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Heavy metals and TPH effects on microbial abundance and diversity in two estuarine areas of the southern-central coast of São Paulo State, Brazil

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ABSTRACT

Coastal areas may be impacted by human and industrial activities, including contamination by wastewater, heavy metals and hydrocarbons. This study aimed to evaluate the impact of hydrocarbons (TPH) and metals on the microbiota composition and abundance in two estuarine systems in the coast of São Paulo: the Santos (SE) and Itanhaém (IE) estuaries. The SE was found to be chronically contaminated by heavy metals and highly contaminated by hydrocarbons. This finding was correlated with the increased density of cyanobacteria in sediments and suggests the possible use of cyanobacteria for bioremediation. These contaminants influence the density and composition of estuarine microbiota that respond to stress caused by human activity. The results are troubling because quantitative and qualitative changes in the microbiota of estuarine sediments may alter microbiological processes such as decomposition of organic matter. Moreover, this pollution can result in damage to the environment, biota and human health.

1. Introduction

Coastal zones are responsible for 30% of biological primary productivity and 90% of fishery resources on our planet (Holligan and Reiners, 1992). They are characterized by increased concentration of nutrients from the land (including rivers and groundwater), as well as from oceans (including upwellings and shifts in the continental shelf) and the atmosphere (Alongi, 1998). Organic material, finer sediments, metals and other pollutants are also trapped in coastal zones (Tam and Wong, 1996, 2000; Che, 1999; Saifullah et al., 2002).

Coastal ecosystems differ in terms of their physical, chemical, and biological characteristics (Burke et al., 2001). Mangrove swamps are particularly unique as they serve as ecotones between land and sea environments along tropical and subtropical latitudes and are biologically more productive and are also subject to variations in temperature and salinity (Schaeffer-Novelli, 1995). They are polluted by different human activities (from factories, ports, and refineries to population increases with inadequate urban planning). These sources include untreated or improperly treated organic effluents, as well as industrial pollutants such as hydrocarbons and heavy metals, all of which negatively impact the environment and the local biota.

The Santos Basin, which is located along the central coast of São Paulo State, is home to the most important Brazilian industrial hub. This region is comprised of 23 industrial complexes, 111 factories and more than 300 sources of pollutants. The Santos Basin is also close to the largest port in Brazil (Pinheiro et al., 2012). All of these industries generate a variety of residues that can affect surrounding environments (Oliveira et al., 2007), which are already affected by illegal and poorly organized urbanization on land (CETESB, 2001).

The environmental area within mangrove swamps that is most susceptible to contamination is the sediment; because it is typically reductant so its finer particles can easily absorb petroleum products and metals and concentrates most of organic and

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inorganic waste (Reber, 1992; Tam and Wong, 1996, 2000; Che, 1999; Saifullah et al., 2002). These environments are therefore difficult to repair. Areas around ports are also subject to significant dredging and tidal fluctuations, which can return some or all contaminants to the water column (Medeiros and Bicego, 2004).

Mangrove swamps stand out for their significant microbial diversity, which is fundamental for maintaining the swamps' productivity, conservation, and recuperation. This diversity also optimizes the efficiency of swamps' nutrient, carbon, and sediment cycling processes. Thus, all organic material that is not expelled is retained in the sediment and subject to decay or to chemical modification (Holguin et al., 2001; Kristensen et al., 1998). According to Alongi (2002), 91% of microbial biomass from mangrove swamps is comprised of fungi, which are involved in various biological processes (such as the transformation of nutrients, atmospheric nitrogen fixation, and the production of metabolites, enzymes, and antibiotics). Therefore, these swamps represent important reserves for products of biotechnological interest (Aniszewski, 2010). Many microbial species including bacteria, yeasts, and cyanobacteria use hydrocarbons as source of carbon, and have the potential to be used as bioremediators and biomarkers for contamination by petroleum products (Zinjarde and Pant, 2002; Roling et al., 2004; Toyoda et al., 2005). The presence of pollutants in mangrove swamps may lead to changes in the microbial community, and may also cover up (or reveal) differences between contaminated and non-contaminated environments.

The aim of this work was to evaluate how the microbial community, including heterotrophic bacteria, yeasts, and cyanobacteria are impacted by concentration of hydrocarbons and metals in mangrove swamps in two estuarine systems in the state of São Paulo with distinct degrees of human impact (the Santos Estuary and the Itanhaém Estuary).

2. Materials and methods

2.1. Area of study: history and location

The Baixada Santista Metropolitan Area, located at southern-central coast of the Brazilian state of São Paulo includes a series of very different environmental features (Oliveira et al., 2007), from preservation areas (such as the 489,000 ha of the Serra do Mar State Environmental Protection Area) to areas characterized by intense industrial activities and large ports. Therefore, the Santos Estuary, which is part of the Santos-São Vicente Estuarine System, or the SESSV (Fig. 1A) and the Itanhaém Estuarine System, known as the SEI (Fig. 1B) were selected as areas of study. The SESSV includes densely populated cities (Santos, São Vicente, and Cubatão; Braga et al., 2000), and the SEI includes a single city with a lower population density.

