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Using Sets of Geochemical Analyses and Toxicity Tests to Assess the Effects of Sewage Disposal in Santos Bay, Brazil

Denis Moledo de Souza Abessa[1]*, Eduinetty Ceci Pereira Moreira de Sousa[2], Bauer Rodarte de Figueredo Rachid[3], Leticia Pires Zaroni[2], Marcia Regina Gasparro[2], Marcos Antonio Hortellani[4], Jorge Eduardo de Souza Sarkis[4]

[1] *UNESP, Praça Infante Dom Henrique, s/n, São Vicente, SP, Brazil. 11330-900*
[2] *USP. Praça do Oceanográfico, 191, São Paulo, SP, Brazil. 05508-900*
[3] *FUNDESPA. Avenida Afrânio Peixoto, 412, São Paulo, SP, Brazil, 05507-000*
[4] *IPEN. Av. Prof. Lineu Prestes, 2242, São Paulo - SP, Brazil, 05508-900*
***Corresponding author**: dmabessa@clp.unesp.br

Abstract:
The submarine sewage outfall of Santos (SSOS) is a significant source of contaminants to the marine ecosystems of Santos Bay. The present study aimed at assessing the influence of SSOS on the sediment quality of Santos Bay. To achieve that sediments were collected at 5 sampling sites located around the SSOS diffusers, then sets of chemical analyses and toxicity tests were conducted. The results indicated that, at the disposal site, sediments tended to be finer, organically richer and exhibited higher levels of surfactants and metals, sometimes exceeding Threshold Effect Level values. The SSOS influence was more evident toward the East, where the sediments exhibited higher levels of TOC, total S and metals. Chronic toxicity occurred in all samples, whereas acute toxicities were more evident in the sediments from station 4 (West). Toxicities correlated to Hg, ammonia, detergents, total N, total S and % mud. Results evidenced that sediments around SSOS are affected by effluent discharges, and that the combined use of sets toxicity tests together with chemical analyses may provide a better overview of the sediment quality.

Keywords:
Integrative Analysis; Marine Pollution; Sediments; Sewage Outfall; Toxicity

1. INTRODUCTION

Urban sewage is the main source of marine and estuarine pollution in Brazil [1]. Most Brazilian coastal cities do not have proper facilities to collect, treat and dispose sewage. In Baixada Santista, situated at the central shore of the State of São Paulo, a sanitation program was conducted between late 1970's and early 1990's, and involved the construction of collection systems in some cities and the installation of four submarine sewage outfalls along the shore. The oldest of them is the submarine sewage outfall of Santos (SSOS), which has been operating since 1978 and serves the cities of Santos and São Vicente. The system was designed to accommodate a maximum population of 1.322 million people [2]. Nowadays, this system receives approximately 95% of the sewage generated in Santos and 40% of the sewage produced in São Vicente.

The SSOS is considered a major source of contamination to the Santos Bay, as it discharges untreated sewage into the sea [2]. This oceanic disposal system is composed of a pre-conditioning plant and its respective sewage outfall. The pre-conditioning consists of a process in which the effluent is chlorinated, double screened (10 and 4 cm length), and sieved (1.5 mm rotation sieve), before being carried into the pipeline. There is not any further treatment (primary or secondary), and the sewage is essentially discharged untreated. The pipeline is built of concrete-covered steel (1.75 m internal diameter). It is 5 km long and its diffusers are situated in the center of Santos Bay, at 4 km from the beach and at 12 m depth [3]. In the last 200 m of the pipeline, there are 40 diffusers, which function to increase the initial dilution of the effluent [4]. The maximum projected capability for the effluent flow is 7 m^3/s but the mean flow ranges between 0.6 to 1.6 m^3/s. The lacking of further treatments in this sewage disposal system has been justified by economic reasons and because they aim, at least theoretically, to improve the water quality at the beaches. Thus other environmental aspects have been historically neglected.

