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Evaluation of the water genotoxicity from Santos Estuary (Brazil) in relation to the sediment contamination and effluent discharges

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Abstract

The genotoxic activity of water samples collected in 9 different sites within the area of the Santos estuary was preliminary evaluated, and related to previous data on the genotoxicity of sediments and the contents of PAHs in both water and sediment samples. The liquid discharge of a steel mill (coke plant), known to be mutagenic, was chemically analyzed to determine its PAH content. For the water evaluation we employed the Salmonella/microsome assay with the strains TA98 and TA100 with and without S9 mix in the plate incorporation method. The water was filtered with an AP20 membrane before being extracted with XAD4 at natural and acidic pH. The industrial effluent was filtered in 0.45 μm membranes before being extracted with the liquid/liquid method. Both membranes containing the particulate material were extracted using ultrasonication. PAHs were found associated with the suspended particles present in the industrial effluent in accordance with mutagenicity data previously reported. In relation to the estuarine waters, sites 1 and 5 presented low levels of mutagenic activity only in the filtered water (liquid fraction) extracts. At site 3, both the filtered water and particulate solids presented also low mutagenicity. Results show that the mutagenic activity observed in water could not be directly related to the genotoxic activity and PAHs contents of the bottom sediments.

Keywords: PAHs; Estuary contamination; Salmonella assay; Suspended particles; Steel mill effluent

1. Introduction

The most important Latin American harbor and the largest industrial complex of Brazil, including steel mill, fertilizer and chemical industries, is situated in Santos estuary which was one of the major examples of coastal degradation by industrial pollution in the 1970s (CETESB, 2001). Several studies have been performed by CETESB, the Environmental Protection Agency of São Paulo State, to evaluate the contamination levels and biota alterations during the last decades (CETESB, 1979, 1981, 1990) in the area of the Santos estuary. Although source control resulted in improved water quality in the last years regarding chemical parameters and toxicity, unfortunately the sediment and biota are still contaminated (CETESB, 2001; Nishigima et al., 2001; Roubicek, 2003; Medeiros and Bícego, 2004; Umbuzeiro et al., 2004). For the maintenance of the port activities, dredging of sediment is required which can resuspend contaminants and consequently affect the water quality and biota.

In relation to the sources of contamination of this estuary, one of the main pollution sources is a steel mill industry that includes a coke plant unit. Coke plants usually produce large quantities of liquid effluents containing suspended solids, high COD, BOD, phenols, ammonia and other toxic substances, especially genotoxic polycyclic aromatic hydrocarbons (PAHs), that can cause serious pollution problems in the receiving waters (Ghose, 2002). Because the solubility of those PAHs in water is very low due to their hydrophobic nature ($\log K_{ow}$ = 3–8) (Kot-Wasik et al., 2004) once in the estuarine water these compounds are preferentially bound to suspended particles or sediment (Nogami et al., 2002; Tam et al., 2001).

Barreto (1995) characterized the genotoxic effect of three samples of the effluent of the steel mill industry that is

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discharged in Santos Estuary. The samples were analyzed using the Salmonella/microsome assay and presented in average 10^5 revertants/liter equivalent, potencies considered high according to Houk's classification (1992). The work also showed that the mutagenic activity was associated with the particulate matter present in the effluent. PAHs such as benzo(*a*)pyrene could be responsible for this effect because this class of contaminants is known to be present in this type of effluent, and they are preferentially bound to particles. Additionally the positive responses observed for the Salmonella/microsome assay were obtained only in the presence of metabolic activation system (S9 mix) which is required for the mutagenic activity of this class of compounds. Unfortunately the study performed by Barreto (1995) did not evaluate chemically the effluent in relation to its PAHs content.

In the estuary, the sediment of the area where this steel mill effluent is discharged showed high levels of mutagenic activity (Roubicek, 2003; Umbuzeiro et al., 2004) and concentrations of total PAHs up to 733 μg/g of dry weight of sediment were detected. Oysters collected near this area showed concentrations of benzo(*a*)pyrene up to 88 μg/kg of body weight, and crabs collected at the site where the effluent is discharged presented concentrations of other PAHs such as naphtalene up to 351 μg/kg. In relation to fishes, due to their high PAH metabolization capacity, low levels of PAHs were detected, as expected. PAHs were not detected in waters collected from this site, despite of the high concentrations levels in the sediment (CETESB, 2001).