The status of the SESSV is extremely critical because of the contamination of its aquatic ecosystems (Abessa, 2002); the current levels of contamination by aliphatic hydrocarbons in the São Vicente Canal and the internal canals in the city of Santos are elevated (Abessa et al., 2001). According to Lamparelli (2001) and Abessa (2005), this contamination comes from oil terminals, factories, and illegal dumping sites, and it results in high and potentially toxic levels of eight PAHs.

The SEI includes the Itanhaém River and its most significant tributary, which is comprised of a 950-km^2 hydrographic basin that measures 50 km in length by 15 km in width (Sant'Anna et al., 2007). Its main tributaries are the Branco da Conceição River, the Preto River, and the Aguapeú River. The current study covers the estuarine region of the Itanhaém River, which was used as a control because of the reduced impact of organic and chemical effluents on the body of water.

2.2. Sampling and processing of Samples

The sediment samples were collected at three points within the intertidal region of the Santos Estuary (SESSV) (P1: 23°59′10″S – 46°17′12″W; P2: 23°59′09″S – 46°17′11″W; and P3: 23°59′08″S – 46°17′11″W) and three points within the Itanhaém Estuary (SEI) (P1: 24°08′25″S – 46°48′11″W; P2: 24°10′23″S – 46°48′13″W; and P3: 24°10′48″S – 46°47′48″W) (Fig 1) using a sterile spatula at the surface and going down 5 cm deep. The samplings were performed at low tide of the spring tide in December 2009 and March and June 2010 and samples were stored in sterile flasks and kept under refrigeration (4 °C) until processing. The water samples were obtained 3 m away from the sediment collection sites. They were also stored in sterile flasks using the aforementioned procedure. In the lab 20 g of each sediment samples were diluted 10-fold with sterile seawater and vortexed during 5 min. Aliquots were used for a direct counting method for bacteria and cyanobacteria according to the process outlined by Hobbie et al. (1977) under a fluorescence microscope. A direct counting method was used to quantify yeasts that were determined using the modified method of Hasek (2006).

Interstitial water aliquots were obtained at the same sampling sites as the sediment using a solution extractor (ESS model), and salinity and pH were also measured using a portable refractometer, and an Inolab pH meter, respectively. Sediment temperature was measured at each sample point using the same portable meter.

2.3. Analysis of contaminants (metals and aromatic compounds)

Each sediment sample was analyzed at the CORPLAB Laboratory for the following parameters: Al, As, Cd, Fe, Hg, Pb and Zn, total petroleum hydrocarbons (TPHs), and BTEX (Benzene, Toluene Etilbenzene and Xylene). Metals were quantified using the mineralization method with HNO_3 at 65%, according to Basset et al. (1981). Analyses were optimized by hollow cathode lamps (LCO) in function of the metallic element analyzed, and samples were read using an atomic absorption spectrophotometer. The concentration of metals in each sample was expressed in micrograms of metal per gram of dry sediment (μg/g), and the minimum detected concentration was represented as μg/g (Cd < 0.01; Pb < 0.05; and Hg < 0.001). Aromatic contaminants (hydrocarbons and solvents) were quantified according to the extraction procedures specified by the EPA (2001), and based on indicative values recommended by Environment Canada (1999). International standards were used because of the absence of these values in Brazilian laws in cases of estuarine sediments. Contamination results of the estuarine areas were compared and are shown in Table 1, which is based on the two categories specified by Environment Canada (1999) and Hortellani et al. (2008): (1) Threshold Effect Level (TEL), the concentration below which adverse biological effects are rarely observed (<10%), and (2) Probable Effect Level (PEL), the concentration above which adverse biological effects are frequently observed.

2.4. Statistical analysis

Statistical analyses were conducted using 'R' version 2.5.0 (Ihaka and Gentleman, 1996). All the environmental and biological parameters were analyzed in order to evaluate normality through a Shapiro–Wilk test (SW) and equality of variances through a Levene test (L). Data with significant normal distribution and homoscedasticity were compared using Student's t test; otherwise, Kruskal–Wallis (KW) was used to compare medians (Zar, 1999). All statistical procedures were employed with a statistical significance level of 5%.

