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INVESTIGAÇÃO DE CONTAMINANTES MUTAGÊNICOS DE MATRIZES AMBIENTAIS EM ÁREA DE RISCO ECOTOXICOLÓGICO

ANDRÉIA TORRES DE LEMOS

Porto Alegre, Agosto de 2011

AVALIAÇÃO DE CONTAMINANTES MUTAGÊNICOS DE MATRIZES AMBIENTAIS EM ÁREA DE RISCO ECOTOXICOLÓGICO

Andréia Torres de Lemos

Dissertação apresentada ao Programa de Pós-Graduação em Ecologia, do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para obtenção do título de Mestre em Ecologia.

Orientadora: Profa. Dra.: Vera Maria Ferrão Vargas

Comissão Examinadora:

Prof[a]. Dr[a]. Tatiana da Silva Pereira

Prof[a]. Dr[a]. Maria Lúcia Kolowski Rodrigues

Prof[a]. Dr[a]. Teresinha Guerra

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RESUMO

A crescente introdução de variados poluentes em matrizes ambientais de áreas urbanas causa prejuízos ao ecossistema e dificulta as medidas de controle ambiental. Essas substâncias, após liberadas, se distribuem e interagem de acordo com suas características e as do meio receptor. Indicadores precoces de contaminação permitem a adoção de medidas preventivas aos danos causados pela poluição ambiental. O presente estudo teve por objetivo investigar a ação de substâncias genotóxicas em diferentes frações de material particulado atmosférico e solos, analisando a presença de contaminantes orgânicos e inorgânicos. Para este fim, foi empregado o ensaio *Salmonella*/microssoma em amostras de áreas urbanas caracterizadas como urbano-residencial e urbano-industrial, na cidade de Rio Grande, RS. Amostras de material particulado atmosférico (PTS e PM2,5) e de solo superficial (composição granulométrica total e fração <0,5mm) foram preparadas por diferentes métodos de extração. Este estudo apresenta uma primeira caracterização de PM2,5, através do ensaio *Salmonella*/microssoma, no Brasil. O estudo de extratos orgânicos e aquosos de PTS e PM2,5 evidenciou a presença de compostos metálicos na fração aquosa, potencialmente biodisponíveis, bem como maior risco de exposição associado à fração de compostos orgânicos das partículas finas. A mutagênese foi detectada, mesmo em amostras de particulados que se encontravam em conformidade com os parâmetros de qualidade recomendados. A análise das extrações ácidas dos solos mostrou que as chuvas da região podem atuar como rota de contaminação ambiental, com risco adicional na disponibilização de substâncias tóxicas nos eventos de precipitação ácida. O estudo mostrou que o emprego do solo com mínima alteração é mais apropriado para os ensaios de mutagênese. A utilização conjunta de diferentes métodos de extração de compostos orgânicos e inorgânicos permite uma avaliação integradora da qualidade de matrizes ambientais complexas, favorecendo a adoção de medidas preventivas para a proteção do ecossistema.

Palavras-chave: Genotoxicidade ambiental, Ensaio *Salmonella*/microssoma, PTS, PM2,5, Mutagenicidade de solos, Chuva ácida, Poluição urbana.

ABSTRACT

The increasing introduction of different pollutants into the environmental matrices of urban areas damages the ecosystem and makes it difficult to perform environmental control. After these substances are released, they are distributed and interact according to their own characteristics and those of the receiving environment. Early indicators of contamination allow measures to be adopted to prevent damage caused by environmental pollution. The purpose of this study was to investigate the action of genotoxic substances in different fractions of atmospheric particulate matter and soils, analyzing the presence of organic and inorganic contaminants. For this, the *Salmonella*/microsome assay was used on samples of urban areas characterized as urban-residential and urban-industrial, in the city of Rio Grande, RS. Samples of atmospheric particulate matter (TSP and PM2.5) and of surface soil (total grain size composition and fraction <0.5mm) were prepared using different extraction methods. This study presents a first characterization of PM2.5 in Brazil, through the *Salmonella*/microsome assay. The study of organic and aqueous extracts of TSP and PM2.5 showed the presence of potentially bioavailable metallic compounds in the aqueous fraction, as well as a greater risk of exposure associated with the fraction of organic compounds of the fine particles. Mutagenesis was detected even in samples of particulates that were in accordance the recommended quality parameters. Analysis of acid extractions from the soils showed that rainfall in the region may act as an environmental contamination route, with an additional risk of making toxic substances available in acid precipitation events. The study showed that the use of minimally altered soil is more appropriate for mutagenesis assays. The joint use of different methods for organic and inorganic compound extractions allows an integrated evaluation of the quality of complex environmental matrices, favoring the adoption of preventive measures to protect the ecosystem.

Keywords: Environmental genotoxicity, *Salmonella*/microsome assay, TSP, PM2.5, Mutagenicity of soils, Acid rain, Urban pollution.

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LISTA DE ABREVIAÇÕES

Português/ Inglês

ABNT – Associação Brasileira de Normas Técnicas

ATSDR - Agency for Toxic Substances and Disease Registry

C.O. - Carbono Orgânico

CETESB – Companhia Ambiental do Estado de São Paulo

CO – Monóxido de carbono

CONAMA – Conselho Nacional do Meio Ambiente

CTC - Capacidade de Troca Catiônica

DCM - Diclorometano

DMSO - Dimetilsulfóxido

FEPAM – Fundação Estadual de Proteção Ambiental Henrique Luís Roessler

HPA/ PAH- Hidrocarbonetos Policíclicos Aromáticos/ Polycyclic Aromatic Hydrocarbons

IARC - International Agency for Research on Cancer

IBGE – Instituto Brasileiro de Geografia e Estatística

ICP-OES - Espectrometria de emissão óptica por plasma indutivamente acoplado/ Inductively coupled plasma optical emission spectrometry

MO - Matéria Orgânica

MOE/EOM – Matéria Orgânica Extraída/ Extracted Organic Matter

MP/ PM - Material particulado atmosférico/ Airborne particulate matter

NAAQS - National Ambient Air Quality Standards

Nitro-HPA/ Nitro-PAH – Nitroderivado de hidrocarbonetos policíclicos aromáticos

NO_x - Óxidos de nitrogênio

O_3 - Ozônio

OMS/ WHO – Organização Mundial de Saúde/ World Health Organization

PM10 – Partículas inaláveis/ Inhalable particles (<10µm diâmetro aerodinâmico)

PM2.5 – Partículas inaláveis finas/ Fine inhalable particles (<2.5µm diâmetro aerodinâmico)

PTS/ TSP – Partículas Totais em Suspensão/ Total Suspended Particles (<50µm diâmetro aerodinâmico)

S9 – Fração de metabolização hepática de mamífero *in vitro*

SO_2 - Dióxido de enxofre

UF - Partículas Ultrafinas

USEPA – US Environmental Protection Agency

2AF - 2 – aminofluoreno/ 2- aminofluorene

2NF – 2 – nitrofluoreno/ 2- nitrofluorene

4NQO – 4 nitroquinoleína 1-óxido/ 4-nitroquinoleine oxide

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1. INTRODUÇÃO

A sociedade moderna, em sua busca constante pelo desenvolvimento econômico e melhora na qualidade de vida, tem levado a uma crescente degradação ambiental. As atividades humanas e industriais liberam no ambiente uma variedade de substâncias químicas prejudiciais ao ecossistema. Estima-se que, diariamente, são utilizadas cerca de 100.000 substâncias químicas, sendo que a produção, distribuição, uso e disposição final desses compostos químicos levam a sua presença, quase inevitável, no ambiente. Após liberadas, essas substâncias podem sofrer transformações, ser transportadas ou permanecer estáveis por longos períodos, sendo difíceis de serem degradadas. Dessa forma, a maioria desses compostos pode persistir no ambiente e/ou sofrer bioacumulação, interferindo no fluxo de energia e nutrientes da cadeia biológica (Holtz, 2000; Tagliari et al., 2004).

Os impactos antropogênicos aos ecossistemas foram classificados por Grantz et al. (2003) em quatro grupos principais, sendo: 1- reestruturação física, como as mudanças resultantes do uso da terra; 2- introdução de espécies exóticas; 3- Uso excessivo dos recursos naturais e 4- introdução de substâncias tóxicas. Estes impactos, caso não removidos ou mitigados, diminuem a capacidade de adaptação dos ecossitemas, bem como modificam sua estrutura e função normal. Este processo de degradação pode levar a uma diminuição da biodiversidade, redução da produção primária e secundária, além de menor capacidade do ecossistema em se recuperar e retornar ao seu estado original. Ainda, podem ocorrer aumentos da prevalência de doenças, redução da ciclagem de nutrientes, e aumento de espécies exóticas e oportunistas (Rapport e Whitford, 1999). Distúrbios causados pela liberação de substâncias tóxicas na atmosfera, solo e água são capazes de causar efeitos agudos e crônicos (Grantz et al., 2003).

1.1. Contaminantes ambientais e genotoxicidade

Entre os contaminantes presentes no ambiente, alguns são capazes de lesar a estrutura ou função da molécula de DNA, sendo denominados de genotóxicos. Estas lesões são corrigidas pelo próprio mecanismo de reparo das células, entretanto, alterações não reparadas ou erroneamente reparadas originam mutações pontuais e/ou cromossômicas (Pfeiffer et al., 1996). Geralmente, essas substâncias encontram-se no ambiente em concentrações abaixo do necessário para causar efeitos agudos. No entanto, mesmo em pequenas concentrações, quando um organismo é exposto a essas condições ele pode tornar-se incapaz de manter a sua

função ecológica (Scott e Sloman, 2004). É possível que substâncias químicas que induzem mutações afetem células somáticas e germinativas, tornando-se potenciais causadores de problemas de fertilidade e indução de câncer (Mortelmans e Zeiger, 2000). Mutações não-específicas são passíveis de se acumular no genoma e potencialmente persistir na população, resultando, eventualmente, em redução da adaptabilidade e do tamanho populacional (Belfiore e Anderson, 2001). Assim, a investigação dessas substâncias genotóxicas torna-se necessária para garantir a integridade das populações expostas e sua função biológica no ecossistema.

Entre os contaminantes ambientais que despertam a atenção devido a seu potencial genotóxico, encontram-se substâncias orgânicas e inorgânicas, salientando-se os hidrocarbonetos policíclicos aromáticos e os compostos metálicos. Apesar do efeito conhecido destas substâncias isoladamente, é importante ressaltar que a ação combinada destes agentes químicos pode alterar suas características iniciais, resultando em misturas complexas cujo efeito é, muitas vezes, desconhecido.

Os hidrocarbonetos policíclicos aromáticos (HPAs) constituem um grupo de substâncias químicas composto exclusivamente de carbono e hidrogênio que contem dois ou mais anéis aromáticos condensados (Barra et al., 2007; Pereira Netto et al., 2000) Os HPAs são contaminantes amplamente distribuídos no ambiente, sendo emitidos quando ocorre a queima incompleta de matéria orgânica, tanto de origem natural quanto antropogênica. As fontes de emissão antrópica compreendem a queima de combustíveis fósseis, derramamentos de óleos e seus subprodutos, incineração de resíduos e fumaça de cigarros. As fontes naturais incluem a queima de biomassa em florestas e erupções vulcânicas (Lopes e Andrade, 1996; Manahan, 2003).

Essas substâncias são encontradas em diferentes níveis de concentração em todos os compartimentos ambientais e na biota. Seus derivados nitrados e oxigenados também possuem ampla ocorrência, mas em geral ocorrem em concentrações ambientais cerca de 100 a 1000 vezes inferiores às dos HPAs (Lopes e Andrade, 1996; Pereira-Netto et al., 2000). Representam um problema global por serem transportadas por longas distâncias através da atmosfera e apresentar características mutagênicas e carcinogênicas (Barra et al., 2007; Meire et al., 2007).

Quando presentes na atmosfera, os HPAs podem estar tanto na fase gasosa quanto associados ao material particulado. A concentração de HPAs em cada fase dependerá da volatilidade e afinidade pelas superfícies das partículas atmosféricas de cada composto. A volatilidade destes compostos depende de seu peso molecular, diminuindo conforme o aumento do peso (Pereira Netto et al., 2000).

No solo, os HPAs encontram-se geralmente adsorvidos a sua matriz, retidos nas camadas superiores. Os compostos de maior peso molecular possuem meias-vidas relativamente elevadas, indicando uma degradação lenta (Pereira Netto et al., 2000). A adsorção de HPAs no solo aumenta com o aumento do teor de carbono orgânico e da área superfícial das partículas (ATSDR, 1995).

Os HPAs são considerados como poluentes prioritários pela Agência de Proteção Ambiental dos Estados Unidos (USEPA), sendo 16 destes particularmente importantes no monitoramento ambiental. Esses compostos apresentam de 2 a 6 anéis aromáticos fundidos entre si, com peso molecular variando entre 128 e 278g/mol (Meire et al., 2007). Alguns HPAs são classificados pela IARC (*International Agency for Research on Cancer*) como possíveis (Grupo 2B), prováveis (Grupo 2A) ou carcinogênicos (Grupo 1) para humanos. Entre as 16 espécies de HPAs listadas como prioritárias segundo a USEPA, oito são classificadas em algum dos grupos citados acima: Acenafteno, acenaftileno, antraceno, benzo(a)antraceno (2B), benzo(a)pireno (1), benzo(b)fluoranteno (2B), benzo(ghi)perileno, benzo(k)fluoranteno (2B), criseno (2B), dibenzo(a,h)antraceno (2A), fenantreno, fluoranteno, fluoreno, indeno(1,2,3-cd)pireno (2B), naftaleno (2B) e pireno.

A legislação brasileira apresenta valores de prevenção e investigação para 10 espécies de HPAs presentes no solo (Brasil, 2009), entretanto, não há regulamentação para a presença destes compostos na atmosfera.

Não há consenso quanto à definição do termo “metais pesados” na literatura. Frequentemente este se refere a um grupo de metais e semi-metais que estão relacionados à toxicidade e problemas ecológicos (Bánfalvi, 2011). A massa atômica ou densidade dos elementos (superior a 55,8 g/mol ou a 5 g/cm^3, respectivamente) também são utilizadas (Pierzynski et al., 2000). De acordo com Rodrigues (2007), embora o termo “metal pesado” não possua uma definição única, em geral, refere-se a elementos associados com poluição e

toxicidade, englobando tanto aqueles causadores de danos ao ambiente e à biota, como aqueles que em baixas concentrações são essenciais aos seres vivos.

Devido a sua capacidade tóxica e de bioacumulação, os metais pesados são de grande significado ecológico. Suas características tóxicas dependem das concentrações e espécies químicas que estão presentes no ambiente (Schnoor, 1996). Ao contrário da maioria dos poluentes, esses não são biodegradáveis, tendo efeitos de longa duração no solo devido à forte adsorção de muitos metais nos colóides húmicos e de argila (Iwegbue et al., 2009). O efeito de bioacumulação apresentado por alguns metais reforça a necessidade de monitorar sua presença no ambiente, bem como sua possível biodisponibilidade (Kouba et al., 2010; Nagajyoti et al., 2010).

Os metais pesados representam maior risco em suas formas catiônicas e quando ligados a cadeias curtas de carbono. Os íons metálicos formam complexos com grande quantidade de ligantes e exercem influência sobre várias funções biológicas (Magalhães, 2005). Alguns elementos como arsênio, cádmio, chumbo, cromo e níquel possuem efeito mutagênico e/ou clastogênico (Tsalev e Zaprianov, 2000; White e Claxton, 2004).

Existem diferentes fontes de metais pesados no ambiente, as quais podem ser tanto de origem natural quanto antropogênica. A industrialização e urbanização têm aumentado a concentração de metais pesados na biosfera. Entre as fontes naturais predominam as rochas e os solos. As principais fontes antrópicas são atividades agrícolas (fertilizantes químicos, adubos de origem animal, aplicação de lodos e pesticidas), metalurgia (incluindo a mineração, fundição e acabamento), queima de combustíveis minerais e fósseis, produção de microeletrônicos e eliminação de resíduos (Bradl, 2005; Nagajyoti et al., 2010). Estas emissões ocorrem por uma variedade de processos e rotas, que incluem a liberação diretamente no ar, nas águas e solos, bem como via escoamento superficial e lixiviação para as águas superficiais e subterrâneas (Bradl, 2005; Jarup 2003). Embora seu destino e fixação final sejam os solos e sedimentos, os metais em sua maioria são transportados por via aérea, na forma de gases ou adsorvidos no material particulado (Baird, 2002; Magalhães, 2005).

A presença de metais nos solos é regulamentada, no Brasil, pela resolução CONAMA n° 420/2009 (Brasil, 2009) que estipula valores de prevenção e investigação de acordo com o uso do solo. Estes elementos não são contemplados nos padrões legais de avaliação atmosférica. Internacionalmente, poucos metais foram incluídos em legislações de qualidade

do ar, como por exemplo, o monitoramento de chumbo em PTS pela agência ambiental norte-americana (USEPA, 2010) e arsênio, cádmio e níquel em PM10 pela União Européia (CE, 2004). Estas normas levam em consideração os teores totais de metais presentes nas amostras, ou determinados após processos de digestão ácida forte.

1.2. Biomonitoramento

O monitoramento ambiental de áreas impactadas geralmente é conduzido utilizando medidas físico-químicas que, devido à natureza complexa dessas amostras, podem não ser suficientes para garantir a segurança biológica (Claxton et al., 1998; Fernandez et al., 2005). Realizar a análise do risco sobre os efeitos de cada substância efetivamente em uso, não é possível. Além disso, os indivíduos raramente estão expostos a um único contaminante, mas a uma mistura deles, que apresentam propriedades tóxicas diferentes dos constituintes originais. Em contrapartida, os bioensaios fornecem uma ferramenta útil na detecção dos efeitos de uma ampla variedade de substâncias químicas e de suas interações, mesmo quando informações detalhadas acerca de sua identidade ou propriedades físico-químicas ainda não sejam conhecidas (Ohe et al., 2004).

Os biomarcadores são respostas biológicas correspondentes à exposição, efeito ou susceptibilidade dos indivíduos aos agentes químicos e/ou estressores ambientais (Van der Oost et al., 2003). Estes marcadores geram respostas funcionais, fisiológicas, bioquímicas em nível celular ou de interação molecular (Souza, 2006). Ao serem mensurados permitem detectar se a contaminação ambiental está em nível suficiente para causar efeitos fisiológicos. Os biomarcadores são considerados excelentes indicadores precoces de contaminação, sendo importantes ferramentas na adoção de medidas preventivas aos danos causados pela poluição ambiental. Isto ocorre porque efeitos em níveis superiores de organização biológica são precedidos por mudanças nos processos biológicos, de forma que os biomarcadores servem como um alerta precoce de efeitos tardios (Bayne et al., 1985; Van der Oost et al., 2003). A utilização de biomarcadores genotóxicos é apropriada para a análise de risco ambiental e vários estudos relacionam danos no DNA com subsequentes alterações a nível molecular, celular e tecidual nos organismos (Ohe et al., 2004).

