Determination of sediment mutagenicity and cytotoxicity in an area subjected to petrochemical contamination

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This study is an evaluation of the mutagenic and cytotoxic activity of sediments in Bom Jardim stream, one of the tributaries of the Caiá River basin, Rio Grande do Sul, Brazil. This stream receives an indirect contribution of treated effluent from a petrochemical plant. The Salmonella/microsome assay, a microsuspension method, was used to evaluate moderately polar extracts of sediment samples at three points along the stream. The grain size analysis showed a lower mean content of fine particles in the principle face (front) of the complex, and this was also the sampling point with the lowest percentage of extracted organics. Low mutagenic activity was observed at the different sites studied, ranging from 3.3 to 8.3%; cytotoxic activity was more important in this area, ranging from 20 to 40%, adding up the results of assays in the presence and absence of external metabolism. In assays without S9mix there were more frequent mutagenic and cytotoxic responses, with frameshift mutations being the most frequent. The results also showed that there was a gradual, seasonal distribution of the responses as the stream mouth is reached, the most compromised points being in front of and downstream of the complex. Mutagenic and cytotoxic activity in sediment samples has proved important to determine environmental quality, despite the complexity of the chemical composition of the environmental matrix. Furthermore, use of the Salmonella assay to monitor mutagenesis and cytotoxicity helped identify the presence of pollutants. This assay is an important tool, aimed mainly at actions to preserve the genetic heritage of the fauna and flora affected by human activity and to improve environmental quality.

Introduction

Compounds for environmental or individual use, such as pesticides, agricultural inputs, food additives, pharmaceuticals and chemotherapeutic agents, as well as a complex samples resulting from the sum of natural and human contributions, have been diagnosed as presenting toxic properties that induce genetic damage. The levels of exposure to these agents are generally low and long-lasting, with a possibility of bioaccumulation and biotransformation, putting the integrity of the environment and human health at risk (Vargas et al., 2001a). Chemical contamination may also result in a population reduction due to the effects of somatic and heritable mutations, as well as to non-genetic toxicity, speeding up stochastic processes in small populations due to the increased frequency of mutations and reducing the fitness of these individuals (Bickham et al., 2000).

A large number of studies, using different approaches, of interstitial surface waters and sediments have verified contamination of these sources with complex mixtures of toxic substances that could induce genetic damage (Loper, 1980; Meier, 1988; Valent, 1990; Stahl, 1991; Houk, 1992; Vargas et al., 1993, 2001b; Claxton et al., 1998). Generally, the contaminants tend to be more associated with the fine portion (silt or clay) than with the larger sediments (sand or gravel). The fine sediments derive partly from suspended particles that adsorb different contaminants in the water column until they are deposited at the bottom. Once they have been deposited, they are overlaid by new layers of sediments and the water column thus loses the original connection to the sources of contaminants (USEPA, 1994). The distribution of chemical contaminants in the sediments depends not only on localized or non-point sources, but also on natural processes and human activities that redistribute the contaminated sediments. Sediment quality in deposition areas may reflect the history of pollution events that occurred over decades or recently in the surface layer (USEPA, 1994). A significant portion of these pollutants is deposited in the sediment by adsorption, flocculation and sedimentation, constituting potential sources of chemical contamination of the water column (Bonnet et al., 2000).

It is very difficult to identify specific chemicals as a genotoxic response in environmental samples because few compounds are present at high concentrations. Rarely, the more abundant substances cause genotoxic activity (Stahl, 1991). Often the action cannot be attributed to specific compounds in the mixture but to a set of chemical properties and interactions of the sample as a whole (McGeorge et al., 1983; Vargas, 1992). This study presents an evaluation of the sediment of Bom Jardim stream, one of the contributors to the Caiá River basin in Rio Grande do Sul (RS), Brazil, which receives an indirect contribution of treated effluents from a petrochemical industry complex. This area was evaluated previously using samples of surface water in a Salmonella/microsome assay and the presence of base pair substitution and frameshift mutagens was diagnosed (Vargas et al., 1988, 1993, 1995; FEPAM, 1997). Sediment evaluation along the stream supplies a diagnosis of the ecotoxicological impact on the area because certain groups of compounds with toxic and genotoxic properties are deposited over time.

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Materials and methods

Chemical substances

The mutagenic substances used in this study were: sodium azide (AZS) (CAS no. 26628-22-8) and dichloromethane (DCM) (CAS no. 75-09-2) from Merck, Brazil; 2-aminofluorene (2AF) (CAS no. 153-78-6) and 1-nitroquinoline-4-oxide (4NQO) (CAS no. 56-57-5), glucose 6-phosphate (G6P) and NADP from Sigma Chemical Co. (St Louis, MO); dimethylsulfoxide (DMSO) (CAS no. 67-68-5) from Riedel and Rödel. A metabolic activation fraction (S9 mix), prepared from Sprague-Dawley rat livers pretreated with Aroclor 1254, was purchased from Molecular Toxicology Inc. (Boone, NC).

