

Blue rayon-anchored technique/*Salmonella* microsome microsuspension assay as a tool to monitor for genotoxic polycyclic compounds in Santos estuary

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Abstract

The most important harbor of Brazil is located in Santos Estuary. In the 1970s, this area was one of the major examples of coastal degradation and although the quality of the environment has improved, the sediment is still contaminated with polycyclic aromatic hydrocarbons (PAHs) and mutagenic activity. Because of sediment dredging and consequently contaminants resuspension, it is useful to have reliable methods to monitor the water quality. Considering that blue rayon (BR) has been successfully used in evaluation of mutagenicity and PAHs content the objective of this work was to verify the applicability and adapt the methodology to monitor the water for mutagenic activity using the BR associated with the *Salmonella* assay. Analysis of three sites with different levels of contamination was performed using a modification of the BR hanging method denominated in this work BR anchored technique. The microsuspension protocol of the *Salmonella*/microsome assay was employed with the strain YG1041. The water from the site 1 the most contaminated and under influence of the steel mill discharge presented the highest potency reaching 36,000 revertants/g of BR with S9. Sites 2 and 3 showed less mutagenicity than site 1 with values ~1000 revertants/g of BR. We conclude that the BR anchored technique associated with *Salmonella* assay using YG1041 is a reliable alternative to monitor estuarine waters, especially in regions where sediment resuspension or acute pollution episodes can occur.

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1. Introduction

Santos estuary, located in southeastern São Paulo State, is economically important considering that it is the major Latin American harbor and the largest industrial complex of Brazil, including steel mill, fertilizer and chemical industries located in this area [1]. In the

1970s, it was considered one of the major examples of coastal degradation [2] and although the quality of the environment has improved in the last years in relation to the chemical parameters and toxicity due to enforcement actions, unfortunately the sediment is still contaminated especially with polycyclic aromatic hydrocarbons (PAHs) and mutagenic activity detected with the *Salmonella* assay [1–5]. In the most contaminated areas total PAHs can reach 347.55 µg/g of sediment (dry weight) [2] and 720,000 revertants/g of sediment (dry weight) [6]. The contaminants present in the sediment, adsorbed or not to particles, can adversely affect

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Table 1

Results expressed in range of mutagenic ratio (MR), that is calculated dividing the mean of the number of revertants per plate for the tested dose by the mean of the negative control, using the *Salmonella*/microsuspension assay in a single dose experiment (0.5 g of blue rayon per plate) for sites 1 and 2 obtained previously by Kummrow et al. [18]

Sampling site	sampling	TA98		YG1041		TA100		YG1042	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Site 1	I			Tox.		NP	NP		
	II								
Site 2	I								
	II								

NP - Not Performed

Tox. - Toxic (MR<0.7)

Mutagenic Ratio ranges:



the sediment-associated community, as well as be resuspended into the water column by navigation and dredging activities affecting the water quality and the aquatic organisms [7].

Umбуzeiro et al. [6] found only low levels of mutagenic activity in the water despite of the high levels of PAHs and mutagenic activity in the sediment of some of the sites evaluated. They considered that the extraction/concentration method (XAD-4) and the *Salmonella* strains used (TA98 and TA100) were not sensitive enough to detect the genotoxic activity suspected to be present in the water of the most contaminated areas. The total PAHs in the water of these sampling sites were below the detection limits of the method applied [2]. In another area from Brazil, Paraíba do Sul river (Rio de Janeiro State), at a site impacted by an important steel mill plant discharge, benzo(a)pyrene was detected in the water in concentrations ranging from 0.03 to 1.2 µg/L [8]. In the sediments of the same area the sum of 12 different PAHs varied from 5.1 to 40.8 µg/g [9].

Considering that the PAHs and other hydrophobic organic compounds present in the aquatic environment will be preferentially adsorbed to the sediment particles, dredging or any resuspension of the sediment can cause adverse effects to the exposed biota or humans [10]. Brazil established a resolution that regulates dredging activities which includes chemical and toxicological criteria (CONAMA 344) [11]. Considering the occurrence of sediment dredging for the maintenance of the port activities in the Santos estuary (around 3 millions m³/year) and possible contaminants resuspension, it is very useful to have reliable methods to evaluate and monitor the water of this estuary for mutagenic activity as well as PAHs among other compounds.

Since the mid-1980s blue cotton, blue rayon and blue chitin have been used as sample extraction/concentration technique specifically for investigations of mutagenic activity where polycyclic compounds were involved or suspected [12]. The blue rayon hanging technique has been successfully used in evaluation of mutagenicity and PAHs content in coastal waters [13–15]. Blue rayon adsorbent is composed of fibers of rayon covalently bound to copper phthalocyanine trisulphonate and it is selective for polycyclic compounds with three or more fused rings [16]. When used in the hanging technique, the fibers stay immersed in the sampling site for 24 h allowing a representative sampling [17].

In Santos Estuary, Kummrow et al. [18] using a combination of a modified blue rayon hanging technique and *Salmonella*/microsome microsuspension assay in single doses with the strains TA98, TA100, YG1041 and YG1042, found that the water of the most contaminated area presented the higher values of mutagenic activity and the YG1041 (Table 1) was the most sensitive strain. The same authors also showed that the benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(e)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene were present in the blue rayon extracts.

The objective of this work was to verify the applicability and adapt the methodological conditions to monitor the water quality of the Santos estuary for mutagenic activity in a 2-year study using, a modified sampling method denominated blue rayon-anchored technique associated with the *Salmonella*/microsome microsuspension assay.

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