1.3. Ensaio *Salmonella*/microssoma

Um ensaio amplamente utilizado em biomonitoramento é o Teste *Salmonella*/microssoma (Teste de Ames), que permite identificar tanto substâncias puras como misturas complexas causadoras de danos genéticos. Este teste foi especificamente desenvolvido para detectar mutagênese quimicamente induzida, tornando-se amplamente utilizado como método de triagem inicial do potencial genotóxico de novas drogas e biocidas, sendo reconhecido pela comunidade científica e agências de controle (Mortelmans e Zeiger, 2000). Já foi estabelecido que existe uma alta correlação entre resposta mutagênica medida no ensaio *Salmonella* e carcinogênese avaliada em roedores, variando de 77% a 90%, dependendo principalmente do grupo químico ao qual a substância estudada pertence (McCann et al., 1975; Mortelmans e Zeiger, 2000; Zeiger, 1998). Esse ensaio baseia-se em linhagens de *Salmonella typhimurium* que contêm uma mutação específica no operon da histidina, o que as torna incapazes de sintetizar este aminoácido (*his* $^{-}$) e crescer em sua ausência no meio de cultura. Novas mutações nesses sítios podem restaurar a função dos genes permitindo que as células sintetizem histidina. Dessa forma, quando semeadas em meio de cultura livre desse aminoácido, apenas as células que reverterem espontaneamente (*his* $^{+}$) formarão colônias. Os valores de mutações espontâneas são relativamente constantes para cada linhagem, entretanto, se uma substância mutagênica é adicionada ao meio de cultura os valores de mutação podem aumentar significativamente (Claxton et al., 1987; Umbuzeiro e Vargas, 2003).

Ao contrário do que ocorre em mamíferos e outros vertebrados, as bactérias são incapazes de metabolizar substâncias via citocromo P450. A função primária do sistema de metabolização hepática é proteger a célula, degradando e detoxificando substâncias estranhas ao organismo. Entretanto, alguns compostos mutagênicos (pró-mutágenos) são inativos a não ser que sejam metabolizados a formas ativas. Por isto, um sistema exógeno de ativação metabólica de mamíferos é incluído aos ensaios. Esse sistema, conhecido como S9 mix, é preparado a partir de células de fígado de ratos *Sprague-Dawley* que foram previamente tratados com um indutor enzimático (Aroclor 1254). Dessa forma, é possível mimetizar o metabolismo de mamíferos e analisar a genotoxicidade dos metabólitos resultantes dos compostos testados. A ativação metabólica das amostras com essas enzimas aumenta a correlação entre a mutagênese observada nesse ensaio e a carcinogênese em mamíferos (Claxton et al., 1987; Maron e Ames, 1983; Umbuzeiro e Vargas, 2003).

Esse bioensaio tem se mostrado apropriado para avaliação rápida da disponibilidade e atuação de contaminantes provenientes de matrizes ambientais complexas. Sua utilização tem se mostrado útil para prevenção e investigação de problemas ambientais. Segundo Claxton et al. (2010), o ensaio *Salmonella* foi essencial para o reconhecimento de agentes mutagênicos em amostras ambientais, permitindo que os pesquisadores descobrissem que grande parte do nosso ambiente possui agentes com atividade mutagênica. Atualmente, esta é a metodologia mais empregada na avaliação da mutagenicidade de matrizes ambientais complexas, contando com aproximadamente 37% do total de estudos em solos (White e Claxton, 2004), 41% em sedimentos (Chen e White, 2004), 37% em ar (Claxton e Woodall, 2007) e 50% em água (Ohe et al., 2004).

Em recente revisão, Claxton et al. (2010) analisaram o número de trabalhos publicados por ano que utilizaram o Teste de Ames em vários tipos de amostras ambientais. Os autores observaram que estudos sobre produtos naturais, água e ar compõem a maioria das publicações atuais, enquanto que relativamente poucos artigos referem-se a amostras de solo e sedimento. White e Claxton (2004) atribuíram o extenso uso do ensaio *Salmonella* em amostras de material particulado atmosférico a características da metodologia, como (a) possibilidade de utilizar pequenas quantidades de amostra, (b) realizar o diagnóstico através do uso de diferentes linhagens e protocolos, (c) simplicidade e difusão em muitos laboratórios, (d) rapidez e baixo custo, se comparado com outros métodos analíticos.

1.4. Material particulado atmosférico

A poluição do compartimento atmosférico vem despertando crescente interesse, uma vez que um número cada vez maior de trabalhos reporta a sua associação com efeitos adversos para o ambiente e a saúde humana (Grantz et al., 2003; Vargas, 2003).

Os poluentes do ar podem ser divididos em primários, que são aqueles lançados diretamente pelas fontes de emissão, e secundários, que são os formados posteriormente, através de reações químicas entre poluentes primários e componentes naturais da atmosfera. Quanto às fontes de emissão, essas podem ser classificadas como fixas ou estacionárias, produzindo cargas pontuais de poluentes, ou móveis, produzindo cargas poluidoras difusas (CETESB, 2011a; Vieira, 2009).

A concentração de poluentes no ar é função das emissões, transporte, dispersão e deposição dos poluentes, bem como da forma como estes reagem entre si, além das condições meteorológicas atuantes. Alguns dos parâmetros meteorológicos importantes são: a temperatura, que influencia as reações fotoquímicas geradoras de poluentes secundários na atmosfera; a precipitação, que atua removendo poluentes do ar; a direção e velocidade dos ventos, que se relacionam com os mecanismos de dispersão (Vieira, 2009). Episódios graves de poluição já foram relacionados a condições meteorológicas desfavoráveis à dispersão dos poluentes (Lippmann, 2009). A topografia local é outro fator que influencia nesta dispersão. Em locais próximos à costa ocorre maior dispersão, enquanto que em áreas cercadas por montanhas, morros, ou dentro de centros urbanos há maior concentração dos mesmos. Outro fator importante é a proximidade de fontes específicas de emissão, uma vez que os níveis de poluentes atmosféricos podem ser mais elevados em localidades como rodovias e indústrias, tornando necessárias medidas especiais de proteção para as populações que vivem no entorno dessas emissões (WHO, 2005).

A Organização Mundial de Saúde considera ser um requisito básico para a saúde e bem estar do ser humano a boa qualidade do ar (WHO, 2006). A determinação da qualidade do ar, geralmente é realizada através do monitoramento de um grupo restrito de poluentes escolhidos devido a sua maior ocorrência, a seus efeitos adversos e aos recursos disponíveis para sua medição. Os poluentes adotados universalmente como indicadores da qualidade do ar são: dióxido de enxofre (SO_2), monóxido de carbono (CO), ozônio (O_3), óxidos de nitrogênio (NO_x) e material particulado (MP) (CETESB, 2011a; FEPAM, 2011; WHO, 2006).

Entre estes poluentes, o material particulado (MP) se destaca por sua complexidade. Este consiste em uma mistura de ampla variedade de substâncias orgânicas e inorgânicas, em estado sólido ou líquido, que se encontram em suspensão na atmosfera (WHO, 2005). Tais partículas diferem quanto ao seu tamanho (de 0,001 µm a 100 µm), origem, mecanismo de formação, composição química e comportamento na atmosfera. A principal forma de classificação destas partículas refere-se a seu diâmetro aerodinâmico (tamanho da partícula), que determina os padrões de transporte, residência no ar e efeitos associados à saúde humana (Englert, 2004; WHO, 2006).

O material particulado é emitido tanto em eventos de origens naturais quanto antropogênicas. As fontes naturais consistem de emissões de cinzas vulcânicas, incêndios

florestais, ressuspensão de poeiras, sais marinhos e materiais biológicos (ex. pólen e bactérias); enquanto as antropogênicas caracterizam-se por atividades industriais, queima de combustíveis fósseis, geração de energia térmica, tráfego de veículos e queima de biomassa (Vieira, 2009).

As partículas totais em suspensão (PTS) compreendem todos os diâmetros de partículas suspensas na atmosfera, entretanto, partículas maiores que 30-70 µm permanecem suspensas por um curto período (Englert, 2004). Assim, para fins práticos, o PTS é definido pela Associação Brasileira de Normas Técnicas (1997) como o particulado em suspensão de até 50 µm coletados em amostradores de grandes volumes de ar. O material particulado com diâmetro inferior a 10 µm compreende a fração inalável do ar, sendo subdividido em: partículas inaláveis grossas (PM10), com diâmetro de 2,5 – 10 µm; partículas inaláveis finas (PM2,5) com tamanho inferior a 2,5 µm; e partículas ultrafinas (UF), menores do que 0,1 µm. As frações menores de particulados estão contidas nas maiores (Englert, 2004).

Os processos de sedimentação e precipitação removem as partículas maiores da atmosfera algumas horas após a emissão, entretanto, as partículas menores que 2,5 µm podem permanecer em suspensão por dias ou mesmo algumas semanas. Consequentemente, essas partículas podem ser transportadas por longas distâncias, enquanto o particulado grosso em suspensão encontra-se mais próximo as fontes de emissão (WHO, 2005).

As frações de PTS e PM10 são compostas principalmente de elementos da crosta terrestre, sais marinhos e elementos biológicos, sendo formadas especialmente através de processos mecânicos de erosão e ressuspensão. Já as partículas inaláveis finas (PM2,5) e ultrafinas (UF) são constituídas primariamente de metais e hidrocarbonetos. Processos de combustão e reações secundárias na atmosfera são os mecanismos predominantes de formação destas partículas (De Kok et al., 2006; Squadrito et al., 2001).

A fração inalável do material particulado atmosférico representa um risco potencial para a saúde humana, devido a sua capacidade de penetrar e depositar-se nas vias aéreas respiratórias. Seus níveis no ambiente têm sido relacionados com a ocorrência de infecções respiratórias agudas, doenças pulmonares e cardiovasculares crônicas, câncer no sistema respiratório e elevação nas taxas de mortalidade na população (Vargas, 2003). A profundidade de penetração das partículas no sistema respiratório é função de seu tamanho aerodinâmico. As partículas maiores que 10 µm (PTS) são filtradas ou depositadas na região extratorácica do

trato respiratório, constituída pelas vias nasal e oral, faringe e laringe. A retenção de partículas na região extratorácica é considerada a primeira forma de defesa contra a penetração mais profunda do MP, mas também torna esta região mais suscetível a infecções, respostas tóxicas e doenças respiratórias. Somente as partículas menores que 10 µm penetram na região intratorácica do sistema respiratório, que é dividida em região traqueobronquial e região alveolar. As partículas PM10 depositam-se principalmente na região traqueobronquial, enquanto as partículas de tamanho <2,5 µm podem atingir a região alveolar (Claxton et al., 2004; Lopes e Andrade, 1996; Squadrito et al., 2001).

Apesar de a saúde humana ser a principal preocupação nas investigações acerca do material particulado, a presença deste poluente na atmosfera ocasiona uma série de consequências adversas ao ambiente, entre as quais podemos citar (Grantz et al., 2003; Van Dingenen et al., 2004):

- Alteração na visibilidade da atmosfera e diminuição da radiação solar incidente na superfície terrestre, podendo influenciar as reações químicas dependentes de luz solar;

- Danos a monumentos e materiais por descoloração, erosão, corrosão e decomposição devido à deposição de partículas na sua superfície;

- Danos à vegetação através de injúrias foliares, redução da fotossíntese ou da captação de nutrientes e redução no crescimento ou reprodução;

- Contaminação do solo com substâncias tóxicas presentes nas partículas, modificações na química do solo e ciclagem de nutrientes após a deposição do particulado.

Na tentativa de minimizar os efeitos adversos provocados pelo material particulado atmosférico, várias agências reguladoras e organizações não governamentais propuseram limites máximos para a concentração deste poluente no ambiente. De acordo com a USEPA (2008), o monitoramento do material particulado fornece dados para propósitos variados da gestão da qualidade do ar, entre os quais são citados: caracterizar sua qualidade; verificar o cumprimento dos padrões legais; apoiar análises de avaliações de exposição, riscos à saúde e ao bem estar; desenvolver e avaliar estratégias de controle de emissões e acompanhar o progresso dos programas de controle da poluição atmosférica.

Com o aumento de estudos demonstrando os efeitos adversos das partículas inaláveis, maior atenção passou a ser dada às frações de PM10 e PM2,5. De fato, a legislação ambiental norte-americana substituiu a regulamentação da concentração de PTS por PM10 em revisão do "*National Ambient Air Quality Standards*" (NAAQS) em 1987. A versão original da NAAQS, que considerava as partículas totais em suspensão, datava de 1971. Padrões de PM2,5 foram adicionados à NAAQS em 1997 (USEPA, 2008).

No Brasil, a resolução CONAMA n° 003/1990 estabelece padrões primários e secundários da qualidade do ar (Brasil, 1990). Os padrões primários referem-se às concentrações de poluentes que, se ultrapassadas, poderão afetar a saúde da população. Já os padrões secundários são mais restritivos, sendo definidos como as concentrações de poluentes abaixo das quais se prevê o mínimo efeito adverso sobre o bem-estar da população, assim como o mínimo dano à fauna, à flora, aos materiais e ao meio ambiente em geral. Os padrões de material particulado atmosférico compreendidos nesta resolução referem-se às partículas totais em suspensão e às partículas inaláveis grossas, inexistindo regulamentações acerca da concentração de partículas PM2,5.

Estudos de genotoxicidade sobre o material particulado atmosférico realizados no Brasil mostraram a presença de compostos mutagênicos em amostras consideradas dentro dos parâmetros permitidos pela legislação ambiental (Coronas et al., 2008; 2009; Ducatti e Vargas, 2003; Pereira et al., 2010). O ensaio S*almonella*/microssoma vem sendo empregado em estudos da qualidade do ar no Estado do Rio Grande do Sul desde 1998 (Vargas et al., 1998). A partir de então, esse bioensaio vem permitindo identificar o potencial mutagênico de PTS e PM10 em áreas urbanas (Ducatti e Vargas, 2003; Kaffer, 2011; Pereira et al., 2010; Vargas et al., 1998; Vargas, 2003) e industriais com influência petroquímica (Coronas et al., 2008; 2009; Vargas, 2003). Contudo verifica-se a ausência de estudos utilizando o Teste de Ames em amostras de particulados PM2,5 evidenciando a necessidade de avaliação deste poluente.

1.5. Contaminação do solo

O solo é uma matriz complexa e dinâmica que se forma na interface da atmosfera, litosfera, hidrosfera e biosfera. É, essencialmente, um agregado de minerais não consolidados e matéria orgânica, produzido por uma combinação complexa de processos físicos, químicos e biológicos (Manahan, 2003; Nortcliff, 2009; White e Claxton, 2004).

O solo possui camadas distintas, conforme o aumento de sua profundidade, chamadas de horizontes. A água da chuva infiltra-se no solo, carregando sólidos dissolvidos e coloidais para porções mais profundas, onde estes são depositados. A camada superior é a de máxima atividade biológica, contendo a maior parte de sua matéria orgânica. Íons metálicos e partículas de argila na superfície estão sujeitos a considerável lixiviação, de forma que esta camada é chamada, algumas vezes, de zona de lixiviação. As porções subjacentes, denominadas subsolo, contêm matéria orgânica em quantidades mais reduzidas do que o solo de topo. O subsolo recebe materiais como sais, partículas de argila, além de matéria orgânica, lixiviados do solo de topo, sendo chamado de zona de acumulação (Brady e Weil, 2008; Manahan, 2003).

A porção mineral dos solos pode ser descrita de acordo com sua granulometria, ou seja, conforme o tamanho de suas partículas e da proporção de ocorrência destas classes (Brady e Weil, 2008). Diferentes sistemas de classificação granulométrica foram criados, separando as partículas de solo em areia, silte e argila. De acordo com a Associação Brasileira de Normas Técnicas (1995), as partículas de areia possuem diâmetro de 2,0 – 0,06 mm, sendo subdivididos em areais grossas (2,0 – 0,6 mm), médias (0,6 – 0,2 mm) e finas (0,2 – 0,06 mm). As partículas de silte possuem diâmetro de 0,06 – 0,002 mm, e as de argila diâmetro inferior a 0,002 mm.

A textura do solo é um fator importante na retenção ou distribuição de substâncias químicas. Assim verifica-se que as partículas de areia não se aderem umas as outras, possuindo muito baixa capacidade de reter a água, substâncias químicas e nutrientes. Por essas características o solo arenoso possui alto potencial de lixiviação de poluentes, baixa resistência a modificações de seu pH, alta aeração e baixo teor de matéria orgânica. O oposto é apresentado por partículas de argila, as quais possuem alta aderência entre si. Solos argilosos caracterizam-se por alta capacidade de reter água, nutrientes, matéria orgânica e substâncias químicas, além de resistir a alterações de pH. Também possuem baixo potencial de lixiviação de poluentes e de aeração. As partículas de silte possuem características intermediárias entre areia e argila (Brady e Weil, 2008).

O declinío da qualidade dos solos é caracterizado por processos de degradação, sendo os principais, conforme referidos por Bone et al. (2010): a erosão pela ação da água ou do vento; o desenvolvimento de reações extremas, como acidificação ou salinização; a degradação

física, como destruição estrutural e compactação; a degradação biológica; mudanças desfavoráveis no regime de nutrientes; diminuição da capacidade tamponante dos solos e a contaminação por fontes naturais ou antropogênicas. A contaminação do solo foi considerada uma das maiores ameaças à sustentabilidade no uso do solo pela Comissão Européia (EC, 2002).

O solo pode caracterizar-se como um filtro ambiental, devido a sua capacidade de depurar e imobilizar grande parte das impurezas nele depositadas. No entanto, essa capacidade é limitada, fazendo com que o solo também funcione como fonte, rota e receptor de contaminantes. O potencial do solo de atuar como fonte de contaminantes é altamente variável, dependendo das suas propriedades, dos produtos químicos presentes e suas concentrações (Bone et al., 2010; CETESB, 2011a).

Atividades industriais, urbanas e agrícolas liberam inúmeras substâncias químicas capazes de comprometer a qualidade dos solos. A contaminação por substâncias tóxicas pode ser resultado de atividades intencionais ou acidentais, através da aplicação de agroquímicos em cultivos, de práticas de irrigação com águas contaminadas, da deposição de resíduos em áreas específicas (aterros, lagoas de tratamento, eliminação de resíduos, entre outros), do descarte indiscriminado de materiais e de vazamentos/derrames durante a produção, transporte ou armazenamento de materiais industriais como óleos e solventes (Rodrigues et al., 2009; White e Claxton, 2004). Ainda, compostos emitidos na atmosfera através da queima de combustíveis fósseis, de emissões veiculares e industriais e da incineração de resíduos, eventualmente se depositam no solo podendo se acumular na sua camada superficial (Nortcliff, 2009; Rodrigues et al., 2009; Watanabe et al., 2008).

A toxicidade de solos contaminados tornou-se um enfoque importante na avaliação de risco ecológico, podendo ser utilizada para definir diretrizes genéricas ou sítio-específicas de qualidade do solo e para orientar o mapeamento e remediação de sítios contaminados (Burns et al., 1996 *apud* Bone et al., 2010).

Essa contaminação afeta a saúde do ecossistema e age de forma direta na perda de diversidade biológica (Gilmore, 2001). Compostos com características tóxicas presentes no solo também podem afetar a saúde humana através da exposição por inalação de poeiras, ingestão de plantas e animais que absorveram estes compostos, ou ainda através da lixiviação para águas subterrâneas e superficiais utilizadas, entre outros usos, para abastecimento

público (Watanabe e Hirayama, 2001). A ingestão direta de solo contaminado por animais e homens, especialmente crianças, e o contato dermal, também representam uma rota de risco (Calabrese et al., 1997; Madrid et al., 2008). Muitos destes compostos são persistentes ou capazes de se bioacumular, representando riscos mesmo se, inicialmente, estiverem presentes em baixas concentrações (Gilmore, 2001).