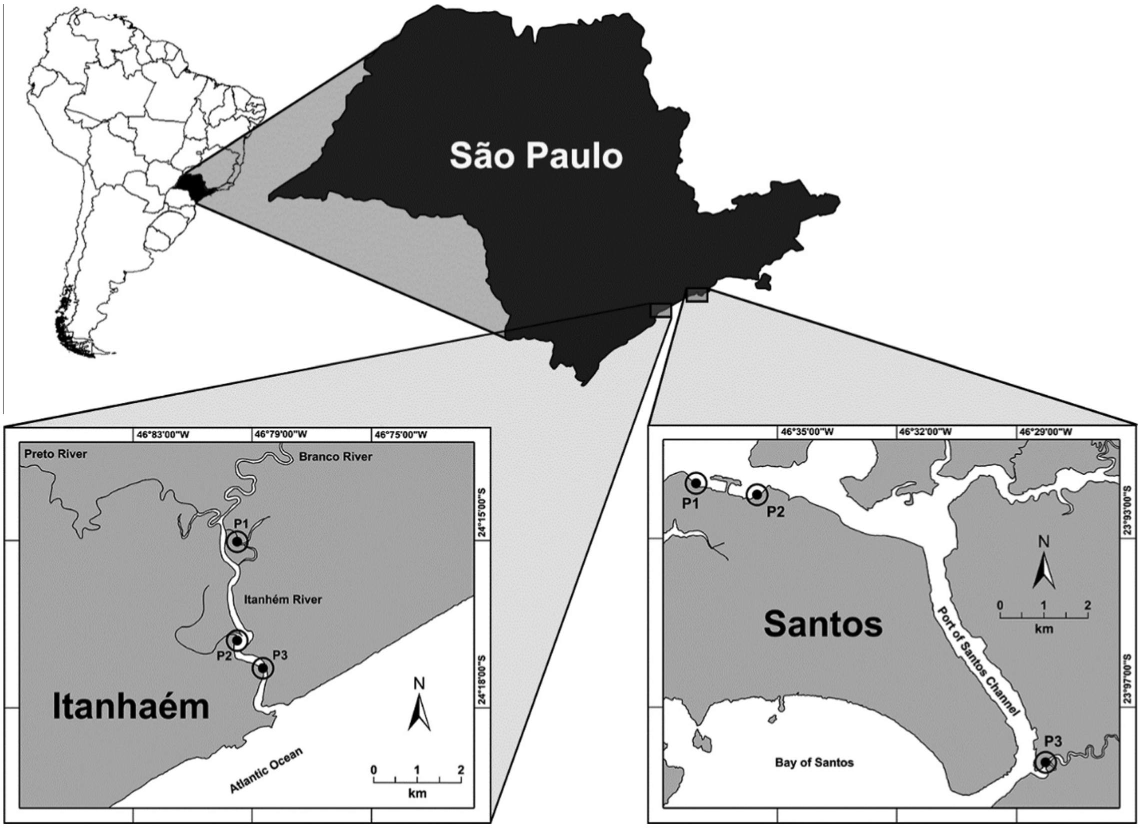

Fig. 1. Sampling points (P1–P3), located in the Itanhaém and Santos Estuarine Systems, in south-central coast of the state of São Paulo, Brazil.

Table 1
Reference values of metal concentrations to Threshold Effect Level (TEL) and Probable Effect Level (PEL) according to Environment Canada (1999).

Metals	TEL (μg/g)	PEL (μg/g)
Arsenic (As)	7.24	41.6
Cadmium (Cd)	0.70	4.21
Chrome (Cr)	52.3	160.0
Copper (Cu)	18.7	108.0
Lead (Pb)	30.2	112.0
Mercury (Hg)	0.13	0.696
Zinc (Zn)	124.0	271.0

3. Results

At the Santos Estuary, high medians values were recorded for the abiotic components of the water (KW > 5.399; $p < 0.05$), as well as in the sediment (KW > 4.600; $p < 0.05$); however, in the case of sediment temperature, the inverse pattern occurred (Table 2). Temperature and pH had much lower coefficients of variation (CV < 10.3%), a finding that contrasted with salinity (CV > 30.0%).

In both estuaries, the concentration of metals was always <PEL. In the case of Cd values were negligible or less than 0.7 μg g^{-1}. In the Santos Estuary the concentration of Hg in the sediment (Points 1 and 2) was 0.19 μg g^{-1}, which was a value between TEL and PEL. At Point 3, the mercury concentration was <TEL, a value that was found at all of the points at the Itanhaém Estuary (Fig. 2). The concentration of zinc in the sediment at Point 3 of the Santos Estuary (144 μg g^{-1}) was between TEL and PEL values (124 and 271 μg g^{-1}, respectively), while at the other Santos sampling points and in the Estuarine Estuary, the values were <TEL. Lead presented similar patterns to those of zinc: lead contamination levels were between TEL and PEL values (30.2 and 112 μg g^{-1}, respectively) at the mouth of the Santos Estuary (Point 3). At Points 1 and 2, lead concentrations were close to TEL values (30 and 34 μg g^{-1}, respectively); meanwhile, at the Itanhaém Estuary, lead concentrations were <TEL at all points. Arsenic concentrations were found to be between TEL and PEL levels (which themselves were 7.24 and 41.6 μg g^{-1}, respectively) only at Point 3 of the Itanhaém Estuary. At the other Itanhaém points and within the Santos Estuary, concentrations were <TEL. In summary, the Santos Estuary was found to have Hg contamination at Points 1 and 2 and Pb and Zn contamination at Point 3. The Itanhaém Estuary was found to have As contamination at Point 3. At the other sample points in each estuary, the concentrations of these metals were <TEL.