Sewage pre-conditioning is recognized as not effective to eliminate of contaminants, therefore, when this approach is elected it is assumed that the water body is capable of naturally dilute and depurate the contaminants.

The discharge of sewage into the sea in shallow waters should involve monitoring programs which assess the environmental impacts of its operation. Historically, the environmental effects produced by the SSOS discharges have not been adequately studied. Investigations regarding the effects are few and far between and have not considered the complexity of the possible effects [2]. In a preliminary study, toxicity was detected in sediments collected close to the outfall diffusers of SSOS [5]. More recent studies detected sediment contamination, toxicity and effects on benthic community in the vicinity of SSOS diffusers [6, 7]; but still the effects of sewage discharges are not totally known and demand further investigations. The objective of this study was to assess the quality of sediments in the vicinity of the Santos sewage outfall, by using geochemical analyses and sets of toxicity tests.

2. MATERIALS AND METHODS

Sampling was conducted in summer 2000, and sediment samples were collected at five stations situated close to the sewage outfall diffusers (**Figure 1**), using a 0.026 m^2 stainless steel Petersen grab sampler.

From the retained material, only the 2-cm surficial layer from the sediment not in contact with the grab sampler walls was composited. Aliquots of the homogenized composite were then sub sampled for chemical, granulometric and ecotoxicological analyses. Control samples were collected at Ilhabela, in Engenho D'água Beach (23.7701°S – 45.3592°W). This choice was supported by the fact that in Baixada Santista the sediments are influenced by anthropogenic activities to varying degrees, thus using reference sediments from that region is not advisable [7]. Moreover, Engenho D'água Beach has been used as reference in previous studies [5–9].

In the whole sediment toxicity tests, approximately 1L aliquots were kept refrigerated at 4 ± 2 °C until the experiments. For the porewater toxicity tests, 3L sediment were separated, conditioned in plastic bags and kept in ice. In laboratory, the porewater was immediately extracted by the suction method [10]. Afterwards, the extracted samples were centrifuged for 20 minutes at 4200g in glass tubes [11, 12],

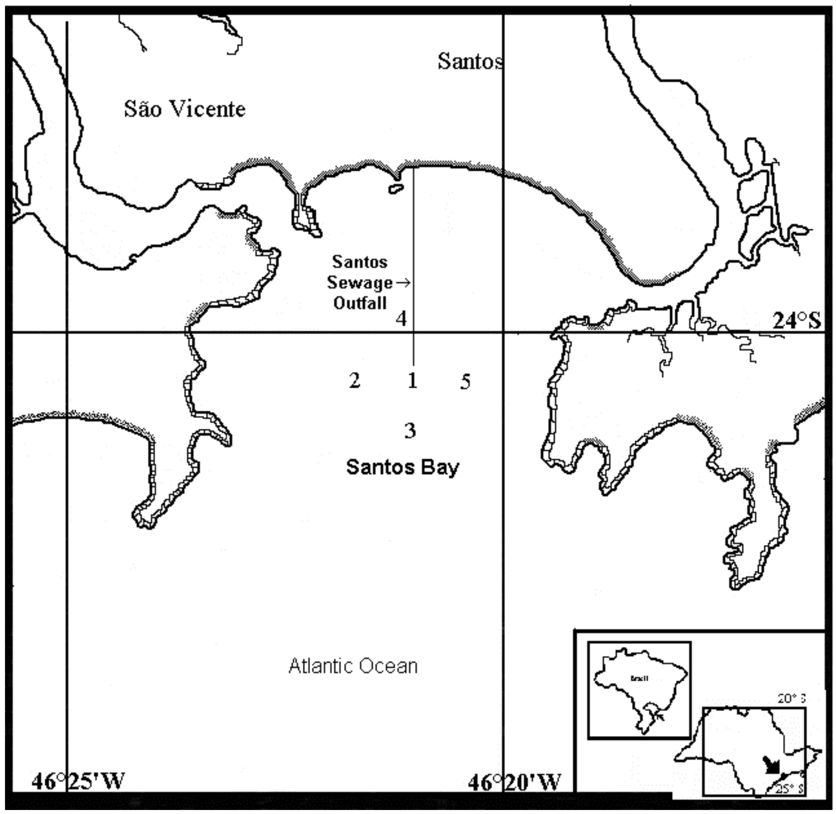


Figure 1. Map showing the sampling sites in the vicinity of the Santos Sewage Outfall System, SP, Brazil