A camada superficial do solo de topo reflete a deposição dos poluentes de origem atmosférica, especialmente aqueles depositados recentemente, bem como poluentes que não se movem em profundidade por estarem adsorvidos as partículas do solo. Nas camadas do subsolo, se encontram poluentes depositados por derramamentos líquidos, deposição de longo prazo de materiais solúveis em água e substâncias enterradas. As chuvas podem mover os poluentes da camada superficial para outras mais profundas ou afastá-los do ponto de deposição por escoamento superficial (USEPA 1992).

Em ambientes poluídos, a água da chuva representa uma significativa rota de contaminação devido à remoção de contaminantes presentes nos solos e atmosfera. A lavagem desses contaminantes do material particulado atmosférico, especialmente dióxidos de enxofre e óxidos de nitrogênio, pode levar à formação de chuvas ácidas, evento comum em áreas localizadas próximas a grandes parques industriais (Holtz, 2000; Mirlean et al., 2000; USEPA, 1980; 1990).

É sabido que a presença de condições ácidas leva a um aumento da biodisponibilidade dos metais que se dissociam mais facilmente, podendo atingir formas tóxicas (Pueyo et al., 2003; Tack e Verloo, 1995). Esta acidificação do ambiente influencia ainda, na solubilidade, especiação, mobilidade ambiental, transferência na cadeia alimentar e toxicidade de vários metais (Scheuhammer, 1991).

A avaliação de substâncias genotóxicas no solo geralmente requer o emprego de uma metodologia de extração, tendo em vista aspectos práticos e/ou necessidade de esterilidade das amostras (Courty et al., 2004). O procedimento de extração representa um estágio decisivo na avaliação das amostras, uma vez que grande variação nas propriedades físicas e químicas dos constituintes, incluindo dos principais compostos genotóxicos, ocorrerá de acordo com o método empregado (Watanabe e Hirayama, 2001; Courty et al., 2004). Embora muitas técnicas e solventes tenham sido utilizados nestas extrações, a maioria dos estudos utilizando o Teste de Ames em amostras de solo avaliou a mutagenicidade dos seus

compostos orgânicos, com pouca ênfase sendo dada à fração inorgânica de seus constituintes (Silva-júnior et al., 2009).

Entre as diferentes metodologias de preparo das amostras, ressaltamos os experimentos de lixiviação. Este processo consiste na remoção das partículas solúveis e/ou coloidais de um solo pela percolação de água (ABNT, 1995). O procedimento de lixiviação mimetiza a transferência de micro-poluentes de uma matriz sólida para o compartimento aquático. Esse procedimento de extração simula os efeitos de chuvas fortes (Mouchet et al., 2006). Diferentes metodologias de extração de lixiviados de amostras de solo para uso no ensaio *Salmonella/*microssoma foram empregadas por Silva-Júnior e Vargas (2009). Os autores evidenciaram que o processo de extração em mesa agitadora com solução de ácido acético, em valor próximo ao pH do solo, foi a metodologia mais eficiente.

A mutagenicidade de solos contaminados foi tema de revisão realizada por White e Claxton (2004), que categorizaram os sítios avaliados em industriais, urbanos e rurais. Os resultados do ensaio *Salmonella/*microssoma, expressos em médias de revertentes/mg solo seco, foram comparadas por análise de variância, mostrando relação significativa entre categoria do sítio e mutagenicidade. Dessa forma, os solos industriais apresentaram os maiores potenciais mutagênicos, seguidos pelos solos urbanos e rurais, respectivamente.

No Brasil, estudos empregando o teste de Ames para avaliação da mutagenicidade de solos foram conduzidos no estado do Rio Grande do Sul em áreas sujeitas a influência de rejeitos de carvão (Silva-Júnior et al., 2009; Silva-Júnior e Vargas, 2009), em solos próximos a rodovias (Silveira, 2002), em potenciais áreas de referência (Meyer, 2008) e em sítios contaminados por preservantes de madeira (Pohren, 2011; Souza, 2008). Não foram encontrados estudos com esta abordagem em solos de áreas urbanas.

O solo forma-se lentamente sendo considerado um recurso vital e não renovável. Embora técnicas de recuperação de áreas degradadas venham sendo desenvolvidas, a remediação do solo é um processo caro e que requer longo período de aplicação. Assim, a qualidade do solo deve ser preservada, sendo necessárias medidas de proteção previstas na legislação (Gilmore, 2001; Nortcliff, 2009; Rombke et al., 2005). No Brasil, a Resolução CONAMA n° 420/2009 “Dispõe sobre critérios e valores orientadores de qualidade do solo quanto à presença de substâncias químicas e estabelece diretrizes para o gerenciamento ambiental de áreas contaminadas por essas substâncias em decorrência de atividades

antrópicas". De acordo com esta resolução, "a proteção do solo deve ser realizada de maneira preventiva, a fim de garantir a manutenção da sua funcionalidade, ou de maneira corretiva, visando restaurar sua qualidade ou recuperá-la de forma compatível com os usos previstos" (Brasil, 2009).

1.6. Área de estudo

O município de Rio Grande localiza-se no extremo sul do Brasil, na península do Estuário da Laguna dos Patos e próximo à sua desembocadura no Oceano Atlântico (Figura 1). A cidade conta com aproximadamente 196.337 habitantes, sendo que 96% da população vivem em áreas urbanas (IBGE, 2009).

Figura 1 - Localização do município de Rio Grande na desembocadura da Lagoa dos Patos ao Oceano Atlântico (Fonte: Fepam, 2010; modificado).

A formação da cidade, hoje com 2710 km^2 de superfície (IBGE, 2009), ocorreu em áreas baixas, onde gradualmente foram sendo criados terrenos, originalmente alagados,

através de aterros (Fragomeni et al., 2010). Desde o inicio de sua ocupação, em 1735, ocorreram seis períodos históricos de formação de aterros, gerando cerca de 60% da área urbana atual (FEPAM, 2010; Mirlean e Oliveira, 2006) (Figura 2). Neste processo, foram misturados aos sedimentos arenosos, diferentes materiais produzidos na época de construção, compreendendo resíduos diversos, entulhos, lixo industrial e doméstico (Fragomeni et al., 2010; Mirlean e Oliveira, 2006).

Figura 2 – Área original da cidade de Rio Grande (linha vermelha) e datas aproximadas dos aterros (Fonte: Garcia, 2010).

Além da criação de aterros, a falta de território levou ao crescimento desordenado da cidade, com as zonas portuária, industrial e urbana posicionadas muito próximas. O município possui um importante complexo industrial, caracterizado, principalmente, por indústrias de fertilizantes, produtos químicos e agroquímicos, alimentos, pescado, extração e refino de óleo vegetal, produção de resinas de madeira e refinaria de petróleo. Conta ainda com os ramos metal-mecânico, têxtil e naval, além de importante zona portuária, usinas termelétricas e uma incineradora de resíduos sólidos. Nas proximidades destes empreendimentos estabeleceram-se lotes residenciais que formam um adensamento urbano junto à área industrial. Estas áreas são

habitadas principalmente pelas populações de baixa renda, compostas frequentemente por operários, pescadores e pequenos agricultores (FEPAM, 2010).

Diversos estudos vêm mostrando a existência de contaminação por elementos traços e substâncias orgânicas em diversos compartimentos ambientais na cidade (Cavalcante, 2002; Mirlean e Oliveira, 2006; Pederzolli, 2006). Quantidades significativas de metais pesados já foram observadas nos solos da região, sendo apontada a presença de chumbo, cromo, mercúrio e zinco como os principais contaminantes metálicos responsáveis por degradar a qualidade na região urbana. No compartimento atmosférico, os elementos cádmio e chumbo apresentaram-se em valores próximos ou pouco superiores aos limites estabelecidos por padrões internacionais de qualidade do ar (FEPAM, 2010). A presença de hidrocarbonetos policíclicos aromáticos (HPAs) em Rio Grande aparece associada com indústrias produtoras de energia por queima de combustíveis fósseis, à refinaria de petróleo, indústrias de óleos vegetais e às atividades portuárias, caracterizando-se como um grupo importante de poluentes atmosféricos na área. A contaminação de sedimentos, águas e material particulado atmosférico por HPAs já foi demonstrada em estudos desenvolvidos na cidade (FEPAM, 2010; Pederzolli, 2006; Pereira, 2008). Foi ainda verificado que Pb, Ni, V, Cd, As e fluoretos oriundos de atividades antrópicas locais contaminaram outras matrizes ambientais devido à ação da dispersão atmosférica (Garcia et al., 2010; Mirlean et al., 2002; 2005; Mirlean e Roisenberg, 2006; Vanz et al., 2003).

Outro dado importante da poluição ambiental foi verificado por Mirlean et al. (2000) que estudou os parâmetros físico-químicos das precipitações em Rio Grande. Os autores observaram a ocorrência de eventos de chuvas ácidas provocadas pelas emissões atmosféricas do parque industrial, sendo 3,6 o menor valor de pH verificado. Foi possível estabelecer que o valor de *background* de pH para a região estaria próximo a 5,2. Essa característica pode influenciar a biodisponibilidade de poluentes.

Além dessas observações, a proximidade das moradias com as fontes de emissão de poluentes ressaltam a importância de se conhecer o impacto dos mesmos na qualidade do ambiente. Com esse propósito, o presente estudo avaliou duas áreas urbanas, uma com característica residencial e outra mista, adjacente a empreendimentos industriais (Figura 3).

Figura 3 – Detalhe da cidade de Rio Grande. 1- Distrito industrial, 2 – Zonas portuárias, 3 – Refinaria de petróleo, 4 – Área urbano-industrial, 5 – Área urbano-residencial. (Fonte: Google Earth, 2010, modificado.)

2. OBJETIVOS

O presente estudo teve por objetivo investigar a presença de substâncias genotóxicas através do ensaio *Salmonella*/microssoma em diferentes frações de material particulado e solos, analisando a presença de contaminantes orgânicos e inorgânicos.

Os objetivos específicos buscaram:

- Avaliar o potencial mutagênico e citotóxico de material particulado atmosférico e amostras de solo de áreas urbanas, com características residenciais e industriais na cidade de Rio Grande, RS.

- Comparar a resposta mutagênica de duas frações de particulados atmosféricos (PTS e PM2,5);

- Avaliar o efeito da granulometria do solo na resposta mutagênica;

- Avaliar o efeito de extrações de solo por lixiviação em diferentes pHs quanto à disponibilização de substâncias genotóxicas, simulando eventos de precipitação;

- Relacionar efeitos biológicos e caracterização química das amostras ambientais estudadas.

3. ARTIGOS CIENTÍFICOS

Considerando os objetivos propostos, a presente dissertação permitiu a elaboração de dois capítulos correspondentes aos artigos científicos resultantes do projeto desenvolvido.

O primeiro artigo *"Mutagenicity of particulate matter fractions in areas under the impact of urban and industrial activities"*, a ser submetido à revista *Chemosphere*, apresenta a comparação do potencial mutagênico de dois particulados atmosféricos (PTS e PM2,5) na cidade de Rio Grande, RS. Ainda, o trabalho realizou esta avaliação em extratos aquosos de material particulado atmosférico, em adição às analises de extratos orgânicos, as quais são predominantes na literatura.

o segundo artigo "*Mutagenicity in urban industrial areas: contribution of acid rain and organic pollutants*", a ser submetido à revista *Science of the Total Environment*, apresenta a avaliação de diferentes extrações ácidas na disponibilização de compostos a partir de duas frações granulométricas de solo em área urbano-residencial e urbano-industrial. O trabalho também analisou o efeito de compostos mutagênicos em extratos orgânicos de solos e particulados PM2,5 da cidade de Rio Grande.

3.1. ARTIGO 1

MUTAGENICITY OF PARTICULATE MATTER FRACTIONS IN AREAS UNDER THE IMPACT OF URBAN AND INDUSTRIAL ACTIVITIES

Artigo a ser submetido para *Chemosphere*

Co-autores: Mariana Vieira Coronas[a,b]; Jocelita Aparecida Vaz Rocha[a]; Vera Maria Ferrão Vargas[a,b]

[a]Programa de Pesquisas Ambientais, Fundação Estadual de Proteção Ambiental Henrique Luís Roessler (FEPAM), Avenida Salvador França, 1707 CEP: 90690-000 Porto Alegre, RS, Brazil.

[b]Programa de Pós-graduação em Ecologia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500, CEP 91501-970, Porto Alegre, RS, Brazil.

ABSTRACT

Since organisms in the environment are exposed to a mixture of pollutants, the purpose of this study was to simultaneously analyze the mutagenicity of organic and inorganic contaminants, comparing biological responses in two fractions of particulates (TSP and PM2.5) and extracts (organic and aqueous). Mutagenic activity of organic and aqueous particulate matter extracts from urban-industrial and urban-residential areas were evaluated by *Salmonella*/microsome assay, through the microsuspension method, using strain TA98 with and without a liver metabolization fraction. Additionally, strains YG1021 and YG1024 (sensitive to nitroderivatives) were used for the organic extracts. The aqueous extracts presented only negative responses for mutagenesis and cytotoxicity was detected in 50% of the samples. In these extracts the presence of potential bioavailable metal compounds were identified. All organic extracts showed the presence of mutagenic compounds with a higher potential associated with PM2.5. This study presents a first characterization of PM2.5 in Brazil, through the *Salmonella*/microsome assay. The strategy used allowed defining the anthropic influence of groups of compounds characteristically found in urban and industrial areas, even in samples with PM values that are acceptable according to quality standards. Thus application of genotoxic approach in areas with different anthropic influences will favor the adoption of preventive actions in the health/environment relation.

Keywords: Environmental genotoxicity, TSP, PM2.5, Organic extraction, Aqueous extraction, Nitrocompounds.

1. INTRODUCTION

Human activities release a variety of chemicals into the environment through production, distribution, use and final disposal, polluting natural resources. Once these substances have been released they may change, be transported and/or bioaccumulate. Pollution of the atmospheric compartment has aroused growing interest, since an increasing number of studies reports its association with adverse effects on the environment and human health (Grantz et al., 2003; Vargas, 2003).

Atmospheric particulate matter (PM) is an air pollutant consisting of a complex mixture of many different organic and inorganic substances suspended in the air. These particles are characterized by a variety of morphological, chemical, physical and thermodynamic properties, but its aerodynamic size is the main form of classification (WHO, 2006). Total suspended particles (TSP) are up to 50 μm in diameter and composed mainly of Earth crust, marine salts and biological elements. They are formed especially through mechanical erosion and re-suspension processes. Particulate matter with a diameter less than 10 μm comprises the inhalable air fraction, which is subdivided into: coarse inhalable particles (PM10), with a 2.5 - 10 μm diameter; fine inhalable particles (PM2.5) less than 2.5 μm in diameter; and ultrafine particles (UF), less than 0.1 μm. The PM2.5 particles are constituted primarily of metals and hydrocarbons. Combustion processes and secondary reactions in the atmosphere are the prevailing mechanisms in the formation of these particles (De Kok et al., 2006).

The occurrence of adverse biological effects associated with exposure to PM has been extensively described in the literature, leading several regulating agencies to adopt restrictive parameters (EC, 2008; USEPA, 2008; WHO, 2006). Despite the complex properties presented, the PM regulatory limits are based only on their mass (particle concentration), according to the aerodynamic size of the suspended particles. However, several studies have shown adverse effects of PM, even at low concentrations (Coronas et al., 2008; Vargas, 2003), demonstrating that there is no threshold below which no effect would be observed (Bonetta et al., 2009; Coronas et al., 2009; Lippmann, 2010; Pereira et al., 2010). Thus, other parameters of the atmospheric particulate must be investigated to improve the evaluation of air quality in order to protect the ecosystem and human health.

Reducing atmospheric particulate material, as proposed by regulating agencies, is an important goal because this will result in a probable reduction of the risk associated with PM.

However, regulatory strategies covering chemical and toxicological characteristics of PM will probably be more effective and efficient (De Kok et al., 2006). In this context, bioassays are valuable tools, because they allow detecting the effects of a wide variety of chemicals and their interactions, even if these chemicals are present at low concentrations. The *Salmonella*/microsome assay is a short duration bacterial test which has proved efficient to investigate the mutagenic potential of samples of atmospheric particulate matter, and this is the method most widely used for this purpose (Claxton et al., 2004; Claxton and Woodall, 2007; Vargas, 2003). Due to the correlation between mutagenicity and carcinogenicity, the presence of potential carcinogens in the environment can be identified by quick assays. In fact, a high correlation was found between a positive mutagenic response in the *Salmonella*/microsome assay and carcinogenesis in rodents. This correlation ranges from 77% to 90%, depending mainly on chemical composition (McCann et al., 1975; Mortelmans and Zeiger, 2000; Zeiger, 1998).

Among the compounds associated with the atmospheric particulates are organic substances whose mutagenic potential was demonstrated by several studies (Claxton et al., 2004; Ducatti and Vargas, 2003; Pereira et al., 2010). Among these substances, special attention is given to polycyclic aromatic hydrocarbons (PAHs) – a group of chemicals composed exclusively of carbon and hydrogen containing two or more condensed aromatic rings. PAHs are emitted when there is incomplete burning of organic matter, and it is one of the main causes associated with adverse effects on human health provoked by air pollution (Pereira et al., 2010; Skarek et al., 2007; Vargas, 2003).

Besides these compounds, the inorganic fraction associated with atmospheric particulate matter can also present genotoxic characteristics (Bonetta et al., 2009). Metals associated with the particulate may be present in highly mobile forms (water soluble) and, therefore, potentially bioavailable (Fernández-Espinosa et al, 2002). In this way, toxic metals associated with PM can be absorbed in lung tissue during breathing (Quitério et al., 2004), influencing biological functions. However, there are few reports in the literature about the evaluation of water-soluble compounds of particulate matter using the *Salmonella*/microsome assay (Brits et al., 2004).

Since organisms in the environment are exposed to a mixture of pollutants, the purpose of this study was to simultaneously analyze organic and inorganic contaminants, comparing

biological and chemical responses in two fractions of particulates (TSP and PM2.5) and extracts (organic and aqueous). For this the present study evaluated the mutagenic and cytotoxic potential of organic and aqueous extracts of atmospheric particulate matter (TSP and PM2.5) in the city of Rio Grande, Rio Grande do Sul, Brazil, using the *Salmonella*/microsome assay, besides chemical analyses of compounds of interest.

2. MATERIAL AND METHODS

2.1. Area of study

The municipality of Rio Grande, in the extreme south of Brazil, has a population of approximately 196,337 inhabitants and an area of 2,710 km^2 with 96% of the population living in urban areas (IBGE, 2009). The municipality is located in an estuarine region, with a major industrial complex whose mainly activities are manufacturing fertilizers, foodstuff, fish processing, extraction and refinery of vegetal oils, oil refinery, besides an important port area. In the vicinity of these plants there are residential areas which form an urban densification next to the industries. The total suspended particles (TSP) in this city were characterized in a previous study regarding mutagenicity, showing a worse air quality compared to an external reference city (Pereira et al., 2008).

2.1.1. Sampling sites:

- Site 1 (32°2’32.98”S, 52°5’16.84”W): Urban-industrial area located in the second quadrant of preferential atmospheric dispersion of an oil refinery.