Study areas

Five samplings were performed at three points in Bom Jardim stream, which drains the effluent disposal area of a petrochemical complex, in 1999 (summer, spring, autumn and winter) and 2000 (summer). The surface sediment samples were collected and stored in a cold chamber (4°C), in the dark until chemical extraction was performed.

The sampling sites are shown in Figure 1: point BJn (6697480 N; 455403 E), one of the ponds that form Bom Jardim stream, located upstream of the area influenced by the deposition of industrial effluents; point BJ002 (6699153.0 N; 462318.4 E), located in front of the area where the treated liquid effluents are sprayed; point BJ000 (6699095.5 N; 464596.0 E), at the mouth of Bom Jardim stream close to the Cai River, which receives drainage water from the final disposal areas of the liquid effluent, from the sludge farm and integrated industrial waste treatment system.

Wet extraction of sediments

The extraction of organic compounds was adapted from White et al. (1998). The samples were homogenized and a 50 g aliquot was removed. Extraction was performed using ultrasound (4 x 3 min cycles), using the pesticide grade solvent dichloromethane (100 ml). The extracts obtained were prefiltered through glass wool and sodium sulfate anhydride and filtered by column chromatography (clean-up performed using sodium sulfate anhydride and Celite 545). Later the sample was concentrated to a volume of ~16 ml using a rotary evaporator (at 40°C) and the mass of extracted organic matter was determined.

Grain size analysis

Grain size analysis used the Wentworth/Krumbein method (Wentworth, 1922; Krumbein, 1934) and that of Stokes (1851). They were classified as gravels, sands, silt and clay. All analyses were performed in the Sedimentology Laboratory of the Federal University of Rio Grande do Sul.

Mutagenicity and cytotoxicity evaluation assays

The Salmonella/microsome microsuspension method assay, also called the Kado test (Kado et al., 1986), was used with tester strains TA97a, TA98, TA100 and TA1535 (Gatehouse et al., 1994). The bacterial culture (1-2 x 10^10 cells/ml) was preincubated with an aliquot of the extract of the sediment sample for 90 min at 37°C. The mixture was later solidified by means of a surface agar solution containing traces of histidine and biotin (Maron and Ames, 1983). The samples were evaluated at least in duplicate in the presence and absence of an exogenous metabolic activation system, S9 mix. In all assays, negative (100 µl of buffer) and positive (5 µg/plate sodium azide, 5 µg/plate 2-aminofluorene or 0.5 µg/plate 1-nitroquinoline-4-oxide) controls were added according to the strain and treatment utilized, accompanied by a cellular viability assay (Maron and Ames, 1983; Vargas et al., 1988). For the viability and cytotoxicity assays the bacterial culture (1-2 x 10^10 cells/ml) was preincubated with an aliquot of the extract of the sediment sample for 90 min at 37°C. Then the mixture was diluted to 1-2 x 10^6 cells/ml with phosphate buffer and plated in a rich nutritive/complete medium. In the cytotoxicity assay (cell viability test) the samples were considered cytotoxic when the percentage survival in the sample was <60% of that observed in the negative control (Vargas et al., 1993). The plates for the mutagenicity and cytotoxicity assays were incubated for 72 h at 37°C in a bacteriological chamber.

Statistical analysis

The sample was considered positive when the induced mutation values reached double the mutagenic activity observed in the negative control, ANOVA test significant (P ≤ 0.05) between the doses and when the elevation in the dose-response curves was evaluated by the SALMONEL test program (Myers et al., 1991) was significant. The sample was considered indicative of mutagenicity when it presented twice the spontaneous mutation or ANOVA and significant dose-response relation.

Results

The mutagenic activities observed in the different sampling periods are compared in Figures 2-5, for assays in the absence (Figures 2 and 4) and presence of exogenous metabolism (Figures 3 and 5), both for mutagenic responses of the frameshift error type (Figures 2 and 3) and base pair substitutions (Figures 4 and 5).

Results indicating direct mutagenic activity of the frameshift error type were observed in the assays with strain TA98 (Figure 2) at BJ000 in sampling II and with strain TA97a at BJ000 in samplings II and III. In assays in the presence of exogenous S9 mix (Figure 3), indicative mutagenic activity was observed in at least one sampling for all points evaluated [BJn (sampling IV), BJ002 (sampling I) and BJ000 (sampling III)] in strain TA98. No mutagenic activity was observed for strain TA97a.