transferred to dark glass flaks and kept frozen at -20 °C. Sub-samples for the geochemical analyses were conditioned in plastic bags and frozen.

Procedures for textural properties and chemical analyses were described in detail in Abessa *et al.* (2005) [6]. Calcium carbonate contents were estimated by acid digestion [13]. Grain size distribution was analyzed by the dry sieving method [14] and samples were classified following the textural classes of particles [15, 16]. The contents of total organic carbon (TOC), nitrogen and sulfur were measured using a LECO CNS 2000 automated analyzer, which uses the Micro Kjeldahl method [17]. The concentrations of Al, Fe, Cd, Cr, Co, Ni, Pb and Zn were analyzed by fast sequential Atomic Absorption Spectroscopy. For Hg, extracted solutions were introduced into a system of Flux Injection for Cold Vapour generation and analyzed by Atomic Absorption Spectroscope. Chemical results were compared to the Canadian Sediment Quality Guidelines [18]. Surfactants concentrations in sediments were estimated by the Methylene Blue Active Substance (MBAS) method [19] after extraction by elutriation in distilled water [20].

Whole sediment acute toxicity tests were conducted using the amphipod *Tiburonella viscana*, according to the procedure described by Melo & Abessa (2002) [21]. Five replicates per test sediment were prepared. One day before the beginning of the test, each sediment sample was thoroughly homogenized and aliquots were distributed into the test chambers (1-L polyethylene beakers). The test chambers were filled to 2 cm depth with the test sediments and filtered seawater up to 750 mL and then maintained overnight at 25 ± 2°C with gentle aeration (maintained by air pumping). On the next day, 10 amphipods were added to each test chamber. The tests were conducted at 25 ± 2°C, under constant aeration and lighting. After ten days, the contents of the test-chambers were gently sieved through a 0.5-mm screen and the surviving amphipods were counted. Missing organisms were considered dead. Mortalities were compared with the control by Student t'-test. The dissolved oxygen concentration, salinity and pH of the overlying water in the test chambers were measured at the beginning and termination of the tests. The water temperature was monitored daily.

Porewater samples were evaluated by the early life stage bioassay with embryos of the sea urchin *Lytechinus variegatus* [22]. Before the test, samples were thawed at 25°C, and the salinity, pH, dissolved oxygen and temperature were checked. The total ammonia concentration was measured by a colorimetric method [23]. Using these data, the unionized ammonia contents were estimated, using the method reported by Whitfield (1974) [24].

Prior to the beginning of the test, adult individuals were collected at rocky reefs, in Ubatuba, a clean site, and taken to the laboratory. The spawning was induced by the injection of 2-3 mL KCl into the coelomic cavities of the animals [22], and gametes of 3 males and 3 females were collected. Ovules were collected by precipitation in beakers filled with filtered seawater, whereas the sperm was collected dry and transferred to beakers kept on ice. The sperm was activated by dilution in filtered seawater and the ovules were fertilized by adding 2 mL sperm solution to the eggs solution. The fertilization success was confirmed by examination under the microscope. The toxicity test was conducted in glass test tubes containing 10 mL test-solution. Four replicates were used for each concentration. Three porewater concentrations were prepared as described in Carr *et al*. (2001) [12]: 100, 50 and 25%. The experiment was kept in a temperature controlled room, at 25 ± 2°C. After 24h, the test was stopped by adding 0.75 mL of 10% buffered formaldehyde to each replicate. The embryos were analyzed microscopically for morphological anomalies and retarded development (100 per replicate). All the embryos which did not reach a well-developed pluteus larvae were considered affected. The results were statistically analyzed by ANOVA, followed by the Dunnett's t'test comparison, with the SAS statistical package.