Taking into account the lack of information in the literature about the levels of PAHs in the steel mill effluent that is discharged in the Santos Estuary and because this effluent has previously shown PAHs-related mutagenic activity, one of the objectives of this work was to determine its PAH content.

Another objective was to evaluate the mutagenic activity, using the Salmonella/microsome assay, of the surface water collected in different locations of this estuary, including the site that is directly under the influence of the steel mill effluent discharge, and compare this activity to the water and sediment PAHs levels previously published.

2. Materials and methods

2.1. Sample collection

2.1.1. Steel mill effluent

An instantaneous sample (1 L) of the final treated effluent from the steel mill industry that is released in front of site 5 (Fig. 1) was collected according to APHA (1998). This effluent had previously shown mutagenic activity with *S. typhimurium* TA98 strain in the presence of S9 mix (Barreto, 1995).

2.1.2. Surface water

Collection sites at the estuary were selected according to their pollution sources and were numbered in this study in order to correspond to the numbers used by CETESB (2001) and Roubicek (2003) (Fig. 1). Sites 1 to 4 are rivers that receive several industrial effluents from different sources such as petrochemical, plastics, fertilizers activities. Site 5, located in the estuary, is considered in previous studies as the most contaminated area and has received discharges from the steel mill plant during the last 50 years. Site 10 is directly impacted with domestic sewage discharges without treatment as well as a landfill. Site 12 is at one of the estuarine channels and is under the influence of a hexachlorobenzene contaminated area. Site 14 is located at another estuarine channel and is less impacted with industrial discharges although it receives untreated domestic sewage. Site 18 is located off shore, with sandy sediment under the influence of a sewage outfall. Volumes of 50 L of surface water were collected in each of the 9 selected sites according to APHA (1998).

Both water and effluent samples were stored in ice until the moment of the extraction procedures.

2.2. Sample organic extraction procedures

2.2.1. Steel mill effluent

In order to determine the PAHs content of the effluent and its relation to the suspended particles a volume of 1 L of effluent was filtered through acetate cellulose membrane 0.45 μm (Millipore) using a vacuum system in order to separate the suspended particles (Turner and Millward, 2002). The membrane containing the particulate matter as well as a clean membrane were extracted by ultrasonication using the same solvent/volume (Grifoll et al., 1990). The filtered effluent (liquid fraction) was extracted by liquid/liquid method with methylene chloride (3 × 60 mL) according to USEPA-Method 610 (US EPA, 1982). Both extracts were solvent exchanged for PAHs analysis.

2.2.2. Surface water

The surface water samples (50 L) were filtered through glass fiber filter AP20 (Millipore) (>0.8 μm) under vacuum and the suspended particles as well as the filtered water were analyzed separately for their mutagenic activity. The filtered water (liquid fraction) was extracted using XAD4 resin at natural and acidic pH. The solvents employed were methanol/methylene chloride for the natural pH extraction and methanol/ethylacetate for the pH 2 extraction. For