All of the aromatic solvent groups (BTEX) were detected in the sediment samples from both estuaries, though at slightly increased concentrations or at levels that do not posed risks to the biota (Table 3). Total petroleum hydrocarbons (TPH) were found at higher values in the Santos Estuary: they increased gradually and were 2.4 times higher at the outermost sampling area compared to the innermost sampling area. TPH values in the Itanhaém Estuary were found to be 1 order of magnitude lower when compared to those of the Santos Estuary, and higher values were recorded at Sampling Point 3, the estuarine mouth (see Fig. 3).

The average of cyanobacteria in Itanhaém waters ($14.3 \times 10^2 \pm 7.4 \times 10^2$ cells ml^{-1}) was 3.3 times higher than in the Santos waters ($4.2 \times 10^2 \pm 1.6 \times 10^2$ cells ml^{-1}) ($F = 14.94$; $p < 0.05$). On the other hand, the average of cyanobacteria in the sediment was 20.6 times higher in the Santos water ($12.4 \times 10^2 \pm 8.9 \times 10^2$ cells ml^{-1}) than in the Itanhaém water, in which it was $0.60 \times 10^2 \pm 0.1 \times 10^2$ cells ml^{-1} ($F = 15.59$; $p < 0.001$, Fig. 4).

The average of heterotrophic bacteria in the Santos waters ($16.9 \times 10^6 \pm 0.87 \times 10^6$ cells ml^{-1}) was 7.5 times higher than in the Itanhaém waters, where it reached $2.3 \times 10^6 \pm 0.12 \times 10^6$ cells ml^{-1} ($F = 2497.16$; $p < 0.001$). Bacteria concentration in

Table 2
Abiotic parameters (pH, temperature, and salinity) measured in water and sediment samples obtained in the estuarine systems of Santos and Itanhaém, on the south-central coast of São Paulo, Brazil. Where: Min, minimum value; Max, maximum value; *x*, mean; *s*, standard deviation; CV (%), coefficient of variation in percentage; KW, Kruskal–Wallis chi-squared.

Environmental matrices	Abiotic parameters	Santos			Itanhaém			KW	*p*
		Min – Max	*x* ± *s*	CV (%)	Min – Max	*x* ± *s*	CV (%)		
Water	pH	7.6–7.9	7.7 ± 0.1	1.5	5.9–6.3	6.1 ± 0.1	2.2	12.92	3.25 e−04
	T (°C)	25.0–33.0	31.0 ± 2.4	7.6	17.5–21.0	18.9 ± 1.1	5.7	13.13	2.91 e−04
	Salinity	0.1–30.0	19.4 ± 11.9	61.5	0.1–0.2	0.2 ± 0.1	30.0	5.39	2.02 e−02
Sediment	pH	7.6–7.9	7.7 ± 0.1	1.3	6.0–6.3	6.1 ± 0.1	1.9	12.83	3.41 e−04
	T (°C)	22.0–26.2	22.5 ± 1.4	6.2	22.0–29.0	25.7 ± 2.7	10.3	7.39	6.54 e−03
	Salinity	0–29.0	19.2 ± 11.5	59.8	0–0.2	0.2 ± 0.1	42.4	4.60	3.19 e−02

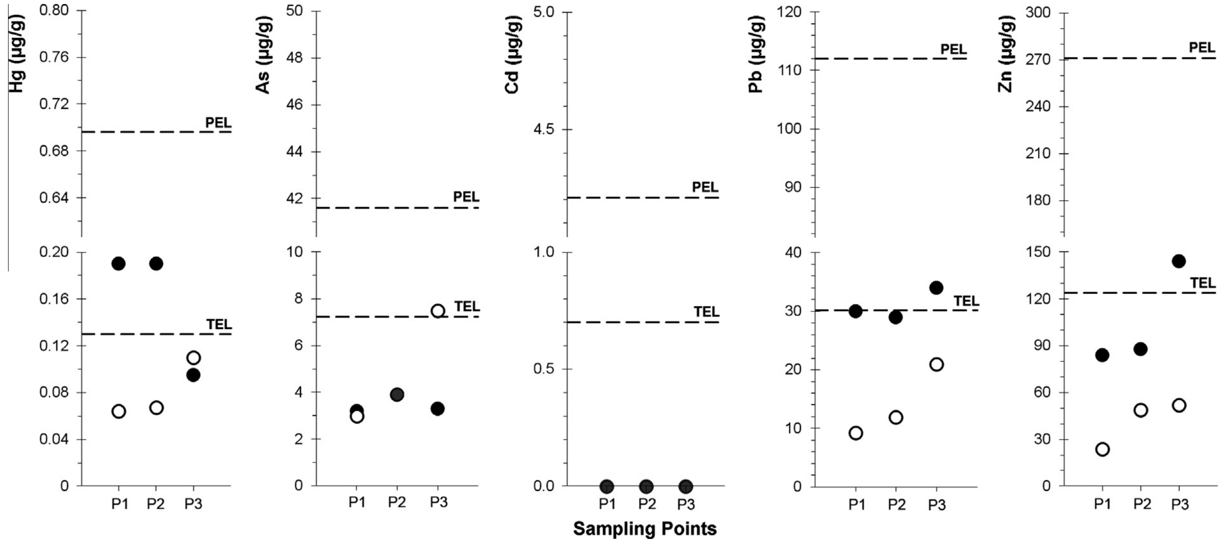

Fig. 2. Metal concentration of Mercury (Hg), Arsenic (As), Cadmium (Cd), Lead (Pb) e Zinc (Zn) in sediment samples from Santos (●) and Itanhaém (○) estuaries. Equal values are show in gray (●). The dashed lines indicate TEL (threshold effect level) and PEL (probable effect level) from Environmental Canada (1999).