The whole sediment toxicity tests with the benthic copepod *Schizopera knabenii* used laboratory cultured organisms. The test protocol for this species was described by Chandler & Green (1996) [25]. The test-chambers consisted of 50 mL glass flasks, containing 3mm sediment layer and 30 mL filtered sea water. pH, salinity and temperature were checked in all the replicates, at the beginning and the end of the experiments. Then, each chamber received 10 ovigerous females. Three replicates were prepared for each sample. Sediment from Engenho D'água Beach was used as reference. After 10 days, the material in each chamber was fixed in alcohol 70% and then colored with Bengal rose dye. Afterwards, the adults and young (nauplii and copepodites) were counted under stereomicroscope. The results were analyzed by the Student t'- test.

Using a similar test protocol, a porewater toxicity test was prepared with *S. knabenii*. In this experiment, 3 replicates were prepared by sample. Whole samples and 25% dilutions (in filtered seawater) were used in this test. After 72 h, the survivors were counted and the results were evaluated by Student t'-test.

Correlations between toxicity, chemical contamination and sediment characteristics were tested using

Table 1. Grain size and organic enrichment and concentrations of metals and anionic tensoactives (mbas) in sediments collected close to the ssos, in the summer 2000 (extracted from [6])

Sampling sites	Texture (%)			Organic enrichment (%)			Surfactants (MBAS) ($mg.kg^{-1}$)		
	Sand	Mud	$CaCO_3$	TOC	S	N			
1	72.23	27.67	8.12	1.01	1.12	0.09	7.33		
2	91.12	8.88	10.23	1.73	0.07	0.01	6.35		
3	64.31	35.69	16.31	0.91	0.79	0.06	4.84		
4	96.09	3.91	7.68	0.96	0.04	<0.01	4.40		
5	32.41	67.39	12.32	1.53	0.45	0.09	4.89		
Reference	95.31	3.69	8.90	1.87	0.22	0.04	5.37		
	Metals (%)		Metals ($mg.kg^{-1}$)						
	Al	Fe	Zn	Ni	Pb	Cd	Cr	Co	Hg
1	3.99	1.78	50.63	12.51	12.31	<0.5	14.34	5.80	0.09
2	3.94	1.78	42.84	11.40	11.34	<0.5	5.46	5.15	0.09
3	4.31	2.02	44.59	12.39	8.92	<0.5	17.97	5.40	0.05
4	4.19	2.18	51.51	14.43	16.29	<0.5	16.99	5.61	0.05
5	5.25	3.25	65.59	20.91	25.95	0.65	38.01	11.15	0.17
Reference	7.14	3.20	78.97	17.37	14.74	0.5	22.03	12.86	0.03

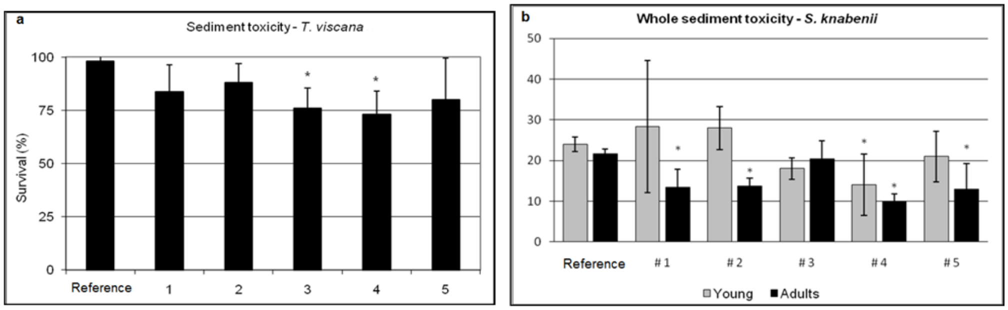
multiple correlation analysis, through the use of the free PAST software.