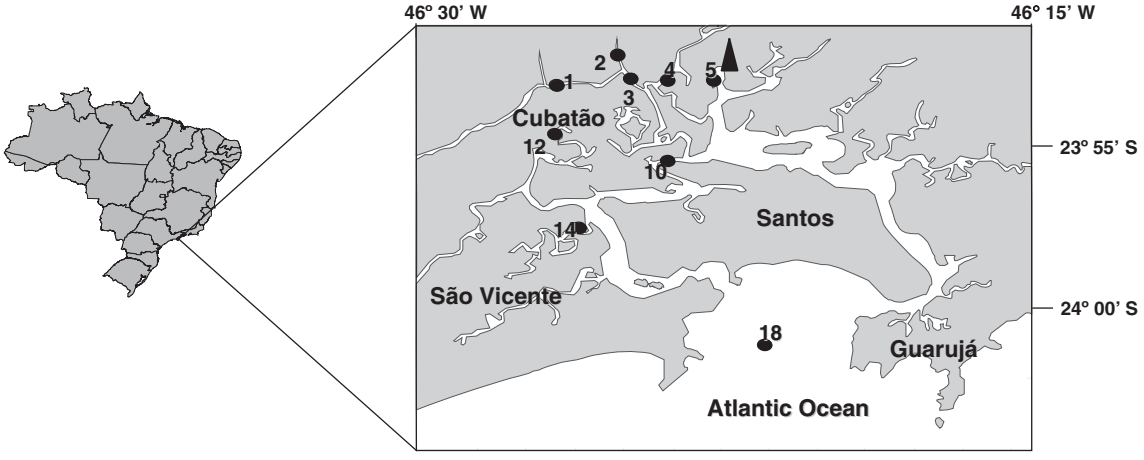

Fig. 1. Map showing the Santos Estuary in São Paulo State (Brazil), the sampling sites evaluated in this study (●) and the location of the steel mill industry (▲).

Table 1
Results of the PAHs content for the filtered and suspended particles of the steel mill industry effluent sample analyzed in this study

PAH	Filtered effluent (μg/L of sample)	Suspended particles		Negative control of membrane (μg/L of extract)
		μg/L	μg/kg	
Acenaphthene	0.21	589	24	–
Anthracene	ND[a]	224	9	<10
Benz[*a*]anthracene	ND	446	18	ND
Benzo[*a*]pyrene	ND	321	13	ND
Benzo[*b*]fluoranthene	ND	391	16	ND
Benzo[*k*]fluoranthene	ND	192	8	ND
Chrysene	ND	527	21	ND
Dibenz[*a*,*h*]anthracene	ND	ND	ND	ND
Fluoranthene	–[b]	1594	64	20
Fluorene	–	709	28	<10
Indeno[1,2,3-*cd*)pyrene	ND	ND	ND	ND
Phenanthrene	<0.01	632	25	<10
Pyrene	0.01	1058	42	ND

[a] ND—Not detected under the analytical conditions.
[b] (–) Not analyzed.

methodology details see Kummrow et al. (2003). Each filter containing the suspended solids was extracted by ultrasonication using methylene chloride/methanol (2.5:1 v/v) for three times (Grifoll et al., 1990; Roubicek, 2003). The volume of solvents employed were the same used for the XAD4 extraction (Kummrow et al., 2003). Both extracts were reduced to 2–3 ml using a rotary evaporator, transferred to small vials, evaporated to dryness with a gentle stream of nitrogen gas and then resuspended in dimethylsulfoxide (DMSO) just before testing in the Salmonella assay.

2.3. PAHs analysis

The determination of PAHs was performed in the steel mill effluent extracts (filtered effluent and suspended particles) according to USEPA-Method 610 (1982). The extract was concentrated and the solvent was exchanged to acetonitrile and analyzed in a programmable gradient system liquid chromatograph (Varian model 9012Q) with fluorescence detector (model Pro Star 360) and an automatic sample injector (AI-200—Hainin Dynamax). The following conditions were used: HPLC column EC 150/4 Nucleosil 100-5 C18 PAH (Macherey Nagel); flow rate: 1 mL/min; injection volume—10 μL; gradient (acetonitrile/water) program 1: 52% A in 10 min; 2: 52% to 80% A in 6 min; 3: 80% A to 100% A in 5 min, held at 100% in 18 min; 4: 100% A to 52% A in 1 min, held at 52% A for 5 min before injection.

2.4. Salmonella mammalian/microsome mutagenicity assay

The Salmonella/microsome assay (Maron and Ames, 1983) was performed in suspended solids and filtered samples extracts, from the 9 sites studied, using the TA98 (*his*D3052, *rfa*, Δ*bio*, Δ*uvr*B, pKm101) and TA100 (*his*G46, *rfa*, Δ*bio*, Δ*uvrB*, pKM101) strains (Maron and Ames, 1983) with and without S9 mix containing 4% (v/v) lyophilized Aroclor-1254-induced rat liver S9 fraction (Moltox Inc.) and cofactors. The samples were tested at 5 doses and 200 mL equivalents per plate was the maximum dose. DMSO was used as negative control. Positive controls were 2-nitrofluorene in assays without metabolic activation (S9), and 2-aminoanthracene with S9 mix, at concentrations of 10 and 2.5 μg per plate, respectively. The background was carefully evaluated for toxicity using a stereomicroscope. Results were analyzed performing an ANOVA test to detect significant positive responses among the tested doses followed by linear regression analysis with the Salanal computer program applying Bernstein model (Bernstein et al., 1982) to calculate the potency of each sample. The potencies were expressed in revertants per liter equivalent of water both for the filtered and the suspended solids in relation to the amount of water filtered.