- Site 2 (32°2’52.23”S, 52°8’24.75”W): Urban-residential area distant from the main industrial parks of the city, but not completely free of the atmospheric dispersion plume of the anthropic sources in the municipality.

The study area and the sampling sites are shown in Figure 1. The samplings took place from October/2009 to January/2010, with the collection of total suspended particulates (TSP) at Site 1 and fine inhalable particles (PM2.5) at both sites. The January/2010 sampling at Site 2 was impaired and therefore excluded from the study.

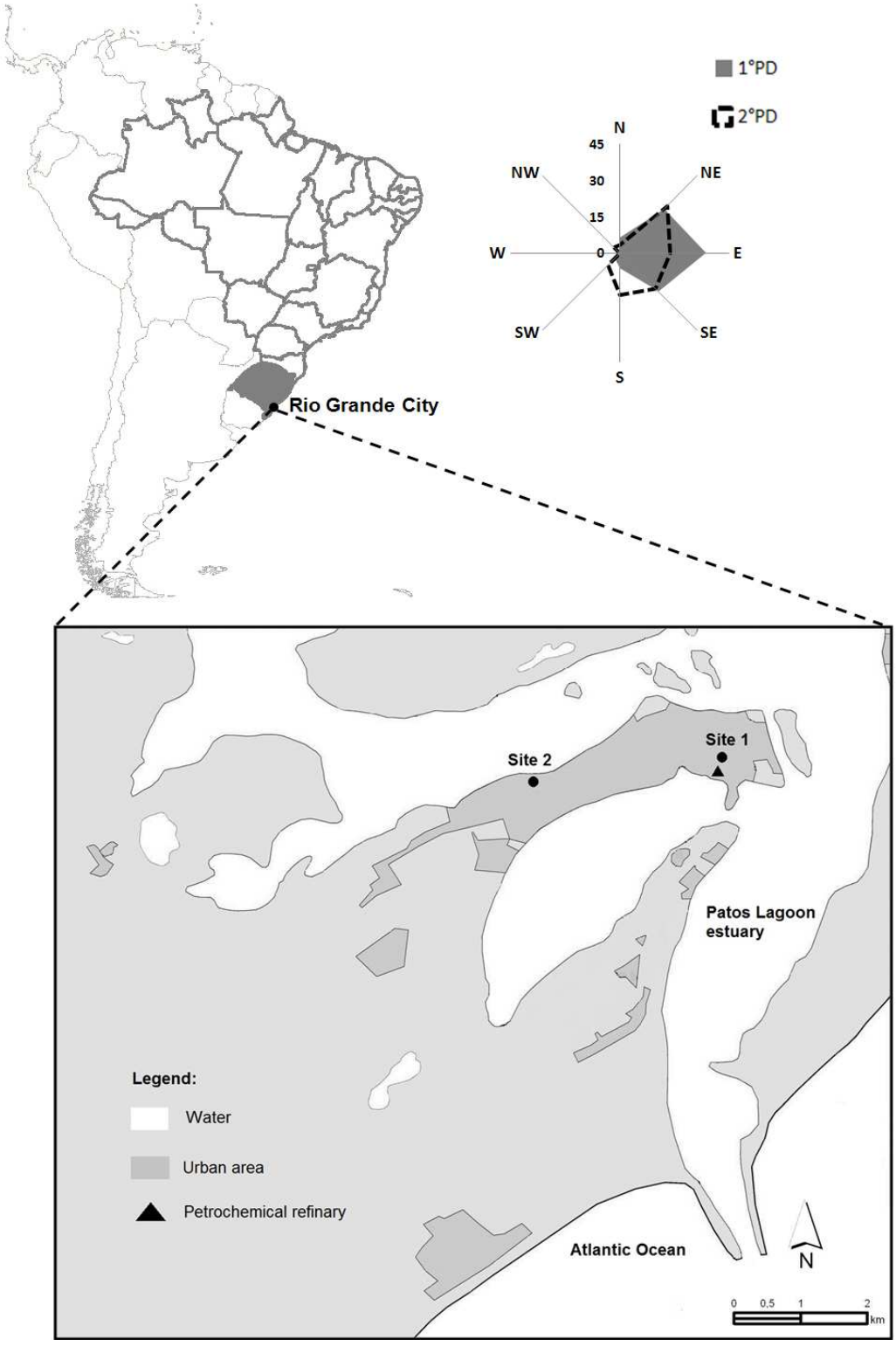

Figure 1 – Location of study area in the city of Rio Grande. Sampling sites: Site 1 – Urban-industrial area, Site 2 – urban-residential area. In detail, Rose diagram of prevailing wind direction (%) during sampling days. 1°PD = first prevailing wind direction; 2°PD = second prevailing wind direction. (Source: FEPAM, 2010; modified).

Climate data observed during the study period were provided by the 8th District of Meteorology of the National Institute of Meteorology (8° DISME – Porto Alegre/INMET, Brazil).

2.2. Sampling of atmospheric particulate matter:

Samples of total suspended particles (TSP <50 μm) and fine inhalable particles (PM2.5 < 2.5 μm) were collected with high volume samplers (General Metal Works Inc. for TSP or AGV MP2.5 1200/CCV Thermo Environmental Instruments for PM2.5), during a 24h period, with a weekly frequency. Glass fiber filters were used (AP 40-810, 20 cm×25 cm Millipore) to sample TSP and Teflon membrane filters (TX40HI20WW, 254X203mm) for PM2.5. The air volume passed through each filter ranged from 1878.1 to 2421.2 m^3 in TSP, with mean values of 2046.1 ± 136.6 m^3; and from 1510.8 to 1840.5 m^3 in PM2.5, with a mean of 1608.5 ± 81.1 m^3. Before and after the samplings, the filters were weighed and stabilized (45% humidity) to determine the concentration of particulates, expressed in units of $\mu g/m^3$ of air sampled (ABNT, 1997). The filters sampled were protected from light, at -20°C, for the extraction procedures.

2.3. Extraction procedures:

A quarter of each filter containing particulate matter was submitted to sequential extraction, comprising a stage of organic extraction followed by an aqueous extraction. The filters of the weekly collections were grouped making up monthly samples. In each sample the organic compounds were extracted using the ultrasound technique with dichloromethane solvent (DCM, CASRN. 75-09-2), as described in Vargas et al. (1998). The remaining filters from organic extraction were dried in a laminar flow hood, at ambient temperature, for total DCM evaporation. For the extraction of soluble compounds in water, the dry filters were submitted to an ultrasound bath for 30 min. Ultrapure water at a sufficient volume for total immersion of the filters was used (80 -100 mL). The soluble compounds were separated from the insoluble ones by centrifuging (5000 rpm for 10 min). The supernatant was removed and filtered in a 0.22 μm membrane. The aqueous extraction method was adapted from Bonetta et al. (2009) and Cavanagh et al. (2009).

The percentage of extractable organic matter (EOM) in $\mu g/m^3$ was calculated from the total amount of EOM per filter divided by the air volume sampled.

Before the bioassays, the organic extracts were dried with nitrogen gas and resuspended in dimethylsulfoxide (DMSO, CASRN. 67-68-5). The aqueous extracts were unfrozen and used directly.

2.4. *Salmonella*/microsome assay:

The mutagenicity of the organic and aqueous extracts was tested using the *Salmonella*/microsome assay (Maron and Ames, 1983), through the microsuspension method (Kado et al., 1983). The *Salmonella typhimurium* strain TA98 was used with and without a liver metabolization fraction (S9 mix), detecting mutagenic substances that cause frameshift error. Nitroderived mutagenic compounds were investigated in the organic extracts in the absence of metabolization using strains YG1021 and YG1024, derived from TA98. These strains present a high level of nitroreductase and O-acetyltransferase enzymes activities conferring greater sensitivity in detecting nitroarenes (YG1021) and dinitroarenes (YG1024), respectively (Watanabe, 1989; 1990).

Six concentrations of each sample were tested generating a dose-response curve with values corresponding to 1.25; 2.50; 10.00; 20.00 and 40.00 µg/plate – for the organic extracts, based on previous studies (Coronas et al., 2009; Ducatti and Vargas, 2003; Vargas et al., 1998). Values corresponding to 320, 640, 1280, 2560, 6400, 12800 µg/plate were used for the aqueous extracts, defined after experimentation in the laboratory, with concentrations ranging from 40 to 12800 µg/plate. Due to the greater volume of sample needed for assays using aqueous extracts, the amounts of agar and NaCl of the surface agar were modified to maintain a constant final concentration, as described in Vargas et al. (1995). All the assays were performed in duplicate, parallel to negative controls (5 µL solvent Dimethylsulfoxide – DMSO or 100 µL sterile ultrapure water; 100 µL liquid nutrient medium) and positive ones (4-nitroquinoleine oxide- 4NQO, 0.5 µg/plate, CASRN. 56-57-5; 2 – nitrofluorene - 2NF, 0.15 µg/plate, CASRN. 607-57-8; and 2- aminofluorene - 2AF, 1 µg/plate, CASRN. 153-78-6 of Sigma Chemical Company, St. Louis, MO) according to strains and extracts used.

Sample cytotoxicity was determined in a cell survival test (strain TA98) performed concurrently with the mutagenesis assays. In brief, this test consisted of diluting cell suspension obtained after the incubation stage at 37°C for 90 min (bacterial culture 1-2 x 10^{10} cells/mL + S9 mix or phosphate buffer 0.1M + solvent or sample), in phosphate buffer (pH = 7.4) up to the concentration of 1-2 x 10^2 cells. After dilution, the bacterial culture was sown in

nutrient agar and incubated for 72h at 37°C. After this period, the surviving colonies were counted manually.

2.5. Data analysis:

The samples were considered positive for mutagenesis when they presented a significant ANOVA ($p < 0.05$) between dosages and positive dose-response curve ($p < 0.05$). The response was considered indicative of a mutagenic effect when only the dose-response was significant. The linear portion of the curves was analyzed by linear regression models or Bernstein (Bernstein et al., 1982). The mutagenic potential of positive and indicative responses was estimated by the regression curve slope and expressed in number of revertants per µg/extract (rev/µg) and revertants per cubic meter of sampled air (rev/m³ = rev/µg X EOM). Statistical analysis was performed with Salanal (*Salmonella* Assay Analysis) software, version 1.0 of the Research Triangle Institute, RTP, NC, USA.

In the cell survival assay, the samples were considered cytotoxic when they presented diminished growth compared to a negative control in at least one dosage, with values below 60% (Vargas et al. 1993).

The number of revertants obtained in *Salmonella*/microsome assay was compared among sites and particle fractions by ANOVA test in blocks ($p< 0.05$). The Mann-Whitney U test was employed to compare particle concentrations between sites ($p< 0.05$). The Spearman correlation coefficient was applied to evaluate relations between variables (TSP, PM2.5, revertants/m³ and climate parameters). Software R (R version 2.9.0., 2009) was used for these purposes.

2.6. Quantification of metals in aqueous extracts:

The metals (Al, V, Cr, Fe, Ni, Cu, Zn, As, Cd, Pb) were quantified in aqueous extracts by inductively coupled plasma optical emission spectrometry (ICP-OES) with the respective detection limits (mg/L): Al - 0.08; V - 0.002; Cr - 0.004; Fe - 0.04; Ni - 0.004; Cu - 0.004; Zn - 0.02; As - 0.02; Cd - 0.002; Pb - 0.01. These analyses were performed in the Environmental Analyses Laboratory, Federal University of Rio Grande do Sul, Brazil, according to the operational conditions established in the Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

3. RESULTS

The climate variables (precipitation, humidity, temperature and wind speed) observed during the study period (October/09 to January/10; Spring and Summer), as well as information regarding the particulate matter concentration are shown in Table 1. Climate data show little pluviosity during the collection days (0 – 3.8 mm), mean temperatures ranging from 18 to 26°C, 76.5 to 86 percent humidity and mean wind speed up to 5.28 ± 1.52 m/s. The prevailing wind direction was from east. TSP concentrations at Site 1 correlated negatively with precipitation ($r= -0.53$, $p= 0.04$). PM2.5 was negatively correlated with humidity ($r= -0.86$, $p< 0.001$) only at Site 2.

TSP concentrations varied from 14.34 to 169.91 µg/m³ with a mean of 65.34 ± 36.84 µg/m³, while PM2.5 varied from 1.74 to 23.11 µg/m³ with a mean of 11.2 ± 5.76 µg/m³ at Site 1, and from 6.01 to 48.55 µg/m³ with a mean of 17.9 ± 11.83 µg/m³ at Site 2 (Table 1). No significant difference was found for PM2.5 between the two sites. The highest TSP concentrations occurred in December/09, while for PM2.5 they were in October/09 for both sites. Only one of the TSP filters investigated in the study (169.91 µg – December/09) was higher than the maximum concentration allowed by Brazilian Law (BRASIL, 1990). Due to the lack of legal parameters for PM2.5 concentration in Brazil, the World Health Organization (WHO, 2006) recommendation of 25 µg was used for comparative purposes. According to this criterion, two PM2.5 filters were above the maximum value recommended for 24 h samplings (48.55 µg – October/09 and 30.31 µg – December/09), both in the residential area (Site 2). The extractable organic matter varied from 0.71 to 2.40 µg/m³ for TSP and from 0.28 to 1.93 µg/m³ for PM2.5 with higher values occurring in TSP.

The mutagenic and cytotoxic evaluation of TSP and PM2.5 aqueous and organic extracts was performed on the samples of urban-industrial area (Site 1), for comparison. The aqueous extracts of both particulates presented negative responses for mutagenesis in all assays. Due to insufficient quantity, the samples of PM2.5 – Dec and TSP - Jan were tested only at four concentrations (ranging from 320 to 2560 µg). In a pilot study the presence of base pair substitution mutagens (TA100 strain) and sensitivity of TA97a strain were also investigated, resulting in negative responses.

Table 1 – Climate variables during the period of study and concentration of particulates sampled in the city of Rio Grande.

	Climate variables *				TSP Site 1				PM 2.5 Site1				PM 2.5 Site 2			
	Precipitation	Temperature	Humidity	Wind Speed	N° of filters	C	∑C	EOM	N° of filters	C	∑C	EOM	N° of filters	C	∑C	EOM
	(mm)	(°C)	(%)	(m/s)		(µg/m³)	(µg/m³)	(µg/m³)		(µg/m³)	(µg/m³)	(µg/m³)		(µg/m³)	(µg/m³)	(µg/m³)
Oct/09	0 ± 0	18 ± 2.30	76.5 ± 7.23	5.28 ± 1.52	4	55.55 57.55 54.31 62.32	229.73	0.93	5	10.98 11.41 6.99 11.49 21.30	62.17	0.44	5	12.36 18.64 19.42 48.55 19.00	117.97	0.73
Nov/09	3.38 ± 5.86	20.3 ± 1.29	85.2 ± 4.04	4.3 ± 1.37	3	91.29 57.23 14.34	162.86	1.33	3	19.06 10.49 1.74	31.29	1.10	3	11.07 6.65 6.01	23.73	0.88
Dec/09	3.7 ± 5.49	22 ± 1.59	85.2 ± 6.35	4.13 ± 1.51	5	75.32 169.91 111.51 48.25 59.04	464.03	0.71	5	11.41 5.25 23.11 7.96 12.86	60.59	0.28	4	9.04 30.31 19.76 14.06	73.17	0.47
Jan/10	1.3 ± 0.83	26 ± 0.31	86 ± 6.06	3.52 ± 1.17	3	32.69 42.96 47.85	123.50	2.40	3	7.41 10.24 6.80	24.45	1.93	-	- - -	-	-

* Mean ± deviation measured on days when atmopheric particulate matter is collected. Source: 8° Distrito de Meteorologia do Instituto Nacional de Meteorologia (8° DISME – Porto Alegre/INMET), Brazil; TSP = Total Suspended Particles. PM2.5 = Fine inhalable particles; C = particle concentration in the filter; ΣC = Total particle concentration. EOM = extractable organic matter.

Cell survival results of PM aqueous extracts are shown in Figure 2. Cytotoxicity was detected in 50% of the samples, always in the absence of metabolization. Only a single sample (TSP- October /09) showed a diminished survival percentage with S9 fraction, reaching values close to the limit for toxicity (61%). The samples of fine inhalable particles (PM2.5) produced most of the cytotoxic responses, present at the higher concentrations. In a pilot study to define the dose-response curve the concentrations used were not cytotoxic.

Toxic substances were also found in organic extracts, but less often than in the aqueous extracts and always in the PM2.5 fraction, both in the presence and absence of metabolization (Figure 3).

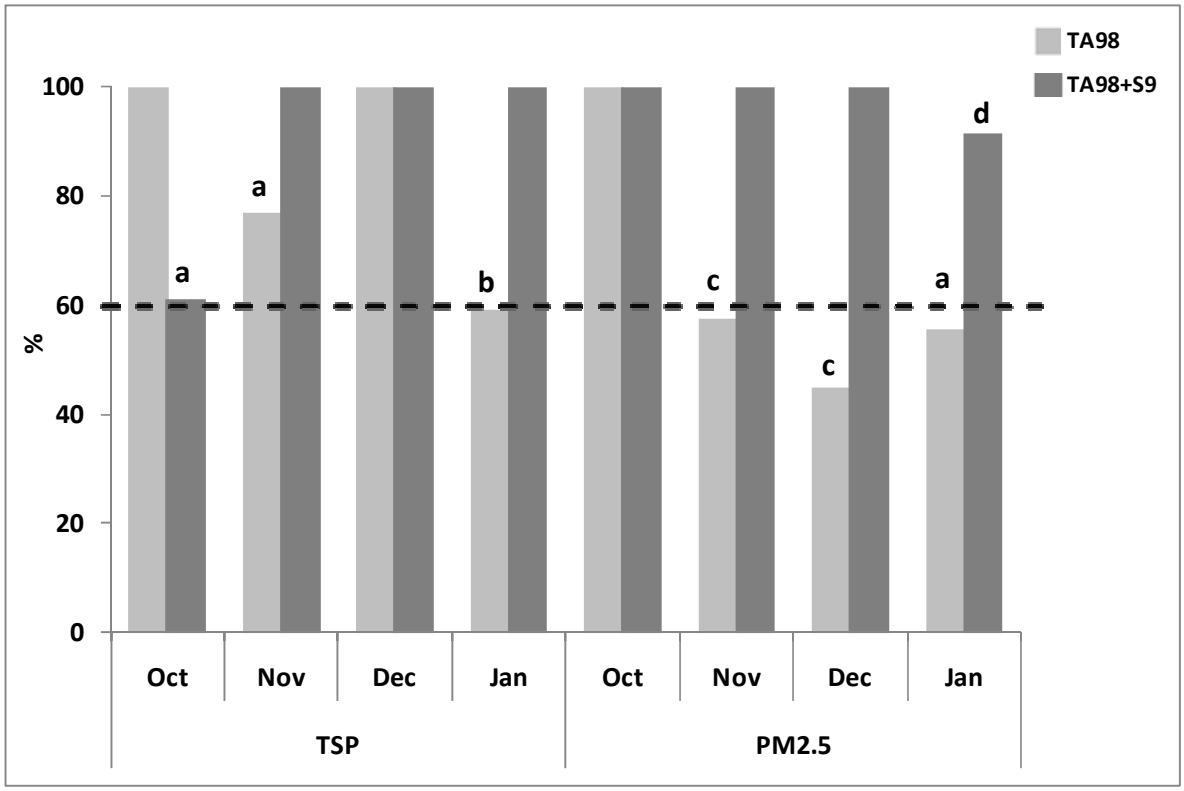

Figure 2 – Percentage of cell survival in the *Salmonella*/microsome assay of aqueous extracts of atmospheric particulate matter in an urban-industrial area (Site 1).