3. RESULTS

Geochemical data were previously presented by Abessa *et al.* (2005) [6]. Sediments from stations 1, 2, 4 and the control were predominantly sandy, whereas sediments from stations 3 and 5 were sandy muds with the greater presence of $CaCO_3$. The highest TOC concentrations were measured in sediments of stations 2 and 5, respectively. The highest concentrations of N and S occurred in sediments from stations 1 and 5 (**Table 1**). Concerning metals, the highest concentrations were observed in sediment from station 5. Concentrations of Cd, Pb and Cr were below the Threshold Effect Level (TEL) proposed by the Environment Canada [18] in all analyzed samples. The highest Hg concentrations were observed in the sediments from stations 4 and 5, exceeding the TEL. Concentration above TEL was observed for Ni, in sediments from station 5 and control sample. High concentrations of Al, Fe and Zn in control sediment were considered natural [6]. The highest concentrations of MBAS were observed in the sediments from stations 1 and 2.

The physical-chemical conditions of the overlying water in the test-chambers during the bioassay with *T. viscana* were considered appropriate [21]. Temperatures ranged between 24.0 and 25.0 °C; pH ranged between 7.6 and 8.6; and salinities ranged from 36 to 37. The concentrations of NH_3-NH_4 ranged from not detectable to 0.25 mg/l. In this toxicity test, the organisms exposed to sediments from stations 3 and 4 exhibited significant mortalities (**Figure 2(a)**).

The results of the whole sediment toxicity test with copepods are presented in the **Figure 2(b)**. The adult survival rates were significantly lower in the sediments from stations 1, 2, 4 and 5, when compared to the control. Regarding the fecundity, only the sediments from the station 4 produced a significant lower rate. The different responses exhibited by adults and offspring probably were due to different exposure routes: young individuals still use energy from the egg and do not feed, thus they are exposed to dissolved



(a) survival rates of *T. viscana*

(b) adult survival and offspring production of harpacticoid copepods *S. knabenii,*

Figure 2. Whole sediment toxicity: a) survival rates of *T. viscana*; and b) adult survival and offspring production of harpacticoid copepods *S. knabenii,* both exposed to sediments from SSOS vicinities; where* = significant difference to the control ($p<0.05$)

Table 2. Physical-chemical characteristics of the porewater samples extracted from sediments collected close to SSOS and estimated concentrations of NH_3

Sample	pH	Salinity (‰)	NH_4-NH_3 (mg.L^{-1})	NH_3 (mg.L^{-1})
Reference	8.2	35	< 0.2	< 0.2
1	10	36	2.5	2.1
2	8.5	37	5.0	1.6
3	8.6	36	2.5	0.8
4	8.6	37	5.0	1.6
5	8.5	36	1.0	0.3

contaminants in porewater; on the other hand, adults feed on biofilm and fine particles [26], thus they integrate exposure through ingestion route and porewater.

For the porewater toxicity tests, salinities ranged between 36 and 37; and pH values ranged between 8.2 and 10 (**Table 2**). The NH_3 estimated concentrations were high, and for the stations 1, 2 and 4 the toxic threshold for sea urchin embryos was exceeded. In this test, only the sample from station 4 (at 100% concentration) was considered toxic to *S. knabenii* (**Figure 3**. On the other hand, all porewater samples produced strong effects on the embryonic development of *L. variegatus*, in all tested concentrations (**Table 3**).