Table 3
Concentrations (mg kg^{-1}) of aromatic solvents registered in samples of sediments in estuaries of Santos and Itanhaém, in south-central coast of state of São Paulo, Brazil. Where: **, out the bounds of quality control by matrix interference.

Aromatic solvents	Santos			Itanhaém		
	P1	P2	P3	P1	P2	P3
Benzene	<0.008	<0.009	<0.011	<0.010	<0.010	<0.017
Toluene	<0.008	<0.009	<0.011	<0.010	<0.010	<0.017
Etilbenzene	<0.008	<0.009	<0.011	<0.010	<0.010	<0.017
m,p-Xylene	<0.016	<0.017	<0.022	<0.020	<0.020	<0.034
o-Xylene	<0.008	<0.009	<0.011	<0.010	<0.010	<0.017
Total Xylene	<0.025	<0.026	<0.033	<0.030	<0.031	<0.050

sediment was quite similar in both estuaries, with an average of approximately 4.3 × 10^3 cells ml^{-1} In Santos, it was found to be 4.6 × 10^3 ± 1.4 × 10^3 cells ml^{-1}; in Itanhaém, it was found to be 4.1 × 10^3 ± 0.8 × 10^3 cells ml^{-1} ($F = 0.70$; $p > 0.05$) (see Fig. 5).

Yeast density in Itanhaém waters (328.7 ± 90.9 cells ml^{-1}) was 2.1 times higher than in Santos waters, where the density was 156.2 ± 145.3 cells ml^{-1} ($F = 9.11$; $p < 0.01$). Yeast concentration in both estuaries were similar and with an average of 3.3 × 10^2 cells ml^{-1}. When yeast density was measured in the sediments, the values were found to be similar between the two estuaries. In Santos, it was found to be 347.1 ± 222.7 cells ml^{-1}; in Itanhaém, it was found to be 313.8 ± 85.9 cells ml^{-1} ($F = 0.18$; $p > 0.05$) (Fig. 6).

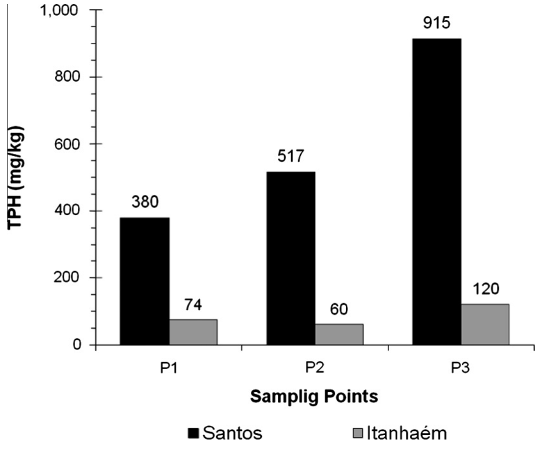

Fig. 3. Concentrations (mg kg^{-1}) of TPH (total petroleum hydrocarbons) in the sediment based on three sampling points in two estuaries (Santos and Itanhaém) in south-central coast of state of São Paulo, Brazil.

4. Discussion

Estuarine systems are influenced by very particular physico-chemical characteristics regarding salinity, temperature, tides and sediment anaerobic conditions (Schaeffer-Novelli, 1995). Among them, salinity is a relevant parameter that is controlled by climate, hydrology, rainfall, topography and tidal range, and

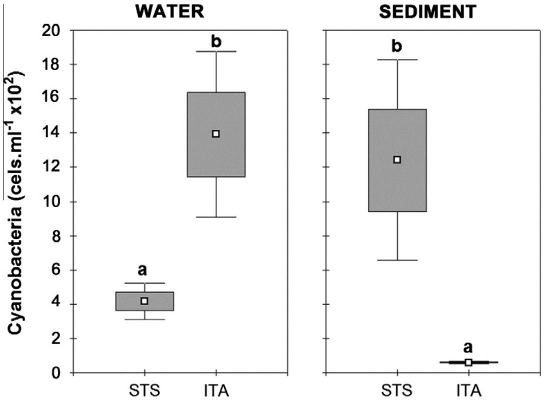

Fig. 4. Density of cyanobacteria (cells ml^{-1} × 10^2) registered in samples of water and sediment obtained in 2009 (December) and 2010 (March and June), at three sampling points in each estuary (STS, Santos; and ITA, Itanhaém). Where: white dot = mean; gray box = mean ± standard error; whiskers = mean ± 5% confidence interval; and letters = means of the same environmental matrix associated with different letters are significantly different ($p < 0.05$).