3. Results and discussion

The PAHs are hydrophobic compounds with two or more fused benzene rings, generated during incomplete combustion of organic mater, naturally present in ecosystems (e.g. natural fires) or associated with human activities. Coke production is one of the major sources of PAHs contamination (Quantin et al., 2005). Several PAHs and derivatives are persistent and potent carcinogens and/or mutagens, therefore studies of their source, occurrence, transport and fate in the environment have been extensively conducted (Zhu et al., 2004).

We decided to analyze the final effluent from the steel mill industry released in the Santos Estuary in order to verify its PAHs content and their association with the suspended particles. These compounds were quantified in the filtered effluent and in the suspended particles separated by filtration. The clean membrane used as negative control showed only very low levels of fluoranthene (Table 1). The results for the effluent sample analyzed are presented in Table 1. Eleven PAHs among the 13 PAHs analyzed were present in the effluent sample, associated to the suspended particles. The concentrations of benzo(*a*)-pyrene were 13 μg/kg of suspended particles (321 μg/L equivalent) (Table 1). In the filtered effluent only small amounts of acenaphtalene, phenanthrene and pyrene were detected (0.21, <0.01 and 0.01 μg/L respectively). Those results could explain the observations of Barreto (1995) that indicated that the mutagenic activity detected with TA98 only in the presence of S9 mix (Table 2) was related to the suspended particles. Sedimentation of this material is probably responsible for the high levels of PAHs already detected in the sediment at site 5 (Fig. 1) that is under the influence of this discharge (Table 3).

Table 3 summarizes the results of filtered water and suspended particles (AP20) and only sites 1, 3 and 5 presented mutagenic activity. Genotoxicity data for sediments (Roubicek, 2003) and concentration of PAHs in the sediment and water samples (CETESB, 2001) were included in the same table for comparison.

The water samples collected at sites 2, 4, 10, 12, 14 and 18 (except 1, 3 and 5) did not present mutagenic activity. PAHs were also not detected in the waters and the sediment presented low levels of PAHs and no mutagenic activity (Table 3). Those results indicated a good water quality in relation to mutagenicity but other genotoxicity bioassays using higher trophic organisms and/or more sensitive extraction procedures could be employed in order to complement the quality evaluation of these sites.

Sites 1, 3 and 5 presented very low levels of mutagenicity (Umbuzeiro et al., 2001; Ohe et al., 2004) and because they correspond to only one sampling the results must be considered preliminary. Site 1 is located at Cubatão river, under the influence of the Billings reservoir from São Paulo city, and has a landfill garbage deposit nearby. The water collected in this site presented low mutagenic activity after

Table 2
Mutagenicity results for the Salmonella/microsome assay using liquid/liquid extraction (source: Barreto, 1995)

Sampling	Number of revertants per liter equivalent		Classification according to Houk (1992)
	TA98 − S9	TA98 + S9	
1[a]	Not detected	93×10^3 (76×10^3–110×10^3)[b]	Moderate
2	Not detected	169×10^3 (125×10^3–213×10^3)[b]	High
3	Not detected	18×10^3 (11.2×10^3–25.3×10^3)[b]	Moderate

[a] The same three samples were analyzed after filtration in 0.45 μm membranes and the filtered effluent showed negative results indicating that the mutagenicity was associated to the suspended particles (Barreto, 1995).
[b] 90% confidence limits.