Sediments from station 4 were toxic in all the tests, exhibiting a critical condition, whereas sediments

Table 3. Results of the porewater toxicity test using *Lytechinus variegatus* embryos

Sample	Mean normal development (%) ± (Standard Deviation)		
	100% PW	50% PW	25% PW
Reference	74.0 ± 2.5	70.3 ± 7.5	72.0 ± 10.8
1	0	0	0
2	0	0	39.3 ± 6.0
3	0	1.0 ± 1.7	49.0 ± 6.6
4	0	0	0
5	0	0	0.3 ±0.6

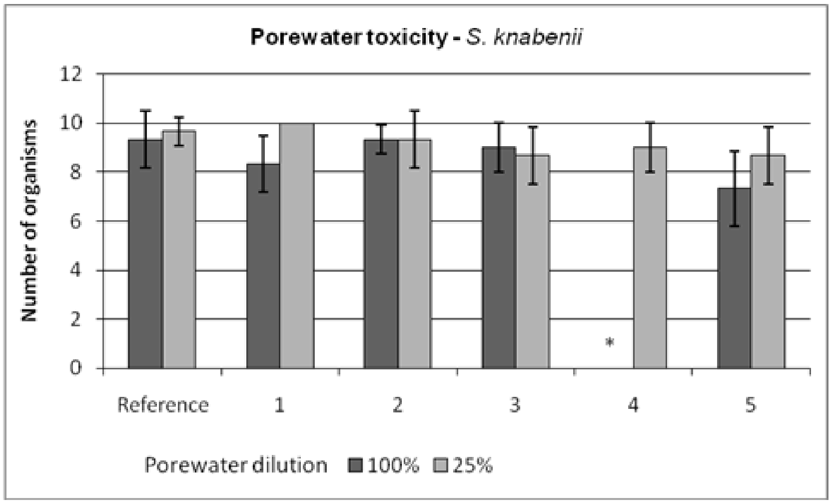


Figure 3. Survival rates of *S. knabenii* exposed to porewater from sediments collected close to the SSOS diffusers, where* = significant difference to the control ($p<0.05$), by the Student t'test

Table 4. Comparative results obtained in sediment toxicity tests for sediment samples collected close to the SSOS diffusers

Exposure form	Whole sediment		Porewater	
Response	Acute	Chronic	Acute	Chronic
Organism	*T. viscana*	*S. knabenii*	*S. knabenii*	*L. variegatus*
1	Not toxic	Toxic	Not toxic	Toxic
2	Not toxic	Toxic	Not toxic	Toxic
3	Toxic	Not toxic	Not toxic	Toxic
4	Toxic	Toxic	Toxic	Toxic
5	Not toxic	Toxic	Not toxic	Toxic

from stations 1, 2 and 5 presented only chronic toxicity (moderate alterations). Sediment from Station 3 presented acute toxicity to amphipods, but was not toxic to copepods in whole sediment assay (**Table 4**).

The results did not show a clear association between sediment toxicity and contamination, at least for the measured contaminants. The more toxic sediment was collected at station 4, whereas the most contaminated was that from station 5. As the levels of metals and detergents were low in the sediment from station 4, the cause of toxicity is probably a contaminant that was not analyzed. Some authors have demonstrated the presence of n-alcanes and poly aromatic hydrocarbons (PAHs) in sediments collected close to the SSOS diffusers [7, 27–31], as well as detergents, steroids and other compounds [6, 32, 33].

For all bioassays, the indicators of absence of toxicity (survival, normal embryonic development, fecundity) negatively correlated with Hg concentrations. Excepting the test with *L. variegatus* embryos, these indicators correlated negatively with NH_3-NH_4 content. These results suggested that probably these two contaminants influenced directly the sediment toxicity (**Table 5**). On the other hand, Al concentrations correlated positively with these indicators, suggesting that this element reduced the bioavailability of the other elements. The concentrations of Zn and Co and the TOC contents correlated negatively with these indicators, in the tests with *T. viscana* and *L. variegatus,* whereas sulphur contents correlated negatively (in the bioassay with copepods). Mud content correlated with the concentrations of Cd, total S, Cr, total N and $CaCO_3$, whereas TOC percentage correlated with Al, Fe, Zn and Co concentrations.