affects estuarine species distribution, productivity and growth (Chapman and Wang, 2001). Likewise, according to these authors, salinity also has an effect on the bioavailability of metals in contaminated environments and is the controlling factor for the partitioning of contaminants between sediments and overlying or interstitial waters. Estuaries with lower saline waters can promote an increase in metals bioavailability by their desorption from sediment particles, with an inverse pattern occurring in more saline areas, but other studies has shown that metals may decrease (e.g., Cd, Zn), increase (e.g., Fe), or be constant (e.g., Ir) when salinity increases (Li et al., 1984; Comans and van Dijk, 1988; Turner and Millward, 1994; Paalmann et al., 1995; Fisher and Reinfelder, 1995). However, Atkinson et al. (2007) has found, in a laboratory experiment, that sediments has a strong salinity-buffering capacity showing that any overlying changes in salinity would be temporary and no significant differences between the rates of metal release was found indicating that salinity would not affect bioavailability of metals in water. In the present study no similar salinity experiments was conducted, but this parameter can be a factor driving the bioavailability of heavy metals on SESSV due their higher salinity range when compared to SEI.

In this study, metals were detected in the sediment samples obtained from the two estuaries that were studied, though at concentrations that was lower than PEL. However, it is important to note that other contaminants were present at concentrations that may affect the local biota. These other contaminants include hydrocarbons from petroleum products. Santos Estuary was more contaminated than the Itanhaém Estuary, a result that is reflected in the overall lower density of microorganisms in the Santos Estuary.

According to CETESB (2001) the high contamination of the Santos-São Vicente Estuarine System was promoted by industrial sources, mainly by five factories: *Presidente Bernardes Refinery* at Cubatão, which belongs to *Petrobras*, in addition to *Carbocloro*, *Liquid Química*, *Cosipa*, and *Dow Química*. Mercury concentrations in the effluents from these factories was reported to be lower than legal limits (CETESB, 1986), though 40% of the sediment samples were found to have concentrations >TEL, and 8% of which were >PEL. According to Fowler (1990), sediments from estuarine and marine areas are considered non-contaminated when the concentration of mercury is less than 0.1 μg g^{-1}, and are considered contaminated when this concentration is above 5 μg g^{-1}. Considering this definition, the results obtained suggest lowered mercury contamination levels in both estuaries studied; however, in some cases, the Santos Estuary was found to exceed the guideline values. This result suggests that mercury likely influences the organisms that live there.

The sediments from the Santos-São Vicente Estuarine System revealed a higher buildup of zinc, particularly in the Santo Amaro River. This river is close to industrial sources (the Cosipa and Dow Química factories), and zinc levels surpassed PEL. The current study confirms zinc contamination close to this river (183–221 μg g^{-1}); zinc concentration was >TEL at Point 3 of the current study, which corresponds to the Santo Amaro region of the CETESB study.

Lead is generally associated with the mining industry, and more specifically, with beneficiation and casting. It is also associated with industrial effluents resulting from petroleum and petrochemical refining processes, among other industries and processes. The highest lead concentrations were registered in the sediments from the Santo Amaro River within the Santos Estuary, which is also close to the Dow Química factory. In results that were consistent with those from the CETESB (2001), a lead concentration of 66 μg g^{-1} was found at Point 3 of the current study, a value which was twice the TEL established by Environment Canada (1999).

Although, in general, metal concentrations were found to be between PEL and TEL, it cannot assure that no effect on biota is

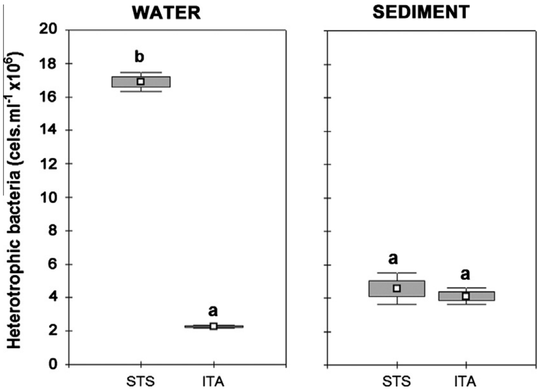

Fig. 5. Density of heterotrophic bacteria (cells ml^{-1} × 10^6) registered in samples of water and sediment obtained in 2009 (December) and 2010 (March and June), at three sampling points in each estuary (STS, Santos; and ITA, Itanhaém). Where: white dot = mean; gray box = mean ± standard error; whiskers = mean ± 5% confidence interval; and letters = means of the same environmental matrix associated with different letters are significantly different ($p < 0.05$).