Values below 60% (broken line) indicate cytotoxicty. TSP = Total Suspended Particles. PM2.5 = Fine inhalable particles. Concentration: a = 12800 µg; b = 1280 µg; c = 2560 µg; d = 6400 µg.

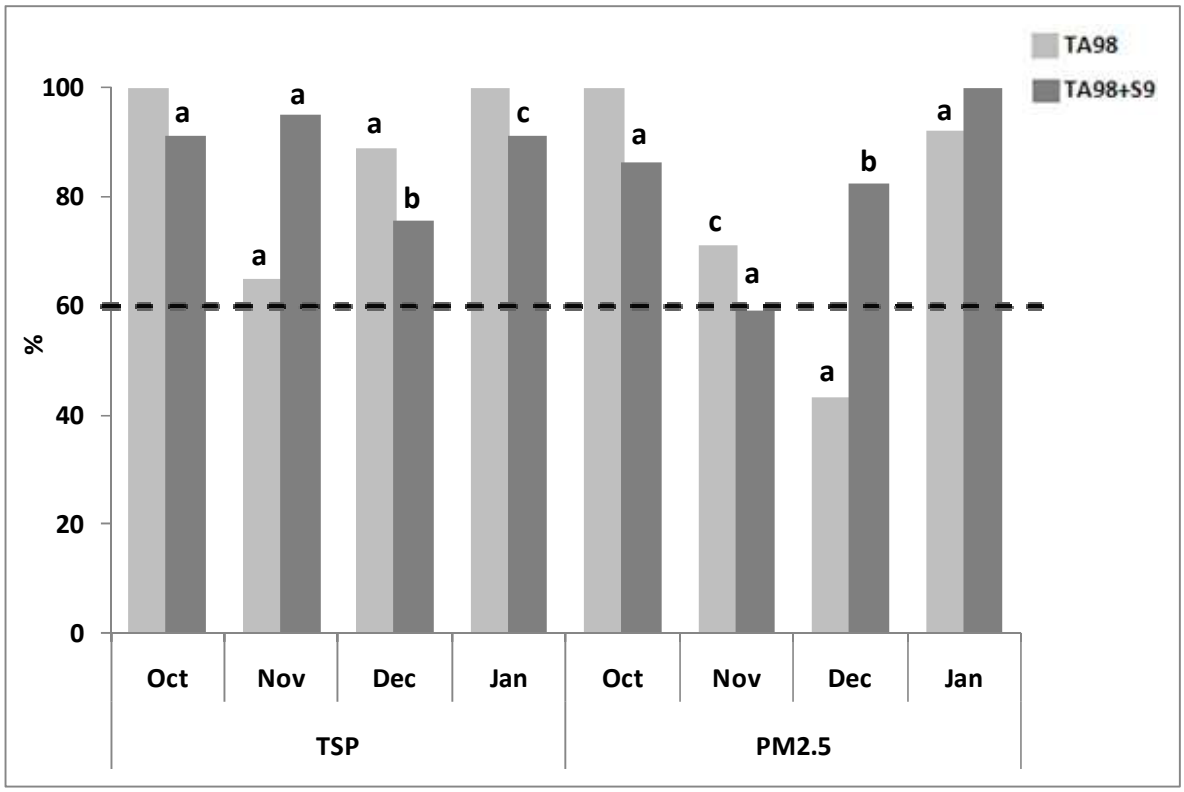

Figure 3 – Percentage of cell survival in the *Salmonella*/microsome assay of organic extracts of atmopheric particulate matter in an urban-industrial area (Site 1).

Values below 60% (broken line) indicate cytotoxicity. TSP = Total Suspended Particles. PM2.5 = Fine inhalable particles. Concentration: a = 40 µg; b = 20 µg; c = 10 µg.

The results of the *Salmonella*/microsome assay for frameshift mutagens in organic extracts showed the presence of mutagenic compounds throughout the studied period (Table 2). The PM2.5 extracts produced positive responses for all months except for a single indicative response (December/09-TA98). However, more than 50% of indicative and negative responses were observed in the TSP extracts. In the assays performed with S9 mix, decreased, similar and increased mutagenic response were observed. The same pattern of direct and indirect responses occurred in TSP and PM2.5 fractions, except for the December/09 samples, when direct action compounds prevailed in TSP and indirect ones in PM2.5.

Table 2 – Mutagenicity in revertants/m³ of organic extracts of atmospheric particulate matter in an urban-industrial area (Site 1)

Sample	TSP		PM2.5	
	TA98	TA98+S9	TA98	TA98+S9
Oct/09	1.56 ± 0.24	0.87 ± 0.18	2.54 ± 0.26	0.98 ± 0.10
Nov/09	—	0.85 ± 0.25•	1.36 ± 0.24	2.29 ± 0.38
Dec/09	0.72 ± 0.12	—	0.27 ± 0.10•	0.47 ± 0.06
Jan/10	—	—	1.49 ± 0.33	1.49 ± 0.25

Values represent mean ± deviation of revertants per m³ of air for samples that are positive and indicate mutagenic activity – indicates negative responses for mutagenesis. • indicates indicative responses for mutagenesis. TSP = Total Suspended Particles. PM2.5 = Fine inhalable particles. Negative control (DMSO): 31.50 ± 15.29 (TA98); 41.69 ± 19.23 (TA98+S9); 64.36 ± 16.08 (YG1021); 46.70 ± 7.94 (YG1024). Positive Control: 4NQO (TA98) 785.28 ± 152.01; 2AF (TA98+S9) 614.20 ± 273.90; 2NF (YG1021) 3955.14 ± 1687.55; 2NF (YG1024) 3312.67 ± 2321.43.

Figure 4 shows the mutagenicity responses of the organic extracts for all strains. The revertant/μg values referring to all months and strains studied, were compared between TSP and PM2.5, showing a significant difference ($F_{1,15}$=36.05 $p<0.001$) with a higher mutagenic potential associated with PM2.5.

The YG family strains allowed the identification of nitrogenated compounds in particulate material of both diameters at Site 1. The predominance of nitroarenes was detected by the larger number of revertants /μg in strain YG1021 throughout the studied period (Figure 4). However, high mutagenesis values were also observed in strain YG1024 compared to its parental TA98 of up to 4.7 times higher. This response also shows the presence of dinitroarenes at the site.

PM2.5 organic extracts were also obtained from the residential area (Site 2) from October to December 2009 (Table 3), enabling comparison with the urban-industrial area (Site 1) results (Table 2). There was a very low cell survival at Site 2, especially in the absence of liver metabolization. Mutagenic potential of samples from Site 1 was the highest in all strains and periods evaluated, except for the December /09 sample for TA98 and YG1021 (Table 4). However, there was no statistically significant difference between the two sites,

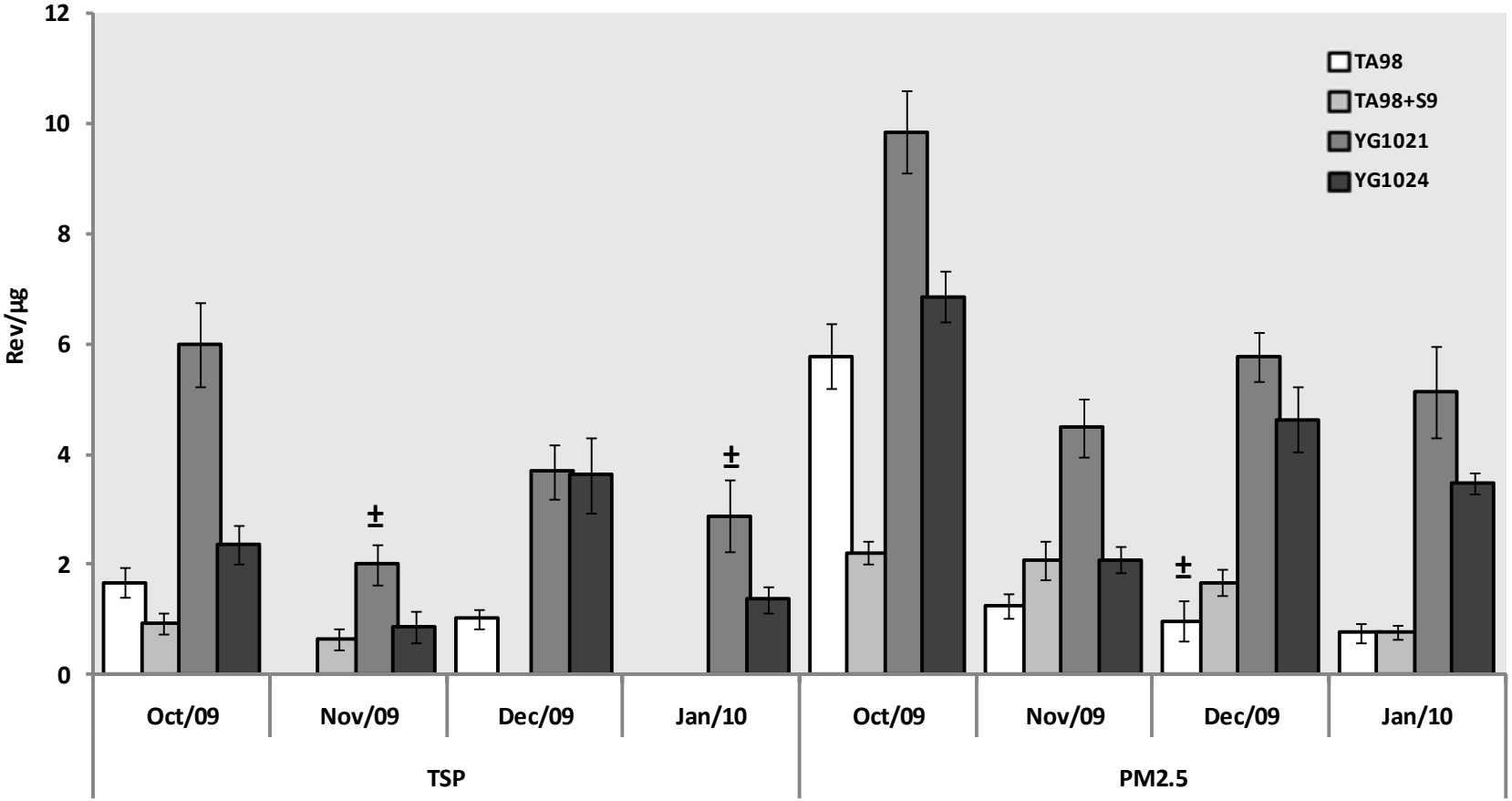

Figure 4 – Mutagenicity (revertants/µg) of organic extracts of atmospheric particulate matter in an urban-industrial area (Site 1).

± shows indicative responses. TSP = Total Suspended Particles. PM2.5 = Fine inhalable particles. Negative control (DMSO): 31.50 ± 15.29 (TA98); 41.69 ± 19.23 (TA98+S9); 64.36 ± 16.08 (YG1021); 46.70 ± 7.94 (YG1024). Positive Control: 4NQO (TA98) 785.28 ± 152.01; 2AF (TA98+S9) 376.40 ± 311.38; 2NF (YG1021) 3955.14 ± 1687.55; 2NF (YG1024) 3312.67 ± 2321.43.

considering the mutagenic potential. Despite this, samples from Site 2 were the only ones that presented negative responses, and also a higher frequency of indicative results of mutagenicity. At Site 2, there was an outstanding contribution of nitroarenes to the responses of the nitrosensitive strains, although the presence of dinitroarenes was also detected, similar to Site 1 results.

Table 3– Mutagenicity and cytotoxicity of PM2.5 organic extracts in an urban-residential area (Site 2).

Sample	Mutagenicity[a]		Citotoxicity[b]	
	TA98	TA98+S9	TA98	TA98+S9
Oct/09	–	1.39 ± 0.23	73.3	61.9
Nov/09	–	0.78 ± 0.12	24.7	60.2
Dec/09	1.88 ± 0.56	0.37 ± 0.15•	25.6	52.5

[a] Values represent mean ± deviation of revertants/m³ of air for positive samples indicative of mutagenic activity . — indicates negative responses for mutagenesis. • indicative responses for mutagenesis. [b]Percentage of cell survival in the concentrations of40µg. Values below 60% indicate toxic responses. Negative control (DMSO): 41.50 ± 11.99 (TA98); 48.33 ± 1.15 (TA98+S9); 89 ± 1.41 (YG1021); 38± 3.61 (YG1024). Positive Control: 4NQO (TA98) 920.50 ± 3.53; 2AF (TA98+S9) 1517 ± 120.21; 2NF (YG1021) 4062 ± 794.80; 2NF (YG1024) 1624 ± 79.19.

Table 4 – Mutagenicity in revertants/µg of PM2.5 organic extracts in an urban-industrial (Site 1) and urban-residential (Site 2) area

Sample	Site 1				Site 2			
	TA98	TA98+S9	YG1021	YG1024	TA98	TA98+S9	YG1021	YG1024
Oct/09	5.77 ± 0.59	2.22 ± 0.22	9.85 ± 0.76	6.85 ± 0.46	–	1.91 ± 0.32	3.64 ± 0.84•	3.40 ± 0.30
Nov/09	1.24 ± 0.22	2.08 ± 0.35	4.49 ± 0.52	2.09 ± 0.24	–	0.89 ± 0.14	2.58 ± 0.42	1.17 ± 0.32•
Dec/09	0.98 ± 0.36•	1.68 ± 0.23	5.76 ± 0.45	4.64 ± 0.59	4.00 ± 1.20	0.78 ± 0.33•	9.01 ± 0.82	3.03 ± 0.80

Values represent mean ± deviation of revertants/µg of air for positive samples indicative of mutagenic activity –. ― indicates negative responses for mutagenesis. • indicative responses for mutagenesis. Negative control (DMSO): 56.82 ± 58.42 (TA98); 42.74 ± 17.73 (TA98+S9); 68.15 ± 17.35 (YG1021); 44.69 ± 8.00 (YG1024). Positive Control: 4NQO (TA98) 815.33 ± 144.52; 2AF (TA98+S9) 566.50 ± 527.03; 2NF (YG1021) 3978.89 ± 1488.97; 2NF (YG1024) 2890.50 ± 2112.17.

Chemical analysis of the aqueous extracts was performed in the samples from October/09 (spring) and January/10 (summer) (Table 5). The highest concentrations were observed for zinc in the samples of PM2.5. Chromium, Arsenic and Cadmium were not detected in any sample. Aluminum, Iron and Copper were found at higher concentrations in the TSP extracts, while Vanadium and Nickel presented similar concentrations in both particulate fractions.

Table 5 – Concentration of metals in aqueous extracts of TSP and PM2.5 in an urban-industrial area (Site 1).

	TSP		**PM2.5**	
	Oct/09	**Jan/10**	**Oct/09**	**Jan/10**
Aluminum	4.29	7.66	<LD	0.86
Vanadium	1.43	0.70	0.79	0.86
Chromium	<LD	<LD	<LD	<LD
Iron	2.38	4.18	<LD	0.86
Nickel	<LD	1.39	0.79	0.86
Copper	2.38	4.18	1.19	0.86
Zinc	<LD	<LD	14.69	35.14
Arsenic	<LD	<LD	<LD	<LD
Cadmium	<LD	<LD	<LD	<LD
Lead	<LD	<LD	0.40	<LD

Concentration of metals in ng/m³. TSP = Total Suspended Particles. PM2.5 = Fine inhalable particles. <LD = below the detection limit.

4. DISCUSSION

The present study used biomarkers to evaluate mutagenicity and cytotoxicity comparing the responses obtained for total suspended and fine inhalable particles for areas under impact of urban and industrial activities. Even at low concentrations, the exposure to atmospheric particulate matter can cause biological damage. Thus the evaluation of air quality from environments under anthropic impact should be complemented with studies of biological approaches. In this context, the use of assays that indicate changes at a genetic level allows the early detection of environmental contaminants, enabling preventive measures to minimize the damage caused by pollution.

The determination of TSP and PM2.5 concentrations in the present study showed that most filters sampled are in accordance with the maximum values allowed by Brazilian law or recommended by WHO, with only three filters going beyond these limits. Concentrations of suspended particles are used worldwide as parameters to evaluate air quality. Limits for the presence of this pollutant in the atmosphere were proposed by several regulating agencies and non-governmental organizations and the maximum values allowed are constantly reviewed and modified. In Brazil, the law regulating the maximum concentration of atmospheric particulate matter is from 1990 with limits only for TSP and PM10. These limits are based on 24h samplings, determining primary standards (240 µg/m³ - TSP or 150 µg/m³ - PM10) and secondary ones (150 µg/m³ - TSP and PM10). Currently in Brazil there are no PM2.5 regulatory parameters. For this reason, the present study adopted the recommendation of 25 µg/m³ suggested by the World Health Organization (WHO, 2006) as a parameter for these particles.

Although most filters studied are above the limits mentioned previously, all samples presented some potential for mutagenicity in the organic fraction. Studies previously performed in the state of Rio Grande do Sul also found a positive mutagenesis in organic extracts of atmospheric particulate matter which were within the legal parameters allowed (Coronas et al., 2008; 2009; Ducatti and Vargas, 2003; Pereira et al., 2010). These data justify the need to include biological parameters in air quality evaluation, thus complementing the diagnoses for environmental management actions in polluted areas.

Other major aspects to be taken into account in the concentration of atmospheric particles are the meteorological conditions that influence transport, dispersion, photochemical

reactions and pollutant deposition. In this study the concentrations of total suspended particles correlated negatively with precipitation, although this was not seen for PM2.5. It was also found that the PM2.5 concentration was negatively correlated with humidity only at Site 2. Wojas and Almquist (2007) suggested that this relation to rainfall can be explained based on the higher susceptibility of larger particles to be carried by rain than smaller ones. Also, water vapor adsorption in the particles could increase their sedimentation rates. This could be enough to diminish the mass observed in the atmosphere according to increased humidity.

Two fractions of particulate matter were evaluated in the urban-industrial area to investigate their mutagenicity and composition. Comparison of the mutagenesis of TSP and PM2.5 organic extracts from the city of Rio Grande showed a higher potency associated with the fine inhalable fraction. This relation has often been mentioned in the literature, with evidence that mutagenicity tends to increase according to the decrease in the aerodynamic particle size (Pagano et al., 1996).

Most PM2.5 extracts presented similar responses to those of TSP extracts, although higher. However, in December/09 a different mutagenicity profile was found between the two fractions, showing that TSP and PM2.5 had a different organic composition in this sample. This may be related to variation in the emissions of the different local sources. Carvalho-Oliveira et al. (2005) showed that the modification of a single source of particle emissions altered the PM2.5 mutagenic potential in an urban area.

Pereira et al. (2008) evaluated the TSP mutagenicity of organic extracts in an area under the influence of atmospheric emissions from the industrial district of Rio Grande. Mutagenic activity verified by these authors for the same season presented similar values to those of the present study, both in the absence (1.6 rev/m^3) and presence (0.2 rev/m^3) of metabolization. Higher values were observed in winter, the rainy season. Higher responses of PM2.5 verified in the present study, reflect the composition of these particles which are comprised mainly by high mutagenic compounds, such as hydrocarbons (De Kok et al., 2006).