Table 5. Variables significantly correlated and their respective Pearson linear correlation coefficients (p = 0.05)

	Al	Zn	Co	Hg	TOC	NH_4-NH_3
T. viscana survival	0.70	0.59	0.59	-0.82	0.84	-0.64
	Hg	MBAS	NH_4-NH_3			
S. knabenii young production	-0.78	0.53	-0.62			
	Al	Hg	S	NH_4-NH_3		
S. knabenii adult survival	0.60	-0.78	0.53	-0.62		
	Hg	MBAS	Mud	$CaCO_3$	N	NH_4-NH_3
S. knabenii survival (25% PW dilution)	-0.56	0.79	-0.60	-0.71	-0.51	-0.99
	Hg	MBAS				
S. knabenii survival (100% PW dilution)	-0.72	0.51				
	Al	Hg	COT			
L. variegatus development (25% PW dilution)	0.57	-0.86	0.50			
	Cd	Cr	$CaCO_3$	S	N	NH_4-NH_3
Mud	0.70	0.64	0,76	0.53	0.89	0.86
	Al	Fe	Zn	Co	Hg	
TOC	0.64	0.55	0.58	0.66	-0.50	

4. DISCUSSION

In Brazil, the disposal of urban effluents in the ocean through outfalls has been adopted as an alternative to sewage treatment, with the purpose protect or restore the bathing quality of beaches [1, 4]. However, studies to identify and estimate such impacts in the coast of São Paulo are sparse and discontinued [3, 6, 7, 34–36].

The primary effect of sewage is the physical alteration of sediment texture in the outfall vicinities, due to deposition of fines. According to Pončano (1985) [37], the sedimentation in SES "is a result of the influence of both continental and marine processes, which interact on each other, producing a complex pattern". Studies made prior or just after the SSOS operation beginning showed a complex sediment distribution in Santos Bay, as a mosaic of textures; however they indicated that, in general, the region was divided in two sectors: one at east, with finer (silty) sediments, and other at west, with sandy sediments [37, 38]. The present study corroborate with literature, as sandy sediments were found at western stations whereas finer sediments occurred in stations positioned at east and south (stations 3 and 5).

Fine particles seemed to be transported from west to east, especially during storms [6, 37]. Other investigations [7, 39] showed that such pattern has been maintained. However, in the central portion of Santos Bay (station 1), the influence of SSOS could be detected, as sediments have become increasingly finer and enriched with S, N and COT, indicating that SSOS discharges are capable of changing some physical and chemical properties of the sediments, by deposition of solids.

Precipitation of particles in areas situated close to sewage outfalls is expected for oceanic disposal systems [2, 34], as part of natural depuration processes which occur in the receptor water body. The sewage effluent from SSOS presents very high contents of suspended solids, with potential to alter grain size distribution and increase levels of organic matter, especially close to the diffusers [2]. Literature presents more examples of organic enrichment and increased amounts of fines in sediments situated close to sewage outfalls [40–43]. Modifications of sediment textures or nutrient levels are important because they relate to geochemical changes and may induce alterations on aquatic communities. Besides, as most of effluent contaminants tend to adsorb to particles [44, 45], they accumulate in the bottom sediments

[41], and produce biological effects, such as toxicity.

Concentrations of metals in sediments collected around the SSOS vicinities tended to be low, and were often below TEL [18], except for Hg (stations 1, 4 and 5) and Ni (stations 1, 3, 5 and control). These results are in accordance to the recent studies for Santos Bay [39, 46], showing higher levels of contamination than the past [47, 48]. These levels are also in the same range than those detected around sewage outfalls worldwide, despite differences due to sedimentary origin, weather regime, geographic and geochemical factors. Sediments from Santos were slightly more contaminated that sediments from Sydney [41], Bilbao [49], Nervión [40] and Rhode Island [50] and less contaminated than those from Southern California [43]. According to Smith *et al* (1996) [18], sediments with Ni concentrations above TEL present only 8% probability of being acutely toxic, whereas those with Hg concentrations above TEL present 24% probability of causing acute toxicity.