Mutagenic activity indicative of base pair substitutions was observed at least once at all sampling points and was diagnosed in
Fig. 2. Mutagenic evaluation by microsuspension assay with frameshift strains (TA98 and TA97a) in the absence of metabolic activation. (a) Dose per plate (µg); (b) mutagenic index = no. of revertants per plate of the sample/negative control revertants per plate; (c) no. of revertants/µg, derived using Salmonel statistical software. Spontaneous revertants: TA98, 38 ± 10.8; TA97a, 146 ± 54.9. The base of the graph indicates regression analysis in the linear portion of the dose–response curve (F test significance): white, non-significant; gray, P ≤ 0.5; black, P ≤ 0.1. The colors of the columns represent the final response: white, non-mutagenic; gray, indicative; black, positive.

Fig. 3. Mutagenic evaluation by microsuspension assay with frameshift strains (TA98 and TA97a) in the presence of metabolic activation. (a) Dose per plate (µg); (b) mutagenic index = no. of revertants per plate of the sample/negative control revertants per plate; (c) no. of revertants/µg, derived using Salmonel statistical software. Spontaneous revertants: TA98, 33 ± 13.7; TA97a, 249 ± 142.9; The base of the graph indicates regression analysis in the linear portion of the dose–response curve (F test significance): white, non-significant; gray, P ≤ 0.5 and black, P ≤ 0.1. The colors of the columns represent the final response: white, non-mutagenic; gray, indicative; black, positive.
Fig. 4. Mutagenic evaluation by microsuspension assay with substitution base pair strains (TA100 and TA1535) in the absence of metabolic activation.
(a) Dose per plate (μg); (b) mutagenic index = no. of revertants per plate of the sample/negative control revertants per plate; (c) no. of revertants/μg, derived using Salmonel statistical software. Spontaneous revertants: TA100, 190 ± 73.4; TA1535, 21 ± 9.1. The base of the graph indicates regression analysis in the linear portion of the dose–response curve (F test significance): white, non-significant; gray, P ≤ 0.5; black, P ≤ 0.1. The colors of the columns represent the final response: white, non-mutagenic; gray, indicative; black, positive.

Fig. 5. Mutagenic evaluation by microsuspension assay with substitution base pair strains (TA1000 and TA1535) in the presence of metabolic activation.
(a) Dose per plate (μg); (b) mutagenic index = no. of revertants per plate of the sample/negative control revertants per plate; (c) no. of revertants/μg, derived using Salmonel statistical software. Spontaneous revertants: TA100, 223 ± 50.0; TA1535, 19 ± 6.3. The base of the graph indicates regression analysis in the linear portion of the dose–response curve (F test significance): white, non-significant; gray, P ≤ 0.5; black, P ≤ 0.1. The colors of the columns represent the final response: white, non-mutagenic; gray, indicative; black, positive.
TA100 at BJn (sampling I) and BJ000 (sampling I) and in TA1535 at BJ0002 (sampling II) in direct assays (Figure 4).

In the presence of S9mix (Figure 5) this type of activity was observed only for assays with strain TA1535 at BJn (sampling V).

Generally, a higher frequency of frameshift mutations can be observed (10%) as compared with base pair substitutions (6.8%) in the responses observed when summing the results for all strains used. The most frequent responses were detected at sampling station BJ000 (8.4%). The seasonal distribution showed a decrease in mutagenic activity throughout the sampling period (Figure 6) and higher activity in the absence of external metabolism (10% without and 6.78% with S9 mix). A change in the type of action of the mutagenic compounds should be noted. More direct activity occurred in the first summer and autumn, while during the other seasons indirect compound activity was seen.

Cytotoxic activity was monitored in all assays and proved to be the most significant parameter in the area studied. Analyzing the data according to season of the year, there was more activity in summer, and it was absent in spring (Figure 7). A gradient of responses, summer > winter > autumn > spring, may be observed. The most affected sampling point was BJ000, presenting a higher percentage of cytotoxic responses. In addition, more activity was observed in the direct assays, i.e. without external metabolism, both with respect to occurrence at sampling points and seasonality.

The results of the grain size analysis (Figure 8A) showed that point BJ002 presented a higher sand content (72.65%) as compared with the other sampling points (BJn, 57.38%; BJ000, 61.53%). This result could possibly account for the lower concentration of active compounds, presenting on average two times less organic material extracted as compared with points BJn and BJ000 (Figure 8B).

Discussion

The results of the grain size analysis show the importance of the matrix that forms the sediment in the percentage of organic compounds adsorbed. Sediments with a lower content of clays and silts, which are more porous materials, showed a lower adsorption of compounds, evidenced by low values of extracted organic mass (Figure 8A). Therefore, physical aspects of the sediment samples are relevant in the diagnosis of contamination of this environmental compartment, because chemical compounds are mainly associated with the fine fraction of sediments (clays and silts) (USEPA, 1994). Further, contaminated sediments may be directly toxic to aquatic life or could be a source of contaminants for bioaccumulation in the food chain (USEPA, 2000).

