5.69	0.41	407.30	25 ± 1
Control C	31	7.94	6.10	0.48	478.68	25 ± 1

18/09/2012 – 14th Day

	Salinity	pH	DO	Unionized ammonia	Total Ammonia	Temperature
P1 A	30	7.77	4.10	0.12	118.15	25 ± 1
P1 B	30	7.49	2.91	0.05	45.66	25 ± 1
P1 C	31	7.75	6.15	0.13	128.34	25 ± 1
P2 A	27	7.10	6.26	0.02	18.01	25 ± 1
P2 B	27	7.14	6.59	0.03	30.84	25 ± 1
P2 C	32	6.78	6.85	0.01	6.98	25 ± 1
P3 A	31	7.66	5.12	0.10	98.35	25 ± 1
P3 B	32	7.36	2.73	0.01	10.11	25 ± 1
P3 C	30	7.70	6.64	0.01	10.83	25 ± 1
P4 A	30	7.87	6.58	0.06	60.48	25 ± 1
P4 B	30	7.57	2.84	0.02	25.46	25 ± 1
P4 C	30	7.61	6.46	0.01	13.82	25 ± 1
P5 A	31	8.01	6.79	0.06	59.92	25 ± 1
P5 B	30	7.84	5.96	0.01	13.91	25 ± 1
P5 C	32	7.91	6.11	0.01	12.59	25 ± 1
Control A	30	7.77	5.73	0.002	2.12	25 ± 1
Control B	31	7.57	4.76	0.01	7.58	25 ± 1
Control C	30	7.75	4.95	0.003	2.62	25 ± 1