Table 3
Results of Salmonella/microsome assay for the filtered water and suspended solids and comparison with sediment mutagenicity (Roubicek, 2003) and water and sediment PAHs levels reported previously (CETESB, 2001) analyzed in the same sites during the same period

Sampling site	Strains	Salmonella/microsome assay			Chemical analysis	
		Data of this paper		Data from Roubicek (2003)	Data from CETESB (2001)	
		Number of revertants per unit of sample			Total PAHs	
		Filtered water (per liter equivalent)	Suspended solids (per liter equivalent)	Sediment (per g of dry weight)	Water (μg/L)[a]	Sediment (μg/g of dry weight) mean of three replicates
1	TA98 − S9	130 (80–180)[b]	ND[c]	ND	<1	1.31 (1.58)[d]
	TA98 + S9	60 (30–90)	ND	ND		
	TA100 − S9	ND	ND	–[e]		
	TA100 + S9	ND	ND	–		
2	TA98 − S9	ND	ND	ND	<1	0.19 (0.09)
	TA98 + S9	ND	ND	ND		
	TA100 − S9	ND	ND	ND		
	TA100 + S9	ND	ND	ND		
3	TA98 − S9	ND	ND	ND	<1	0.42 (0.29)
	TA98 + S9	50 (21–74)	70 (30–100)	70		
	TA100 − S9	ND	ND	ND		
	TA100 + S9	ND	ND	ND		
4	TA98 − S9	ND	ND	ND	<1	3.30 (1.83)
	TA98 + S9	ND	ND	ND		
	TA100 − S9	ND	ND	ND		
	TA100 + S9	ND	ND	ND		
5	TA98 − S9	90 (40–130)	ND	2100[f]	<0.01	347.55 (274.60)
	TA98 + S9	100 (40–170)	ND	31,000		
	TA100 − S9	ND	ND	20,000		
	TA100 + S9	ND	ND	720,000		
10	TA98 − S9	ND	ND	ND	<1	0.47 (0.24)
	TA98 + S9	ND	ND	ND		
	TA100 − S9	ND	ND	ND		
	TA100 + S9	ND	ND	ND		
12	TA98 − S9	ND	ND	ND	<1	0.037 (0.04)
	TA98 + S9	ND	ND	ND		
	TA100 − S9	ND	ND	ND		
	TA100 + S9	ND	ND	ND		
14	TA98 − S9	ND	ND	ND	<1	0.03 (0.08)
	TA98 + S9	ND	ND	ND		
	TA100 − S9	ND	ND	ND		
	TA100 + S9	ND	ND	ND		
18	TA98 − S9	ND	ND	ND	<0.01	0.04 (0.06)
	TA98 + S9	ND	ND	ND		
	TA100 − S9	ND	ND	ND		
	TA100 + S9	ND	ND	ND		

Values correspond to one sample unless specified in the table.
[a] The detection limit for the water samples varied from 0.1 to 1 μg/L depending on the complexity of the sample analyzed.
[b] 90% confidence limits.
[c] ND—Not detected under the test conditions.
[d] Mean standard deviation ($n=3$).
[e] (–) Not analyzed.
[f] Mean of the number of revertants/g ($n=3$). For confidence limits and individual values see Roubicek (2003).

filtration, and PAHs were not present in detectable levels (Table 3). Sediment sample of this site did not present mutagenic activity and PAHs were found in low levels (mean of 3 replicates, 1.31 μg/g dry weight). Therefore the dissolved mutagens in the water column probably belong to a different class of compounds than PAHs.

The sample collected at site 3, which is downstream the Cubatão river, presented low levels of mutagenic activity (120 revertants/L, considering the sum of the filtered water and suspended particles) only in the presence of S9 mix. Although this could indicate the presence of PAHs, only 0.42 μg/g of PAHs were found in the sediment and no PAHs were detected in the water sample analyzed under the analytical conditions employed (Table 3). In this case the mutagenic activity was present in the filtered water as well as in the suspended solids. Considering that the sediment was also mutagenic (70 revertants/g of dry weight) (Table 3) it could be the source of this contamination along with industrial discharges.