The results show that there is mutagenic and cytotoxic activity dispersed throughout the area of study. The main activity in this region is petrochemical processing and the points in front of and downstream from the area where the greatest deposition of treated effluents occurs presents the greatest degradation of environmental quality (BJ002 and

![Fig. 6. Percentage of positive responses for mutagenic activity and its seasonal distribution.](https://academic.oup.com/mutage/article-abstract/19/6/445/1053267)

![Fig. 7. Percentage of positive responses for cytotoxic activity (%) and its seasonal distribution. The percentage of responses was calculated based on all assays performed and the number of cytotoxic responses. In spring there was no cytotoxicity. The results represent mean activity between treatments with and without metabolic activation.](https://academic.oup.com/mutage/article-abstract/19/6/445/1053267)
BJ000 defined by the presence of mutagenic and cytotoxic activity. The results observed at the mouth of the stream (BJ000) agree with previous studies on surface water (Vargas et al., 1993; FEPAM, 1997), being the site of highest incidence of positive responses in the Cai River basin. They also agree with results observed in water samples collected at the same sites and on the same collection dates, and evaluated by the classical Salmonella/microsome test. In this associated study (Cardozo et al., 2001; Vargas et al., 2003) a higher percentage of mutagenic samples was observed at the two sites with the greatest environmental degradation.

In the present study activity indicating mutagenicity was also observed at the reference point upstream of the complex area (BJn). This activity may be related to atmospheric pollution generated by the complex, since the area investigated is in the main pollutant dispersion plume for this area (Ducatti et al., 2001). Chemical extraction of the samples using a specific solvent enables characterization of some types of contaminants, as in a study by Grifoll et al. (1990), which related the mutagenic activity in sediments extracted with moderately polar solvents, containing compounds such as phthalic esters, aromatic amines and ketones, and extracted with polar solvents, containing substances such as nitro-poly cyclic aromatic hydrocarbons, azaarenes and aromatic anhydrides. Fernandez et al. (1992) showed that the sediments extracted using solvents that were not highly polar or non-polar presented a higher number of compounds such as chlorinated pesticides and hepta and octachlorodibenzo-p-dioxins. These authors also showed that the origin of these compounds was oxygenation of polycyclic aromatic hydrocarbons during combustion and/or photooxidation of fossil materials and wood. He also revealed that other compounds with mutagenic activity extracted by moderately polar solvents belong to the nitroarenes group and are due to fuel combustion in internal combustion engines. They are more active in the presence of external metabolic activation.

The mutagenic activity observed in this study did not have high indices, varying from 0.28 to 2.9 times the values observed in the negative control, with responses that are only indicative of mutagenicity. A linear regression analysis (Myers et al., 1991) allowed the estimation of values of 1.38 revertants/µg extract.

According to Vargas et al. (1995), different chemical extraction processes do not preserve volatile compounds present in the samples. In a study of water samples from the Cai River, an area under petrochemical influence, using a specific process to extract volatile compounds, they showed that these substances could be the main agents responsible for the mutagenic activity observed. Therefore, the low mutagenic activity observed in the sediments in this area may be related to this factor. Despite this, differences could be observed in mutagenic activity between the upstream point and the stream mouth, with activity at the latter being more frequent in assays with metabolic activation. It should also be mentioned that different types of mutagenesis were found, indicating different contributions in the areas sampled.

Cytotoxicity proved to be the main marker of pollution for sediment samples in the area, showing a rising gradient of responses as the industrial complex was approached and downstream of the complex. This response was higher during those periods of the year with high and low mean temperatures and was lower or absent when the weather was mild. Following up these analyses with chronic toxicity assays has supplied responses that are complementary to this study, showing evidence of this action of sediment samples from the same sites and collection dates in Daphnia magna (Felden et al., 2002).

Thus, it is clear that the stream is undergoing ecotoxicological degradation as the first area of impact of the petrochemical complex on the environment. Mutagenic and cytotoxic activity in sediment samples proved an important tool to determine the degradation of environmental quality. The chemical composition of this environmental matrix is complex and not fully understood, allowing multiple interactions between the biotic and abiotic components, generating synergistic, antagonistic and/or toxic effects. The Salmonella/microsome assay monitoring data at the molecular level served as a bioindicator, helping to determine impact. This property of the assay and its use in areas under environmental threat allows diagnosis of the presence of low concentrations of pollutants that cause mutagenesis and/or cytotoxicity, serving as a warning of environmental contamination and preventing damage to the genetic heritage of the fauna and flora affected by human activity.

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