Organic solvents for extractions of atmospheric particulate matter are widely used to investigate genotoxic compounds associated with particles. There are a variety of solvents available and many extractions techniques have been described (Claxton et al., 2004; Claxton and Woodall, 2007). However, few studies have evaluated the genotoxicity of the inorganic compounds associated with PM (Claxton and Woodall, 2007). Different aspects of this PM

inorganic fraction were investigated using acid and aqueous extractions (Park et al., 2008; Heal et al., 2005). In this study a sequential extraction procedure was performed, with an aqueous stage carried out after extraction of the organic compounds of the sample. Aqueous extraction was preferred because it allows the analysis of compounds considered more easily bioavailable (Cavanagh et al., 2009), and also because they can be mobilized by rainfall. Moreover, the extraction of soluble compounds in water can be compared to the substance mobilization process that occurs in pulmonary cells (Calcabrini et al., 2004; Hetland et al., 2004). In an attempt to obtain results even closer to the pulmonary processes, some studies chose to use saline solutions (Voutsa and Samara, 2002), but Heal et al. (2005) argue that the procedure using water is simpler and more universal, besides avoiding the introduction of chemical variables.

None of the aqueous extracts evaluated presented any mutagenic activity in the *Salmonella*/microsome assay. However, 50% of these samples presented cytotoxicity, which occurred with a greater frequency that in the organic extracts. Cavanagh et al. (2009) used the neutral-red uptake assay in a mouse macrophage cell line (RAW-264.7), to evaluate the cytotoxicity of organic and aqueous extracts of particulate matter. Similarly to the present study, the authors observed a greater frequency of cytotoxicity in the aqueous extracts, besides toxic responses of the organic fraction that occur at much lower concentrations than those found for aqueous extracts. Also, toxic responses without metabolization were already observed in leukocytes and lymphocytes exposed to aqueous suspensions of PM10 (Brits et al., 2004).

In this study the effects provoked by the organic extracts occurred at lower doses than those found in the aqueous fraction. Moreover, mutagenic responses were found only for the organic fraction. The joint analysis of these data indicates that the organic components were more important in the induction of biological damage.

As to the chemical analysis of the aqueous extracts, higher concentrations of aluminum, iron and copper were found in total suspended particles. Sato et al. (2007) also found these metals predominantly in the TSP and PM10 particles compared to PM2.5. Lead and zinc were detected only in the fine inhalable particles, with zinc presenting the highest concentrations among all metals evaluated in both particulates diameters. Lead was already found at a higher proportion in the fine fraction (Sato et al., 2007; Vanz et al., 2003; Wojas and Almquist,

2007) and zinc with a similar distribution in both fractions, but with greater solubility in the fines (Sato et al., 2007). The nickel and vanadium elements were found in similar amounts in TSP and PM2.5. The anthropic emission of these elements was related to the petrochemical industry and fuel burning (ATSDR, 2005; 2009; Bradl, 2005; Fernández-Espinosa et al., 2002). These are major sources in the studied city. Chromium, arsenic and cadmium were not detected in any of the aqueous extracts evaluated. The absence of chromium can be ascribed to its predominantly insoluble characteristic, but the same does not apply to arsenic and cadmium which are highly water soluble (Beni et al., 2010; Sato et al., 2007).

The concentrations of metal elements evaluated in this study concern the available fraction that varies according to metal solubility. The values of these fractions are lower than the total concentration of these elements, the latter being the most commonly reported in the literature. Generally, metals from anthropogenic sources exist mainly in easily water-soluble forms (Fernandez-Espinosa et al., 2002). Metal in anthropogenic particles consists of metal-dominated abrasion or hot-vapor condensation particles or metals that have condensed onto the surface of other particles, and thus tend to be more labile than metal bound within crustal material (Heal et al., 2005).

Other studies were performed to evaluate the air quality in the city of Rio Grande during a period concomitant to this study (FEPAM, 2010). Part of this evaluation used the quantification of metal elements (lead, copper, cadmium and arsenic) in PM10 and PM2.5 samples after a hot acid extraction process. As expected, the monthly concentrations of metals obtained were a lot higher than the values found in the aqueous extracts of this study. The mean PM2.5 values, based on four months of samplings (October/2009 to January/2010) were 4.80 ± 3.33 μg/m³ for copper, 1.60 ± 0.93 μg/m³ for lead, 0.11 ± 0.09 μg/m³ for cadmium and 0.07 ± 0.06 μg/m³ for arsenic. As to these metal groups, in the aqueous extracts evaluated in this study, copper (TSP, 4.18 ng/m³; PM2.5, 1.19 ng/m³) also had the highest concentrations, followed by lead (PM2.5, 0.40 ng/m³).

Although the *Salmonella*/microsome assay produced only negative responses for mutagenicity in the evaluation of aqueous extracts, there were cytotoxicity effects. Reactivity with DNA has been reported for elements detected in these samples. According to the agency for toxic substances and disease registry (2004, 2005, 2007a, 2007b, 2008, 2009), aluminum, vanadium, copper and lead showed *in vitro* and *in vivo* genotoxicity, especially in eukaryote

organisms. Nickel and zinc presented evidence of clastogenicity. The release of these potential bioavailable elements based on particulate matter is important, since the main exposure route to atmospheric particles occurs during breathing at the air/pulmonary fluid interface (Voutsa et al., 2002).

Few studies were performed to characterize the fine inhalable particulates in Brazil. Although studies investigated physical and chemical aspects of their composition (Braga et al., 2005), no study evaluated PM2.5 mutagenicity in Brazil. In order to characterize this, the organic extracts of PM2.5 in the urban industrial area (Site 1) were compared to samples collected during the same period in a residential area (Site 2) in the city. Positive responses for mutagenicity were observed at both sites, but about 83% were higher at Site 1, showing worse air quality there.

The YG family strains enabled the identification of nitro-PAHs presence in all samples from the urban-industrial and residential area. Nitro –PAHs are powerful mutagenic agents in bacteria and mammals, and do not need to be metabolically activated (Pereira Netto et al., 2000). These compounds are associated with combustion sources, but may also occur as a result of secondary reactions between PAHs and nitrogen oxides in the atmosphere (Zwozdziak et al., 2001). All organic extracts showed an revertants /µg increment in strains YG1021 and YG1024 compared to the parental TA98, indicating the existence of a mixture of mono and dinitroarenes. This increment in the mutagenic potential was up to 6.6 times greater in the YG1021 strain, and up to 4.7 times in YG1024.

PM Investigations in petrochemical industrial areas have shown the greater sensitivity of strain YG1024 to detect mutagenicity (Coronas et al., 2009; Pereira et al., 2010), although an alternance with YG1021 has also been observed depending on the period studied (Coronas et al., 2008). On the other hand, responses indicating the preponderant presence of mononitroderivatives (YG1021) have been observed in urban areas (Ducatti and Vargas, 2003; Kaffer, 2011; Vargas et al., 1998; 2003). Pereira et al. (2008), in a previous study performed in the city of Rio Grande also showed a mixture of nitrocompounds, since there was a small difference in the mutagenic potential among strains YG1021 and YG1024, but with a preponderance of dinitroarenes. Hence, the use of these two strains is appropriate to evaluate areas where there are both industrial and urban atmospheric contaminants.

TA98 strain is appropriate for the environmental diagnosis of impacted areas due to its capacity to detect mutagenics belonging to a wide class of compounds. Mutagenicity without metabolization of this strain is related to the presence of nitro-PAHs (Claxton and Woodall, 2007). However, it should be emphasized that the use of nitro-sensitive strains allowed detecting the mutagenic potential of some samples that presented negative responses in the parental strain. Therefore, it is important that, whenever possible, specific strains to chemical compounds of interest in the investigated area be included in studies on environmental diagnosis and monitoring.

5. CONCLUSION

The comparative investigation between organic and aqueous extracts of TSP and PM2.5 showed a higher risk of exposure associated with the organic fraction of the fine particles. The extraction methodology used allowed identifying the presence of potential bioavailable metal compounds. The *Salmonella*/microsome assay was not sensitive to mutagens possibly present in the aqueous concentrations extracted, although the associated cytotoxicity shows the presence of substance with an adverse effect. The strategy used allowed defining the anthropic influence of groups of compounds characteristically found in urban and industrial areas.

This study presents a first characterization of mutagenic substances in inhalable particles using the *Salmonella*/microsome assay in Brazil. Application of this approach in areas with different anthropic influences will favor the adoption of preventive actions in the health/environment relation.

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3.2. ARTIGO 2

MUTAGENICITY IN URBAN INDUSTRIAL AREAS: CONTRIBUTION OF ACID RAIN AND ORGANIC POLLUTANTS

Artigo a ser submetido para *Science of The Total Environment*

Co-autores: Jocelita Aparecida Vaz Rocha[a]; Vera Maria Ferrão Vargas[a,b]

[a]Programa de Pesquisas Ambientais, Fundação Estadual de Proteção Ambiental Henrique Luís Roessler (FEPAM), Avenida Salvador França, 1707 CEP: 90690-000 Porto Alegre, RS, Brazil.

[b]Programa de Pós-graduação em Ecologia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500, CEP 91501-970, Porto Alegre, RS, Brazil.

ABSTRACT

The purpose of this study was to assess the mutagenicity of soils and atmospheric particulate matter in areas under urban and industrial influence. The effect of the pH of rains on the availability of compounds in soil was also investigated, as well as the presence and distribution of mutagenic organic compounds in soil and air. Surface soil samples (0-5 cm) and atmospheric particulates (PM2.5) were collected at two sites, one urban-residential and another urban-industrial. The soils were assessed as to total grain size composition and in fraction <0.5 mm, through acid extracts with different pHs simulating those found in local rainfall. Metals were quantified in these extracts. Organic extracts of these soils and of PM2.5 were also analyzed. Extract mutagenicity was analyzed using the *Salmonella*/microsome assay, microsuspension method, in strain TA98, in the presence/absence of hepatic metabolization. The nitro-sensitive strains YG1021 and YG1024 were also used in the organic extracts. The results showed distinct biological responses in the samples of total soils and in fraction <0.5 mm, suggesting that the use of minimally altered soil is more appropriate for mutagenesis assays. Soil extraction at pH 3.6 presented greater toxicity, variety and concentration of metals. Extraction at pH 5.3 improved the detection of mutagenic compounds. Thus, rainfall in the region may be an environmental contamination route, with an additional risk of releasing toxic substances during acid precipitation events. Mutagenic activity was found in all samples of PM2.5, which, together with the responses obtained in soils, helped improve the characterization of anthropic influence at the two sites.

Key-words: Soil genotoxicity, PM2.5, Bioavailable compounds, Leached extracts, Organic compounds.

1. INTRODUCTION

Historically, soil quality assessment prioritized productivity measures for agronomic aspects. However, the importance of this environmental compartment goes beyond food production. It acts as a filter for several substances, a deposit and supplier of water, as well as a medium to degrade wastes and a source of nutrients and gases through biological activity (Doran and Zeiss, 2000; Stenberg, 1999). Hence, a broader concept of soil quality or health was suggested by Doran and Parkin (1994), as being the capacity of a soil to support biological productivity, maintain environmental quality and promote the health of plants and animals, within the ecosystem limits.

Industrial, urban and agricultural activities release many chemical substances into the atmosphere, soils and water bodies which may compromise environmental quality. Soil contamination by toxic substances may be the result of intentional or accidental activities, by applying agricultural chemicals on crops, waste disposal in specific areas (landfill, treatment lagoons, etc.), the indiscriminate disposal of materials and leakages/spills during production, transport or storage of industrial materials (White e Claxton, 2004).

Besides these forms of direct contamination, compounds emitted into the atmosphere are sometimes deposited in the soil, and can accumulate in its surface layer (Watanabe et al., 2008). Atmospheric particulate matter (PM) is outstanding among air pollutants, because it represents a complex mixture of a wide diversity of organic and inorganic substances that remain in suspension. Particles with an aerodynamic size of up to 2.5 µm (PM2.5) are constituted primarily by metals and hydrocarbons, the main mechanisms in their formation being the combustion processes and secondary reactions in the atmosphere (De Kok et al., 2006; Squadrito et al.,2001).

Contaminant exchange on the soil-atmosphere interface involves dry and wet deposition processes from air to soil, and volatilization from soil to air (Cousins et al., 1999). The following compounds involved in this process were studied: polycyclic aromatic hydrocarbons (Wang et al., 2008), the organochlorated pesticides (Harner et al., 2001) and polychlorinated biphenyls (Cousins and Jones, 1998).

Besides volatilization, the substances present in the soil can also be mobilized by leaching and surface runoff processes (Silva-Júnior and Vargas, 2009), and may contaminate

ground and surface waters (Chakraborty and Murkherjee, 2009; Ohe et al., 2004). Rain water is an important factor in these processes, and it is characterized as a contamination route (Holt, 2000). Washing the contaminants present in the atmospheric particulate matter can lead to acid rain formation, a common event in areas located close to large industrial areas (Holtz, 2000; Mirlean et al., 2000). Acid conditions may increase the bioavailability of metals which become more easily dissociated, and may take on toxic forms (Pueyo et al., 2003; Tack and Verloo, 1995).

Soil contamination affects the health of the ecosystem and acts directly on the loss of biological diversity (Gilmore, 2001). Compounds, in the soil, with toxic characteristics can also affect human health by exposure through the inhalation of dusts, ingestion of plants and animals that have absorbed these compounds, or else, by leaching into ground and surface waters used for supply (Watanabe e Hirayama, 2001).

Some of these substances have genotoxic effect, as reported in various assessments performed on industrial, urban, residential, agricultural and forest soils (Courty et al., 2008; Pohren, 2011; Silva-Júnior and Vargas, 2009; Watanabe et al., 2008). Among the residues that contaminate soils are heavy metals and organic compounds. The polycyclic aromatic hydrocarbons (PAHs) are widely distributed organic compounds that form when biomass is burned, both from natural and from anthropogenic sources, and they are intensively studied due to their mutagenic and carcinogenic properties (Barra et al. 2007). Studies have shown that the soil is a PAH reservoir (Wang et al., 2008). Differently from the organic toxic compounds, metals are not biodegradable and may remain for a long time in the soil due to the high adsorption to the humic and clay colloids in the soil (Iwegbue et al., 2009). Some of these elements, such as arsenic, cadmium, lead, chromium and nickel have a mutagenic and/or clastogenic effect (Tsalev and Zaprianov, 2000; White and Claxton, 2004).

It is not easy to assess genotoxic compounds in soils, since, as in the other environmental matrices there are a great variety of substances present. When these chemicals interact with each other and with the environment, they may present different toxic characteristics from those of their original constituents alone. Moreover, soil characteristics such as texture, amount of organic matter, spatial heterogeneity and microbial activity provide further challenges for the interpretation of results (White and Claxton, 2004).

Since these mixtures are complex, assessment using traditional chemical and physical analyses is a limited approach to forecast their toxicity. For this purpose, assessment using bioassays allows detecting the biological effect that integrates all the components of the mixture, even without identifying the compounds present. According to reviews of literature, the *Salmonella/*microsome assay is the most used method to investigate mutagenicity in soils (Watanabe and Hirayama, 2001; White and Claxton 2004) and atmospheric particulate matter (Claxton et al., 2004).

The present study used the *Salmonella*/microsome assay, associated with chemical analyses, to assess urban and industrial influences through *(i)* analysis of the effect of different acid extractions, simulating changes in the background pH of rain, that can act on the availability of compounds present in the soil *(ii)* analysis of the presence of mutagenic organic compounds and their distribution in soil and air.

2. MATERIAL AND METHODS

2.1. Area of study

The city of Rio Grande is located in the extreme south of Brazil, in the estuarine area of Patos Lagoon, close to the mouth where it flows into the Atlantic Ocean. The city, now with 2710 km^2 (IBGE, 2009), was formed in lowlands, where terrain was gradually created by landfill in areas that were originally flooded. The city began to be occupied in 1735, and since then there were six historical periods of landfill formation. In this process, different materials produced at the time of construction were mixed to the sandy sediments. It comprises different types of residues, industrial and domestic wastes and rubble (Mirlean e Oliveira, 2006).

The municipality has a major industrial complex, characterized mainly by the manufacture of fertilizers, chemicals and agrochemicals, food, fish, extraction and refinery of vegetal oil, production of wood resins and an oil refinery. There are also the metal-mechanical, textile and naval sectors, besides an important port area, thermopower plants, and a solid waste incinerator. Residential developments have been built in the vicinity of these companies.

Mirlean et al. (2000), studied the physical-chemical parameters of rainfall in this city for over 12 months. The authors observed acid rains that occurred due to atmospheric emissions from the industrial park. The lowest pH found was 3.6. The background pH value found for the region was close to 5.2.

2.1.2. Sampling sites

Two sites with distinct characteristics (urban-industrial and urban-residential) were selected for sampling (Figure 1). At each of them soil and atmospheric particulate matter (PM2.5) were collected. The soil samplings occurred in August/2010, and PM2.5 was collected in July and August/2010.

- Site 1 – Urban-industrial area (32°2’32.98”S, 52°5’16.84”W):

- Soil: located in a landfill area which dates from 1936-1937. The soil at the collection site was characterized by Garcia et al. (2010) as an area under the influence of emissions from the local oil refinery.

– PM2.5: Located in the second quadrant of preferential atmospheric dispersion from an oil refinery, where air quality is also monitored by the State Environmental Agency (FEPAM).

- Site 2 – Urban –residential area (32°2’52.23”S, 52°8’24.75”W):

– Soil: Urban area with residential characteristics, located in the original emerged area of the city, without landfills.

– PM2.5 – Urban area with residential characteristics and far from the main industrial parks of the city. However, it is not completely free of the atmospheric dispersion plume from anthropic sources in the municipality.

Climate data observed during the study period were provided by the 8th District of the National Institute of Meteorology (8° DISME – Porto Alegre/INMET, Brazil). They show mean values for precipitations of 3.66 ± 11.02 mm, for a humidity of 81.50 ± 7.77% and for temperature of 13.48 ± 3.69°C.