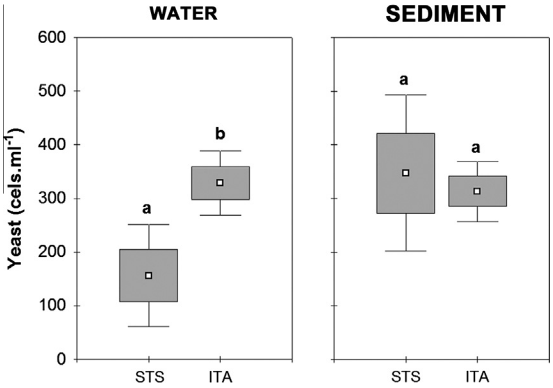

Fig. 6. Density of yeast (cells ml^{-1}) registered in samples of water and sediment obtained in 2009 (December) and 2010 (March and June), at three sampling points in each estuary (STS, Santos; and ITA, Itanhaém). Where: white dot = mean; gray box = mean ± standard error; whiskers = mean ± 5% confidence interval; and letters = means of the same environmental matrix associated with different letters are significantly different ($p < 0.05$).

occurring since reference values are based on international guidelines and their application may not fully address local particularities of each environment (Choueri et al., 2009). As recommended by Chapman et al. (2005): "there is a need to develop estuarine-specific sediment quality guidelines that more appropriately account for low and variable salinities".

In natural ecosystems, natural oscillations in diversity and number of microorganisms occur as a function of environmental changes (Frostegard et al., 1996; Oliveira et al., 2007; Pinto and Oliveira, 2011). However, it is easy to imagine that the increased concentration of metals in the environment as a result of human activity could further alter the microbiological environment (Abaye et al., 2005; Hemme et al., 2010) through both their lost-lasting presence and their toxic potential.

The current study did reveal differences in densities among the three microorganisms within the different environmental matrices (the sediments from the Santos and Itanhaém Estuaries). In the water samples, the three microorganisms (cyanobacteria, heterotrophic bacteria, and yeasts) were found at higher overall densities in Itanhaém than in Santos. Though the heterotrophic bacteria and yeasts in the Santos Estuary were found at lower densities in the water samples, the cyanobacteria densities were found to be higher in the Santos sediment samples probably due to higher contaminants concentrations.

There are few studies assessing changes in bacterial communities in non-contaminated and contaminated areas. Vishnivetskaya et al. (2011) found significant differences in bacterial communities between contaminated and non-contaminated sites in streams' sediment of Tennessee, that were strongly correlated with higher concentrations of Hg and MeHg (methylmercury). Other studies also have shown MeHg production to be related to seasonal effects, with the highest rate occurring in summer and increasing microbial activity (Gilmour and Henry, 1991; Stoichev et al., 2004). Previous work in estuarine sediments with different levels of anthropogenic contamination, especially heavy metals and PAHs, has highlighted that changes in microbial community and diversity are strongly associated with contaminants concentration (Sun et al., 2012). It also have shown microbial community to be more sensitive to anthropogenic contamination than to environmental variation related to latitude, salinity or pH, already identified as an influence on bacterial community in previous studies (Fierer and Jackson, 2006; Fuhrman et al., 2008; Lozupone and Knight, 2007 The authors state that Pb, Cu, Zn and PAH are responsible for driving changes in bacterial community composition and diversity and microbial community have great potential to be use as an indicator of contamination in estuarine sediments.

The influence of metals may or may not be additive, and it can lead to a decrease in the genetic population of the microbial population when the metals occur at high concentrations (Hirsch et al., 1993). Metals can also inhibit the activities performed by bacteria, fungi, and yeasts, and can thus significantly alter the ecological balance of ecosystems (Reber, 1992; Landmeyer et al., 1993; Rassmussen and Sorensen, 1998; Jankowska et al., 2006).

The presence of heavy metals in the environment (particularly in the sediment) can lead to a drastic decrease in population abundance and diversity. Heavy metals can interfere in microbiological processes involved in the transformations of nitrogen compounds (Hassen et al., 1998; Munn et al., 2000), reduce the mineralization of the organic material in the sediment (Reber, 1992), provoke the denaturation of proteins, and block sites that are active in connecting enzymes (Siqueira et al., 1994). However, it is important to note that some metals are used in reduced quantities in the metabolism and growth of some microorganisms (e.g., Zn, Cu, Ni, Co, Cd and Cr), which is why they are referred to as essential metals. They differ from non-essential metals (e.g., Pb, Hg, As, etc.), which have no function in biochemical processes (Chaudri et al., 1992).

Some of the sample points in the current study were found to have values of metals that were lower than guideline values, though their continuous release into the water promotes their absorption into the sediment. The sediment functions as both a filter and a trap (Fan et al., 2002; Eggleton and Thomas, 2004), which results in a buildup of these pollutants and others in the environmental matrix. This buildup can become a serious problem. It occurs because microorganisms are able to use chemical processes (reduction and oxidation) to transform metals into compounds that are even more toxic to the environment through the conversion of metals into organic forms, and vice-versa (Eggleton and Thomas, 2004). As studied by Paul and Clark and Hungria and Urquiaga, one example is the methylation of mercury by aerobic bacteria, anaerobic bacteria, fungi, and yeasts. This process results in the formation of more toxic molecules that are more rapidly absorbed by animal tissues. These molecules include monomethylmercury (CH_3Hg) and dimethylmercury (CH_3HgCH_3). Mercury, lead, and zinc at levels above TEL limits in the sediments from the Santos-São Vicente Estuarine System are likely to have negative effects on the local biota, which includes the microorganisms that are found there. The data obtained reinforce the possibility of contamination by individual or diffuse sources. Human involvement in this contamination needs to be investigated further in terms of both the extent of the contamination and the negative effects on the biota and microbiota of these environments.