The water sample from site 5 presented also low levels of mutagenicity. Those results were not expected considering the direct influence of the steel mill industry discharge in this site (Fig. 1) and the high contamination of the sediment (Table 3). This sediment presented high levels of mutagenicity with a remarkable increase in the presence of S9 and showed in average 347.55 μg/g of PAHs (Table 3). Despite the high levels of contaminants in the sediment, the water showed low mutagenic activity. The mutagenicity was detected both in the presence

and absence of S9, in similar potencies, indicating that direct-acting compounds, such as substituted PAHs, which do not need S9 mix to be mutagenic in the Salmonella/microsome assay, could explain this mutagenicity (Table 3). PAHs could also account for the detected mutagenicity in the presence of S9 although these compounds were not detected in the water from this site (Table 3). DaSilva et al. (2001, 2003) showed that fungi collected from site 5 (Fig. 1) were able to metabolize PAHs. Those organisms could be biotransforming the PAHs present in the sediment in more soluble compounds, in a way that they could be transferred to the water column. Substituted PAHs could also be generated by photochemical reactions. Those compounds, which are usually more polar than non-substituted PAHs, could explain the direct-acting activity observed. More chemical analysis should be performed in order to identify those compounds in waters collected from this site.

The low levels of mutagenicity observed in surface water and the high levels of sediment contamination lead us to the hypothesis that other concentration/extractions procedures could be used in order to try to detect PAHs as well as mutagenic activity, in the water, related to PAHs. Blue rayon hanging technique, where the adsorbent fibers stay immersed in water for 24 h, was successfully applied by Kira et al. (1995) in the extraction and detection of PAHs in sea water. The use of additional fractionation procedures (Grifoll et al., 1990) combined with different Salmonella strains followed by chemical analysis of the Blue rayon extracts could also be used to better evaluate the water in relation to genotoxic compounds and indicate to which class of compounds they belong.

4. Final considerations

The mutagenicity of the steel mill industry effluent detected previously by Barreto (1995) could be explained by the presence of PAHs bound to the suspended particles. Considering that this effluent is released in the estuary in front of site 5 (Fig. 1), the sedimentation of those particles could be responsible for the high levels of PAHs and mutagenicity detected in the sediment samples previously analyzed (Roubicek, 2003).

In relation to the water samples analyzed in the estuary, only sites 1, 3 and 5 presented mutagenicity. These activities do not seem to be explained by the presence of PAHs because they were not detected in the water under the analytical conditions. At site 5 the mutagenicity can be related to the steel mill industrial discharge. For sites 1 and 3 other sources of mutagens should be investigated. Although based in only one sampling, the results show that the weak mutagenic activity detected in the water column, which reflects current inputs, is different from the activity found in the bottom sediment. More studies are being performed, using more efficient concentration/extraction procedures and specific strains of Salmonella in order to elucidate which compounds could be the cause of the detected mutagenic activity. For the other sites analyzed both PAHs in water and sediment were found in low concentrations and the mutagenicity was not detected in water as well as in the sediment.

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References

APHA Standard methods for examination of water and wastewater. 20th ed. Washington (DC): American Public Health Association; 1998.

Barreto JCN, 1995. Caracterização de toxicidade de efluentes de usina siderúrgica mediante bioensaios com microrganismos. São Paulo: Faculdade de Saúde Publica, Universidade de São Paulo; 1995. p. 71.

Bernstein L, Kaldor J, McCann J, Pike MC. An empirical approach to the statistical analysis of mutagenesis data from the *Salmonella* test. Mutat Res 1982;97:97–267.

CETESB (Companhia de Tecnologia de Saneamento Ambiental). Relatório Técnico, Poluição das Águas na Baía de Santos e Estuários de Santos e São Vicente; 1979.

CETESB (Companhia de Tecnologia de Saneamento Ambiental). Relatório Técnico, Metais pesados na Baía de Santos e Estuários de Santos e São Vicente; 1981.

CETESB (Companhia de Tecnologia de Saneamento Ambiental). Relatório Técnico, Contaminantes na Bacia do rio Cubatão e seus Reflexos na Biota Aquática; 1990.

CETESB (Companhia de Tecnologia de Saneamento Ambiental). Relatório Sistema Estuarino Santos São Vicente—Agosto de 2001. Available from <www.cetesb.sp.gov.br/Agua/agua_geral.asp/>; 2001.