Although the contamination levels are not critical, they evidence that sewage discharges are negatively affecting the quality of environment. Some factors favor the contaminants retention in the sediments from the vicinities of SSOS diffusers, and they include the high average flow rate, and SSOS location, as the discharges are made within a bay, where hydrodynamic processes are attenuated and may favor the precipitation of solids and contaminants released by the outfall.

Till recently, sewage effluents were expected to present high concentrations of total solids, nutrients and TOC, and lower amounts of metals, hydrocarbons and pesticides [34]. However, such effluents have been reported as containing higher levels of metals [41, 51], detergents, insecticides, deodorizers [50], hormones and personal care compounds [52], among other substances. In addition, sewage may receive contribution from stormwater and urban drainage waters containing high concentrations of metals, PAHs, PCBs and ammonia [53], increasing their toxic potential.

The chemical composition of SSOS effluent was previously analyzed [2, 6], and, concentrations of metals, PCBs, organochlorines and PAHs were low. Solids concentration was considered high, as well as the concentrations of oil and greases, ammonia and sulfides, which exceeded the legal standards for Brazil. Rachid (2002) [2] evaluated the SSOS effluent by using the Toxicity Identification and Evaluation (TIE) approach, and observed that the substances responsible for the toxicity were the suspended solids (SS), ammonia, volatile compounds (as chlorine), oxidant substances and apolar organic compounds.

The toxicity associated to suspended solids is due the capacity of SS of adsorbing contaminants [45]. As the suspended solids precipitate around SSOS diffusers, they increase the sediment contamination. Further studies should consider the analysis of the chemical composition of the suspend solids from SSOS, as well as the modeling of solids behavior in the SSOS plume.

Detergents may occur in sewage as well [54–56], and accumulate in the sediments [57, 58]. In this study, the higher concentration of detergent was observed in sediments from station 1 (SSOS diffusers). Similar distribution was observed for linear alkylbenzene detergents in sediments [28] and in bottom waters [35].

Toxicity of porewater was possibly influenced by ammonia and sulfides [2]. Although ammonia and sulfides have been considered confounding factors in toxicity tests [12, 59], in the case of sewage, they may be considered contaminants [6, 7, 60]. In this study, NH_3 concentrations were above the toxic threshold for *L. variegatus* [61] that is 0.05 mg/L. The test with copepods indicated only one sample as toxic (station 4). The lower sensitivity of *S. knabenii* to ammonia was probably due to the adaptation of this species to high levels of ammonia. According to Rachid (2002) [2], ammonia was the main responsible for the toxicity of interstitial waters extracted from sediments from SSOS vicinities, followed by suspended solids. In addition to ammonia, sulfides had probably an influence on the sediment toxicity, as they may be toxic [62, 63] and are discharged by SSOS in large quantities [2]. Abessa *et al*. (2008) [7] found very high concentrations of sulfides in sediments collected around SSOS diffusers.

In addition to ammonia and sulfides, Hg and detergents correlated to the toxicity, indicating that toxicity was a result of a combination of different contaminants.

5. CONCLUSIONS

The SSOS is a source of alteration of sediments at the central portion of Santos Bay, causing changes in texture and chemical and ecotoxicological characteristics. Such changes are more intense in the proximity of diffusers, where enrichment by fines, TOC, total N and total S occurs due to the discharge of fine particles by SSOS.

The contamination by metals in sediments tended to be low in the region around SSOS diffusers, with concentrations often below TEL. Concentrations above TEL were detected for Hg and Ni. Apart from station 1 (SSOS diffusers), concentrations of detergents were low. The majority of sediments produced chronic toxic effects on tested organisms, and sediment from station 4 was consistently toxic in all tests. Ammonia seemed to be one of the main substances related to the toxicity, but negative biological effects also correlated with Hg, total S and detergents, suggesting that toxic effects were due to the combination among them.

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