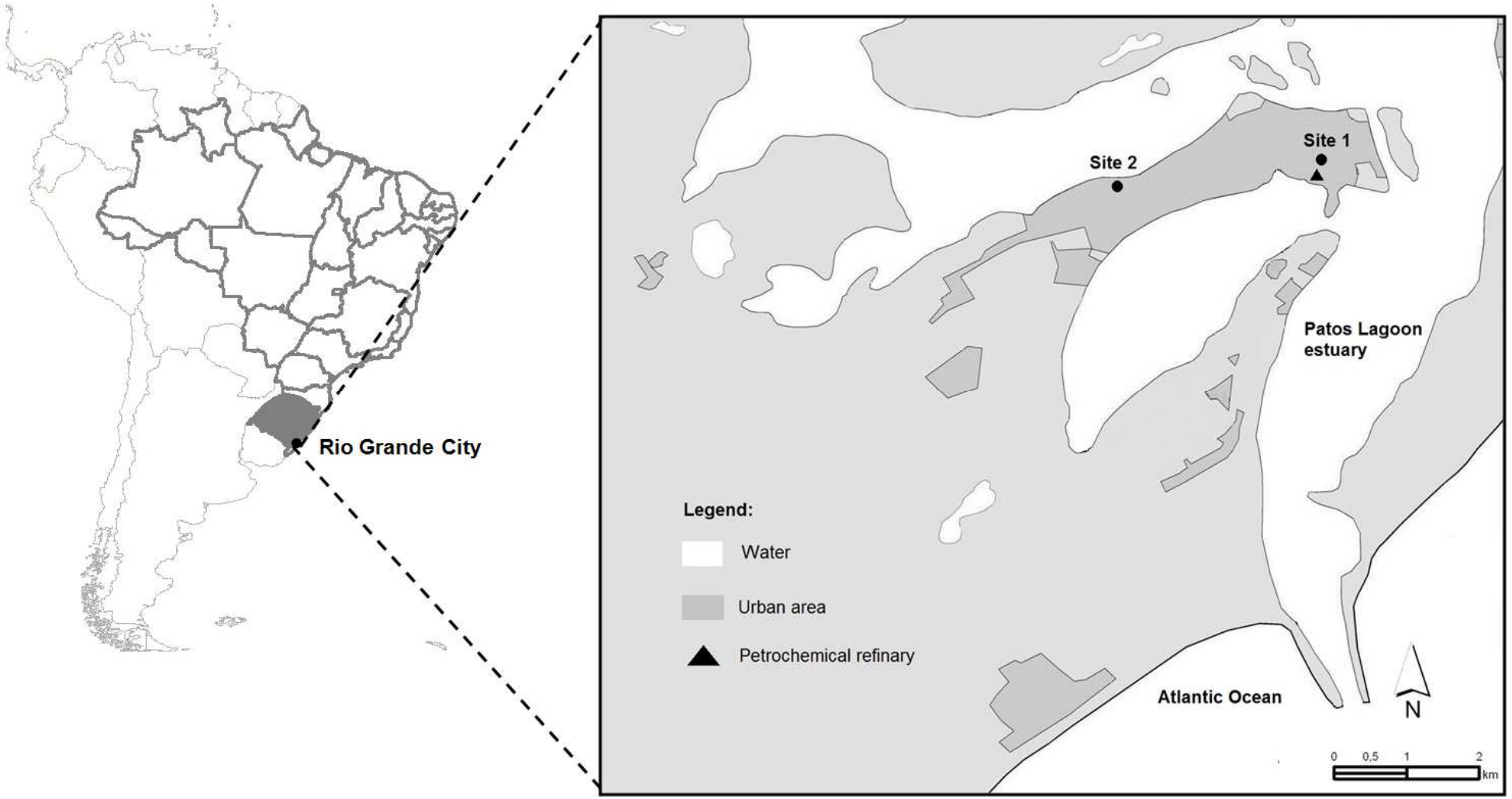

Figure 1 – Location of the study area in the city of Rio Grande, Brazil. Collection sites: Site 1 – urban-industrial area; Site 2 – urban-residential area (Source: FEPAM, 2010; modified).

2.2. Sampling procedures

2.2.1. Soil

At each site, the sampling areas were demarcated as triangles with 20 m sides. Soil sampling was performed in the first centimeters of the surface layer (0-5 cm), at the points corresponding to the triangle vertexes, generating three subsamples. These were homogenized resulting in a sample composed of approximately 1 kg per site. Excess plant and stone residues were removed. The samplings were performed after 7 days without rain. Stainless steel spatulas were used for sampling, and glass flasks to store the samples that remained protected from light at 4°C.

In the laboratory, part of the soils collected was immediately stored and called Total Soil. The remainder was sieved in stainless steel mesh, 0.5 mm, and called Soil <0.5. The sample drying process was not carried out to prevent possible loss or contamination of the compounds (Cousins et al., 1997). The samples were kept at -20°C and protected from light until the time of extraction.

Grain size analysis of the soils was performed at the Sedimentology Laboratory of the Center of Studies on Coastal and Ocean Geology (CECO) of the Federal University of Rio Grande do Sul. The sieving method according to Wentworth (1922) and Krumbein (1934) was used to separate the coarser fractions. The fine fractions were separated by the pipetting method, based on the Stokes Law of sedimentation (1851). Soil characterization analyses were performed in the Laboratory of Environmental Analyses at the Federal University of Rio Grande do Sul, Brazil. The percentages of organic matter and organic carbon, as well as the cation exchange capacity (CEC) were higher in the samples from Site 2 and in the samples of Total Soils (Table 1). The pH values were similar in all samples, varying from 5.6 to 5.9.

Table 1 – Characterization of the soils sampled

		O.M[a]	O.C[b]	pH	CEC [c]
		%	%		$cmol_c/dm^3$
Site 1	Total Soil	3.6	2.1	5.6	10.6
	Soil <0.5	3.2	1.9	5.8	10.2
Site 2	Total Soil	4.3	2.5	5.9	12.9
	Soil <0.5	3.4	2.0	5.9	12.1

[a] O.M.. = organic matter by wet digestion; [b] O.C. = organic carbon (Walkey Black – LD: 0.01%); [c] CEC = cation exchange capacity at pH 7.0.

2.2.2. Atmospheric particulate matter – PM2.5

Atmospheric particulates up to 2.5 μm (fine inhalable particles - PM2.5) were collected using high volume air samplers (AGV MP2.5 1200/CCV Thermo Environmental Instruments), in Teflon membrane filters (TX40HI20WW, 254X203mm). The samplings occurred for 24 hours in the first and second fortnight of each month. The air volume that passed through each filter varied from 1343.8 to 1589.9 m^3, with mean values of 1517.7 ± 82.6 m^3. Before and after the samplings, the filters were weighed and stabilized, under controlled temperature and humidity conditions, to determine the concentration of PM2.5, expressed in units of $\mu g/m^3$ of air sampled (ABNT, 1997). The filters sampled were kept protected from light, at -20°C, for the extraction procedures.

2.3. Extraction procedures

Acid and organic extracts were obtained from the samples of Total Soil and Soil <0.5, while in the atmospheric particulate only organic extraction was performed.

2.3.1. Acid extraction of soils

For acid extraction from the soil samples, solutions of acetic acid (CASRN. 64-19-7) and sodium hydroxide (CASRN. 1310-73-2) were used, prepared according to the Brazilian standard NBR10005 (ABNT, 2004), modified for pH 3.6 (pH of the more acid rains at the study location); and pH 5.3 (background pH of the rain at the study area). Each of these

solutions was used individually, resulting in two acid leachate extracts per sample. Leachates were obtained submitting the proportion of 1 g of soil: 2mL of solvent to agitation, for 24h at 20°C, on a shaking table, and then centrifuging at 13,000xg for 15 min at 4°C as described in Silva-Júnior and Vargas (2009). After filtration in a 0.45 µm, the leached extracts were divided into aliquots and stored in the dark for up to 24 h to be used in the *Salmonella*/microsome assays and for metal determination.

2.3.2. Organic extraction of soils

The soil samples were homogenized for 15 minutes and extracted (15 g of dry weight) using pesticide grade (DCM, CASRN. 75-09-2) dichloromethane solvent (200 mL/cycle), in ultra sound for two 10 minute cycles. The resulting extracts were filtered in a chromatographic column of sodium sulphate and celite, and concentrated in a rotavapor at 40°C. Then they were stored protected from light at -20°C, for up to 30 days, in order to perform the mutagenicity assay. The amount of extracted organic matter from these samples (EOM) was calculated dividing the total organic matter by the respective volume of soil extracted.

2.3.3. Organic extraction of PM2.5

A quarter of each filter containing the atmospheric particulate matter was grouped composing monthly samples. Each sample was submitted to extraction in ultrasound (3 cycles of 10 min, 40°C) with dichloromethane solvent (DCM, CASRN. 75-09-2), filtered in a 0.5 µm teflon membrane and concentrated in a rotavapor up to 15 mL, as described in Vargas et al. (1998). The amount of extracted organic matter (EOM) in µg/m³ was calculated from the total amount of organic matter in the extract divided by the respective volume of air sampled. Before the bioassays, appropriate amounts of the extracts were dried with nitrogen gas and re-suspended in dimethylsulphoxide (DMSO, CASRN. 67-68-5).

2.4. *Salmonella*/microsome assay

The different acid and organic extracts produced in the study were evaluated for mutagenicity using the *Salmonella*/microsome assay (Maron & Ames, 1983), microsuspension method (Kado et al. 1983). The classic strain of *Salmonella typhimurium* TA98 was used to evaluate the mutagenic potential of all extracts studied due to its capacity

to detect a broad class of mutagenic compounds, and it is appropriate for environmental studies. These assays were performed in the absence and presence of an exogenous fraction of liver metabolization in mammals (S9 mix), detecting direct and indirect mutagenic substances that cause frameshift errors. In the organic extracts, in addition, the presence of nitroderivate mutagenic compounds was investigated, in the absence of metabolization, through strains YG1021 and YG1024. These strains derived from TA98, presented high activity of the nitroreductase and O-acetyltransferase enzymes, respectively, conferring greater sensitivity on the nitroarenes (YG1021) and dinitroarenes (YG1024) (Watanabe, 1989; 1990).

Based on previous studies by our research group, different concentrations of each sample were tested, generating dose-response curves as follows:

- Soil samples: 25, 50, 75, 100, 150 and 200 mg equivalents of dry soil, for the acid extracts of pH 5.3; 25, 50, 75 mg equivalents of dry soil, for the acid extracts of pH 3.6; 10, 20, 40, 80, 120 and 160 mg equivalents of dry soil for the organic extracts. The control analysis of the acid solutions of pH 5.3 and pH 3.6 was performed for mutagenicity and cytotoxicity in strain TA98. The volumes of solvent needed for each concentration equivalent of soil (1 g of soil: 2 mL of solvent) were tested in the absence of sample. The pH 5.3 solution presented toxicity with 400 µL only in the presence of S9mix, and therefore the 200 mg concentration was excluded in this treatment. The pH 3.6 solution presented toxicity in the larger volumes, leading to the reduction of sample concentrations tested in these extracts, for non-toxic equivalent volumes. In this way, only three concentrations could be tested. The final volumes of acid solutions used in this work were those that did not produce any cytotoxic effect.

- Samples of PM2.5: 1.25; 2.50; 10.00; 20.00 and 40.00 µg/plate – for the organic extracts.

All the assays were performed in duplicate in the presence of negative controls (5 µL solvent Dimethylsulphoxide – DMSO in the organic extracts or 100 µL acid solution in the acid extracts; 100 µL liquid nutrient medium) and positive ones (4-nitroquinoleine oxide-4NQO, 0.5 µg/plate, CASRN. 56-57-5; 2 – nitrofluorene - 2NF, 0.15 µg/plate, CASRN. 607-57-8; and 2- aminofluorene - 2AF, 1 µg/plate, CASRN. 153-78-6 of Sigma Chemical Company, St. Louis, MO) according to the strain and extract tested.

Sample cytotoxicity was determined in a cell survival test in strain TA98, performed in parallel to the mutagenesis assays. In brief, the test consists of diluting the suspension obtained after the incubation stage at 37°C for 90 min (bacterial culture 1-2 x 10^{10} cells/mL + S9 mix or phosphate buffer 0.1M + solvent or sample), in buffer phosphate (pH = 7.4) up to the concentration of 1-2 x 10^2 cells. After dilution the bacterial culture was seeded in nutrient agar and incubated for 72h to 37°C. After this time the surviving colonies were counted manually.

2.5. Analysis of the results

The samples were considered mutagenic when they presented a significant ANOVA ($p < 0.05$) between the dosages and positive dose-response curve ($p < 0.05$). When only the dose-response was significant, the response was considered indicative of a mutagenic effect. The linear portion of the dose-response curves was analyzed by the linear regression models or Berstein (Bernstein et al., 1982). The mutagenic potential of the positive and indicative samples was estimated by the slope of the regression curve and expressed in number of revertants per gram of soil equivalent (rev/g); revertants per µg of PM2.5 extract (rev/µg) and revertants per cubic meter of air sampled (rev/m^3 = rev/µg X EOM), according to the sample analyzed. The statistical analyses were performed using Salanal (*Salmonella* Assay Analysis) Software, version 1.0, from Research Triangle Institute, RTP, NC, USA.

In order to compare the number of revertants obtained in the *Salmonella*/microsome assay between the sites, the blocked analysis of variance (ANOVA) was used, with a level of significance of $p < 0.05$. For this purpose *Software* R (R version 2.9.0., 2009) was utilized.

In the cell survival assay, the samples were considered cytotoxic when they presented diminished growth compared to the negative control in at least one dosage, with values equal or inferior to 60% (Vargas et al. 1993).

2.6. Quantification of metals in acid extracts

Metals (Al, V, Cr, Fe, Ni, Cu, Zn, As, Cd, Pb) were quantified in the acid extracts of soils by inductively coupled plasma optical emission spectroscopy (ICP-OES), with the respective limits of detection (mg/L): Al – 0.08; V – 0.002; Cr – 0.004; Fe – 0.04; Ni – 0.004; Cu – 0.004; Zn – 0.02; As – 0.02; Cd – 0.002; Pb – 0.01. These analyses were

performed in the Laboratory of Environmental Analyses, Federal University of Rio Grande do Sul, Brazil, according to the *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005). The results were expressed in mg/kg of dry soil. Further, to estimate the expected metal concentrations in Soil <0.5 mm, based on the Total Soil measures, a correction was used based on soil grain size (EC = [COT x 100]/ %P, where: EC= Expected concentration; COT= Concentration observed in Total Soil; %P = Percentage of sieved fraction in Total Soil).

3. RESULTS

3.1. Characterization of soil grain size

The grain size classification of soil samples is presented in Figure 2, showing that the soils from Site 1 and 2 are sandy, with predominance of fine sands and low percentages of clay and silt. Soils <0.5 corresponded to the fractions smaller than the coarse sands (0.5 mm), and represented approximately 89% (Site 1) and 98% (Site 2) of the original grain size composition of the soils.

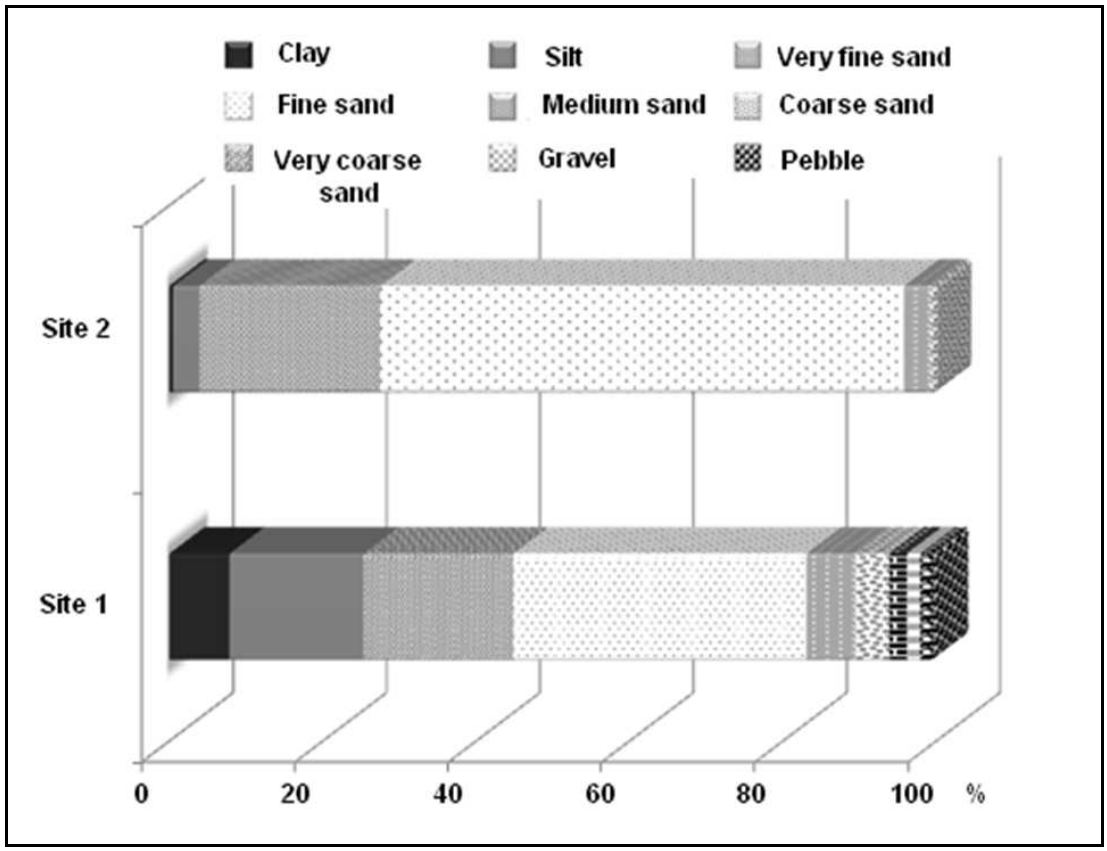

Figure 2 – Grain size classification of the soils sampled

3.2. Acid extractions of the soil at different pHs: mutagenic activity and analysis of metals

The acid extracts of pH 3.6 were more frequently cytotoxic than those of the pH 5.3 solution, both in assays in the presence and in the absence of S9 fraction. In the pH 5.3 extracting solution, the responses were observed only in the presence of metabolization. Generally, cytotoxicity predominated in Soils < 0.5 mm (Table 2).

Table 2 – Percentage of cell survival of acid extracts of soil samples through the *Salmonella*/microsome assay

		Acid extracts							
		pH 5.3				pH 3.6			
		TA98-S9		TA98+S9		TA98-S9		TA98+S9	
		%	Conc.	%	Conc.	%	Conc.	%	Conc.
Site 1	Total Soil	78.3	75	62.4	100	**52.1**	50	70	75
	Soil <0.5	100	200	**56.3**	75	**44.8**	50	**52.6**	75
Site 2	Total Soil	100	200	91.4	25	**55.5**	25	100	75
	Soil <0.5	86.2	150	**59.1**	150	100	75	**29.2**	75

Percentages of cell survival below 60% (in boldface) show cytotoxicity. Cytotoxic samples were recorded at the lowest concentration of toxic effect observed; for non-cytotoxic samples the lowest percentages of survival observed were recorded.

Conc. – concentration. Tested in mg equivalents of dry soil with: 25, 50, 75, 100, 150, 200 – acid extracts pH5.3(with S9 addition the concentration 200 was not tested); 25, 50, 75 – acid extracts pH 3.6.

The results of the mutagenic activity of the acid extracts observed in the pH 5.3 of soils in the urban-industrial and urban-residential areas are shown in Table 3. Considering the presence/absence of metabolization the responses obtained showed a difference in the type of mutagenic action in the Total Soils and Soils <0.5 from Site 1. The greatest mutagenic potentials and diversity of responses were observed in the samples of Site 2, and the

metabolization fraction diminished the mutagenic potency of Total Soil and elevated that of Soil <0.5.

Table 3 – Mutagenicity in revertants/g of dry soil in acid extracts at pH 5.3

		Mutagenicity[a]		Citotoxicity[b]	
		TA98-S9	TA98+S9	TA98-S9	TA98+S9
Site 1	Total Soil	ns	204.65 ± 47.08[i]	–	–
	Soil <0.5	310.66 ± 67.44	ns	–	+
Site 2	Total Soil	905.85 ± 87.54	349.81 ± 69.32	–	–
	Soil <0.5	117.23 ± 43.6[i]	508.96 ± 99.85	–	+

[a] mean ± deviation of revertants/g of dry soil; [i] responses indicative for mutagenesis, ns – non significant; [b] cytotoxicity: +present in at least one concentration, - absent. Negative Control (Acid pH 5.3): 43.55 ± 16.04 (TA98); 52.50 ± 8.78 (TA98+S9). Positive Control: 4NQO (TA98) 838.20 ± 140.58; 2AF (TA98+S9) 422.75 ± 44.85.