DaSilva M, Umbuzeiro GA, Pfenning LH, Canhos VP, Pothuluri JV, Cerniglia CE, et al. Pyrene metabolism by filamentous fungi isolated from estuarine sediment. 9th International symposium on microbial ecology, interactions in the microbial world Final program and abstracts; 2001. p. 373.

DaSilva M, Umbuzeiro GA, Pfenning LH, Canhos VP, Esposito E. Filamentous fungi isolated from estuarine sediments contaminated with industrial discharges. Soil Sediment Contam 2003;12(3):345–6.

Ghose MK. Complete physic–chemical treatment for coke plat effluents. Water Res 2002;36:1127–34.

Grifoll M, Solanas AM, Bayona JM. Characterization of genotoxic compounds in the sediments by mass spectrometric technics combined with *Salmonella*/microsome test. Arch Environ Contam Toxicol 1990;19:175–84.

Houk VS. The genotoxicity of industrial wastes and effluents. Mutat Res 1992;277:91–138.

Kira S, Taketa K, Nogami Y, Hayatsu H. A simple technique for monitoring mutagenicity and Benzo(*a*)pyrene contents in mussels and their ambient water. Environ Toxicol Water Qual 1995;10:167–72.

Kot-Wasik A, Debska J, Namiesnik J. Monitoring of organic pollutants in coastal waters of the Gulf of Gdansk, Southern Baltic. Mar Pollut Bull 2004;49:264–76.

Kummrow F, Rech CM, Coimbrão CA, Roubicek DA, Umbuzeiro GdeA. Comparison of the mutagenic activity of XAD4 and blue rayon extracts of surface water and related drinking water samples. Mutat Res 2003;541:103–13.

Maron DN, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res 1983;113:173–215.

Medeiros PM, Bícego MC. Investigation of natural and anthropogenic hydrocarbon inputs in sediments using geochemical markers. I. Santos, SP—Brazil. Mar Pollut Bull 2004;49:761–9.

Nishigima FN, Weber RR, Bícego MC. Aliphatic and aromatic hydrocarbons in sediments of Santos and Cananéia, SP, Brazil. Mar Pollut Bull 2001; 42(11):1064–72.

Nogami Y, Imaeda R, Ito T, Kira S. Benzo(*a*)pyrene adsorbed to suspended solids in fresh water. Environ Toxicol 2002;15:500–3.

Ohe T, Watanabe T, Wakabayashi K. Mutagens in surface waters: a review. Mutat Res 2004;567:109–49.

Quantin C, Joner EJ, Portal JM, Berthelin J. PAH dissipation in a contaminated river sediment under oxic and anoxic conditions. Environ Pollut 2005;134:315–22.

Roubicek DA, 2003. Estratégias para avaliação da genotoxicidade de sedimentos, Departamento de Toxicologia e Análises Toxicológicas. São Paulo: Faculdade de Ciências Farmacêuticas, Universidade de São Paulo; 2003. p. 125.

Tam NFY, Ke L, Wang XH, Wong YS. Contamination of polycyclic aromatic hydrocarbons in surface sediment of mangrove swamps. Environ Pollut 2001;114:255–63.

Turner A, Millward GE. Suspended particles: their role in estuarine biogeochemicals cycles. Estuar Coast Shelf Sci 2002;55:857–83.

Umbuzeiro GA, Roubicek DA, Sanchez PS, Sato MIZ. The *Salmonella* mutagenicity assay in surface water quality monitoring program based on a 20-year survey. Mutat Res 2001;491:119–26.

Umbuzeiro GA, Roubicek DA, Stoppe NC, Rodrigues PF, Rech CM, Coimbrão CA, et al. Caracterização de amostras de sedimento hídrico do estuário de Santos utilizando analises de toxicidade aguda e genotoxicidade e quantificação de bactérias dos ciclos biogeoquímicos. Mundo Saúde 2004;28(4):436–42.

US EPA Method 610—Polynuclear Aromatic Hydrocarbons, Environmental Monitoring and Support Laboratory Cincinnati OH 610/1–610/10; 1982.

Zhu L, Chen B, Wang J, Shen H. Pollution survey of polycyclic aromatic hydrocarbon in surface water of Hangzohou, China. Chemosphere 2004; 56:1085–95.