When it comes to aromatic solvents (BTEX), all of the groups were detected in samples from the two estuaries studied; however, their concentrations were not as high or did not present a risk at the local biota. Meanwhile, the presence of TPH at all of the sample points may be associated with chronic sediment contamination, which was more evident in the Santos Estuary. In this estuarine system, the origin of TPH is certainly the petroleum refineries close to the Cubatão River, as well as the oil and other fuels dumped into the Santo Amaro River by sea vessels. In the case of the Itanhaém Estuary, the presence of TPH may be due to small deposits released by sea vessels that navigate the Itanhaém River as a part of ecotourism activities. Other researchers have confirmed the association between the density of some microorganisms (such as cyanobacteria) and areas with higher levels of pollution by petroleum products (Oliveira et al., 2007). This association can be explained by the use of carbon in microorganism growth (Al-Hasan, 1994; Barth, 2003). In addition, the main predators of cyanobacteria are drastically affected by the presence of toxicity and petroleum products. As a consequence, there is a greater potential for cyanobacteria growth in these contaminated environments (Sorkhoh et al., 1995), and also for an increased degradation rate of these hydrocarbons (Chaillan et al., 2004).

Waters from the SESSV are influenced by numerous activities, which may contribute to the increased density of the microorganisms that are present. Among these activities, port-related functions stand out, as does the release of domestic effluents into the estuarine region. These effluents, which can be released *in natura* or which can be partially or completely treated, carry with them a variety of pathogenic microorganisms (e.g., bacteria, viruses, and protozoa). The presence of these microorganisms would explain the high densities of bacteria registered in the water samples from the Santos Estuary. The environmental destruction of the SESSV has been outlined in technical reports (CETESB, 1979, 1986, 2001). It has had significant impacts on society and public health as a result of its association with industrial and wastewater pollutants from the Port of Santos, as well as from other cities in the region. This situation has been further aggravated by the illegal dumping of solid waste (from both industrial and domestic sources), as well as by the frequent oil spills and other accidents with toxic substances in the region's watercourses (Tommasi, 1979). These factors impede the analysis of any correlation between the density of bacteria and the presence of petroleum and/or petroleum products.

According to the literature, the highest yeast densities are associated with the least polluted environments. The findings of the current study differ: the highest yeast densities were registered in both the water and sediment from the Itanhaém Estuary, which were the samples which were found to be less contaminated. This situation occurs because marine yeasts are extremely versatile biodegradation agents; they participate in various biological processes in aquatic environments, particularly in estuaries and coastal environments (DeSouza and D'Souza, 1979; Kobatake, 1992). According to Kutty and Philip (2008), yeast populations in estuarine sediments tend to increase in the presence of oil because they posses hydrocarbonoclastic properties that make them more effective than yeasts in non-contaminated areas. Thus, there is a tendency toward a significant reduction in yeast densities in non-contaminated areas, particularly in estuarine areas (Hagler et al., 1982; Zinjarde and Pant, 2002).

The densities of different microorganisms in environmental matrices react differently to the levels of contamination by heavy metals and other pollutants, depending on the source of the pollution (industry vs. ports) and the release of wastewater (both industrial and organic) into the areas studied. This contamination has different effects on the survival and mortality of microorganisms.

5. Conclusion

Microbial communities can be affected by the presence of contaminants in the environment: proximity to ports and industrial hubs results in chronic contamination, which is reflected in the composition of the microbiota. Thus, microorganisms that are able to degrade hydrocarbons and that use them as a source of carbon in their metabolic process can be used both in studies and in the development of bioremediation techniques. The results of this study suggest that cyanobacteria possess a greater potential for breaking down hydrocarbons in estuarine regions that are contaminated by heavy metals and organic material. The presence of these contaminants seems to have a negative influence on the survival of yeasts. In the case of organic contamination, competition and/or synergism can occur among the microorganisms introduced through domestic wastewater and the yeasts that are present in the environment.

Thus, the breakdown of hydrocarbons by microorganisms in estuarine areas is extremely dependent upon both environmental conditions and human activity. The latter should be taken into consideration when certain species or microbial consortia are selected for bioremediation studies.

This study corroborates the idea that the presence of heavy metals or other pollutants in the environment (particularly in the sediments) can lead to drastic changes to the abundance and diversity of microbial populations, and can also interfere in microbiological processes that are extremely important to maintaining the health of estuarine systems, the health of organisms that live in those systems, and public health in general.

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