The evaluation of acid extracts of pH 3.6 (data not shown) presented a majority of negative responses for mutagenicity with only two indicative responses. These occurred in the Total Soil of Site 1, in the presence of S9 (TA98+S9 = 716.86 ± 291.18), and in the Soil <0.5 of Site 2, in the absence of S9 (TA98 = 177.34 ± 81.81). These responses were higher than those found in the corresponding acid extracts of pH 5.3.

The metal concentrations obtained in the acid extracts are shown in Table 4. Considering both soil extracts, some metals presented higher concentrations, depending on the site studies, namely: copper and nickel at Site 1; zinc, iron and cadmium at Site 2; chromium with a similar occurrence at both sites. Aluminium has a different concentration depending on the extractor solution. It was higher at Site 2, pH 5.3 and at Site 1, pH 3.6. Lead, arsenic and vanadium were not detected. The metal concentrations observed in the soil extracts <0.5 were similar to the values obtained by calculating the correction through grain size. These values were not significantly different from those observed in Total Soils.

In general, higher concentrations and a greater variety of metal elements were seen in the acid extracts of pH 3.6 (Figure 3). A higher occurrence of metal elements was also observed at Site 2 in both extractor solutions.

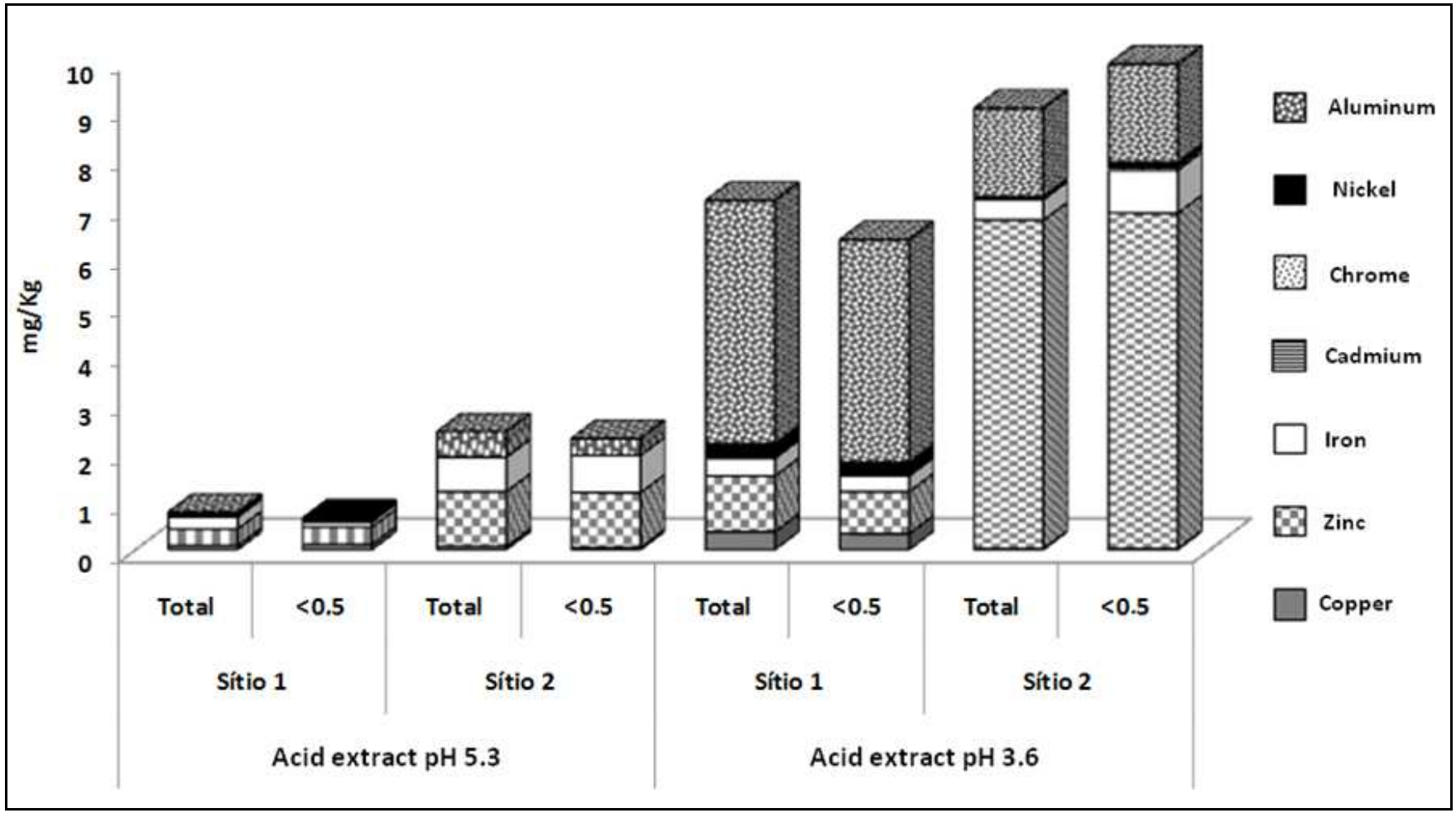

Figure 3 – Sum of metal concentrations in different acid extracts of soil (Total and < 0.5)

Table 4 – Concentration of metals in acid extracts of soils from urban areas in the city of Rio Grande, Brazil

	Acid extract pH 5.3						Acid extract pH 3.6					
	Site 1			Site 2			Site 1			Site 2		
	Total Soil	Soil <0.5	EC*	Total Soil	Soil <0.5	EC	Total Soil	Soil <0.5	EC	Total Soil l	Soil <0.5	EC
Copper	0.08	0.10	0.09	0.06	0.04	0.06	0.36	0.32	0.40	0.02	0.02	0.02
Zinc	0.34	0.36	0.38	1.12	1.12	1.13	1.14	0.86	1.27	6.70	6.84	6.78
Iron	0.24	0.10	0.27	0.70	0.76	0.71	0.36	0.32	0.40	0.42	0.88	0.43
Cadmium	<LD	<LD	-	<LD	<LD	-	<LD	<LD	-	0.02	0.02	0.02
Chrome	<LD	<LD	-	<LD	<LD	-	0.02	0.02	0.02	0.02	0.04	0.02
Nickel	0.06	0.08	0.07	<LD	<LD	-	0.28	0.26	0.31	0.02	0.10	0.02
Lead	<LD	<LD	-	<LD	<LD	-	<LD	<LD	-	<LD	<LD	-
Aluminum	0.04	<LD	0.04	0.52	0.34	0.53	4.96	4.54	5.54	1.8	2.00	1.82
Arsenic	<LD	<LD	-	<LD	<LD	-	<LD	<LD	-	<LD	<LD	-
Vanadium	<LD	<LD	-	<LD	<LD	-	<LD	<LD	-	<LD	<LD	-
Sum	1.06	0.64	0.85	2.70	2.56	2.43	7.40	6.56	7.95	9.28	10.18	9.11

Values expressed in mg/Kg of dry soil. <LD –below the detection limit. Correction by soil grain size: *EC – Expected concentration = [COT x 100]/ %P, where COT= Concentration observed in Total Soil; %P = Percentage of the sieved fraction in Total Soil. Soils <0.5 represent 89% (Site 1) and 98% (Site 2) of the grain size composition of Total Soils.

3.3. Extractions of organic compounds in soils and in atmospheric particulate material: mutagenic activity

3.3.1. Soil

Cell survival found in the assays with organic extracts of soils from Sites 1 and 2 is shown in Table 5. The organic extracts presented toxic compounds with direct action, with increased survival after adding the metabolization system. At Site 1, higher toxicity was associated with Total Soil, since this occurred at lower concentrations and attained values of 16.4% at the higher concentration. In fraction <0.5 mm of the soil, only the highest concentration was toxic. The opposite was seen in the samples from Site 2, which induced lower survival in Soil <0.5. In this fraction, toxicity occurred from 80 mg onwards, reaching percentages of 10.8% at the highest concentration.

Table 5 – Percentage of cell survival of organic extracts from soil samples through the *Salmonella*/microsome assay

	Site 1				Site 2			
	TA98-S9		TA98+S9		TA98-S9		TA98+S9	
	%	Conc.	%	Conc.	%	Conc.	%	Conc.
Total Soil	**57.1**	80	76.9	160	**47.3**	120	72.7	160
Soil <0.5	**43.1**	160	64	120	**47.9**	80	86.6	80

Percentages of cell survival below 60% (in boldface) mean cytotoxicity. Cytotoxic samples were recorded at the lowest concentration with a toxic effect observed; for non-cytotoxic samples, the lowest percentages of survival observed were recorded. Conc. – concentration. Tested in mg equivalents of dry soil with: 10, 20, 40, 80 120 and 160mg.

As to mutagenic activity, the samples from the urban-industrial site (Table 6), showed a higher response. The soil in this area was characterized by organic compounds with different mutagenic actions in the two fractions studied, with an inverse profile to the corresponding acid extracts of pH 5.3. Direct action compounds occurred only in Total Soil, while indirect

mutagenics occurred in the sieved soil. In the urban-residential area (Site 2), mutagenic responses were observed only in the total fraction, with a similar number of revertants/g in the absence and presence of hepatic metabolization.

As to the assays performed with nitrosensitive strains, most of the results (62%) were negative, only three responses (38%) presenting greater sensitivity than the parental strain (Table 6).

Table 6 – Mutagenicity in revertants/g dry soil in organic extracts

		TA98[a]		YG1021[a]	YG1024[a]	Citotoxicity[b]	
		-S9	+S9			TA98-S9	TA98+S9
Site 1	Total Soil	397.60 ± 143.5[i]	ns	ns	ns	+	–
	Soil <0.5	ns	429.33 ± 106.21	ns	103.96 ± 39.19[i]	+	–
Site 2	Total Soil	88.08 ± 31.38[i]	87.91 ± 32.15[i]	737.63 ± 156.64	116.91 ± 44.44[i]	+	–
	Soil <0.5	ns	ns	ns	ns	+	–

[a] mean ± deviation of revertants/g of dry soil; [i] responses indicative of mutagenesis, ns – non-significant; [b] cytotoxicity: +present in at least one concentration, -absent. Negative Control (DMSO): 45.83 ± 16.79 (TA98); 39.00 ± 8.32 (TA98+S9); 83 ± 4.36 (YG1021); 42.33 ± 12.50 (YG1024). Positive Control: 4NQO (TA98) 757.33 ± 159.52; 2AF (TA98+S9) 294.33 ± 123.87; 2NF (YG1021) 4070 ±636.40; 2NF (YG1024) 2466 ±373.35.

3.3.2. Atmospheric particulate matter

The concentrations of fine inhalable particles (PM 2.5) among the sites sampled varied from 12.6 to 32.67 µg/m³ (Table 7). Of all filters analyzed, only one in the urban-industrial area (Site 1–– Jul/10: 32.67 µg/m³) surpassed the maximum limit of 25 µg/m³ of particles recommended by the World Health Organization in 24-hour samplings (WHO, 2006).

Table 7 – Concentration of particles, extracted organic matter and mutagenicity of organic extracts of fine inhalable particles in an urban-industrial (Site 1) and urban-residential area (Site 2) in the city of Rio Grande, Brazil.

	Sample	N° of filters	C PM2.5 (µg/m³)	Mean C [a] (µg/m³)	EOM [a] (µg/m³)	TA98 [a] -S9 (rev/m³)	TA98 [a] +S9 (rev/m³)
Site 1	Jul/10	2	17.64 32.67	25.15 ± 10.63	3.47	17.80 ± 2.01	6.90 ± 0.76
	Aug/10	2	15.48 13.07	14.27 ± 1.70	1.25	3.85 ± 0.29	1.66 ± 0.46 [i]
Site 2	Jul/10	2	18.18 18.49	18.33 ± 0.22	2.88	9.70 ± 0.86	4.00 ± 0.34
	Aug/10	2	19.28 12,26	15.77 ± 4.96	4.21	1.98 ± 0.63 [i]	4.12 ± 0.93

[a] mean ± deviation; [i] responses indicative for mutagenesis; C = concentration of particles; EOM = organic matter extracted; Negative Control (DMSO): 48.33 ± 4.93 (TA98); 43.17 ± 18.00 (TA98+S9); Positive Control: 4NQO (TA98) 1153.50 ± 203.80; 2AF (TA98+S9) 297.25 ± 181.70.

Mutagenicity evaluation of organic extracts of particulate matter resulted in positive or indicative responses in all samples and strains tested (Table 7 and Figure 4). The mutagenic potential in number of revertants/m³ of air sampled was, generally, higher in the absence of metabolization, indicating the predominance of direct action substances in the samples (Table 7). As to the period evaluated, it could be observed that the samples corresponding to the month of July/10 presented greater mutagenesis. The participation of nitrogenated compounds in direct mutagenic activity is evidenced by the increment of revertants/µg observed in the YG family strains, in relation to their parental TA98 (Figure 4). This increment was observed in all samples evaluated, for nitroarenes (YG1021) and dinitroarenes (YG1024). However, the

YG1021 strain proved more sensitive, evidencing the predominance of nitroarenes at both places. The comparison between the mutagenic potential associated with the urban-industrial and residential sites was performed through the set of responses of every month and strain assessed. In this way, the urban-industrial site (Site 1) presented a significantly higher mutagenic potential than the urban-residential area (Site 2) ($F_{1,7} = 7,11$; $p < 0.05$).

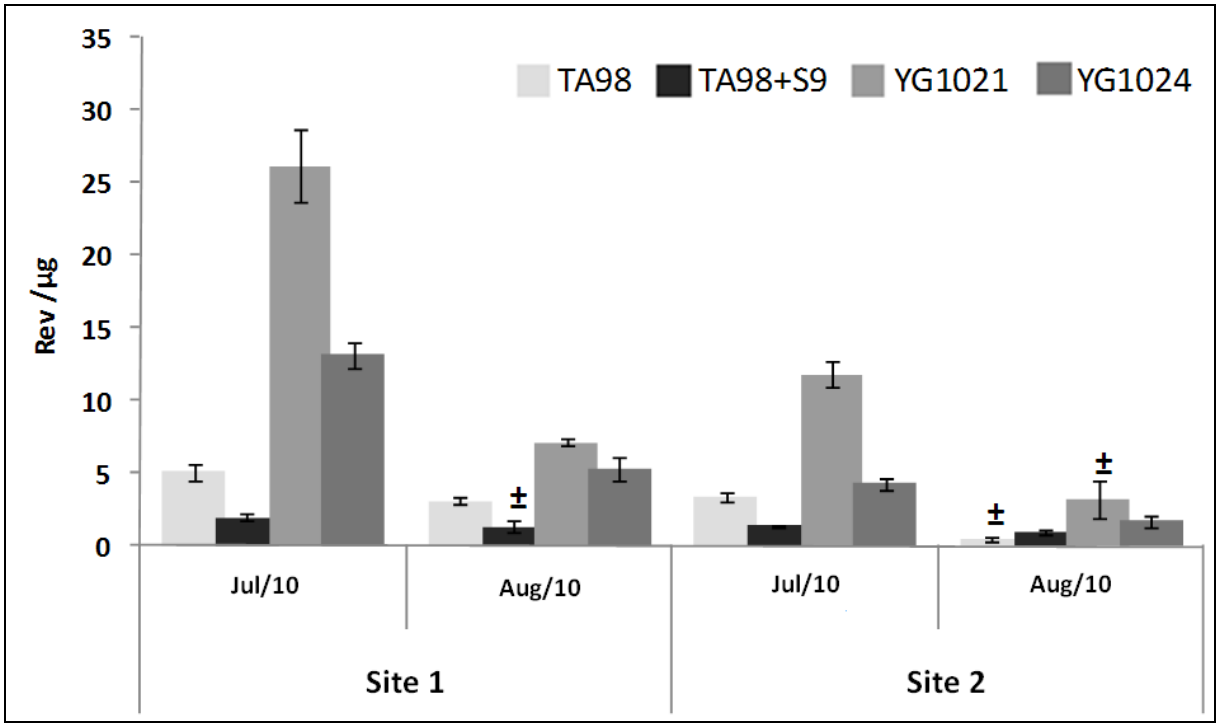

Figure 4 – Mutagenicity in revertants/µg (Rev/µg) of organic PM2.5 extracts in an urban-industrial (Site 1) and urban-residential area (Site 2).

± indicative responses. Negative Control (DMSO): 48.33 ± 4.93 (TA98); 43.17 ± 18.00 (TA98+S9); 62.87 ± 16.86 (YG1021); 45.67 ± 3.61 (YG1024). Positive Control: 4NQO (TA98) 1153.50 ± 203.80; 2AF (TA98+S9) 297.25 ± 181.70; 2NF (YG1021) 4613 ± 1498.70; 2NF (YG1024) 3374 ± 500.63.

The cell survival test in the PM2.5 extracts resulted in toxic responses only in the sample at Site 1 in August/10, in the absence of metabolization (59.2% - concentration 10µg). The other samples presented over 70% survival (data not shown).

4. DISCUSSION

A major part of the chemicals released by anthropic activities ends up in the soil (Courty et al., 2008). The surface layer of the soil reflects the deposition of atmospheric pollutants, especiallly those deposited recently, as well as pollutants that do not move into the deeper layers because they are attached to soil particles. In the deeper layers are pollutants deposited by liquid spills, long term deposition of water-soluble materials and buried substances. Rains can move the pollutants from the surface layer to other deeper ones, or take them away from the point of deposition by surface runoff (USEPA,1992).

In this study, only the 0-5 cm surface soil layer was analyzed, enabling the evaluation of the recent atmospheric contribution, the elements that could become available at the next rainfall events, and minimization of the effects of possible contaminants remaining from landfills.

Although it is recommended that the sample preparation stage be performed as simply as possible to avoid alterations, different stages of pre-treatment (drying, grinding, sieving, storage, extraction) are needed to analyze soil samples (Lamé, 1995; Michalke, 2003). Frequently, when evaluating the contaminated soils, a sample sieving stage is performed (Belkessam et al., 2005). This often occurs to exclude the coarser fraction (>2 mm), or to concentrate the pollutants that have adhered preferentially to the finer soil particles.

The smaller diameter particles have larger area of surface contact that causes an increase in the sites available for binding of pollutants. Thus, it would be expected that the smaller diameter particles would have a greater biological effect. Some studies consider the larger fractions of the soil as inert or less important, thus performing the sieving of the fine fractions to improve chemical detection of metal contaminants present in the soil (Bian and Zhu, 2009; Garcia et al., 2010; Vasiluk et al., 2011). The preferential concentration of metals in the smaller soil particles has also been demonstrated (Bian and Zhu, 2009; Luo et al., 2011; Madrid et al., 2008). This aspect raises the question of their influence also on the genotoxic responses.

Grain size separation may influence the final result of analysis, since this physical process may interfere in solubility, interconversion of the chemical species or favor an increased loss of volatile species (Zoumis et al., 2001 *Apud.* Rodrigues et al., 2008). Thus,

sieving the samples may alter the balance between the chemical species, resulting in different conditions from those observed in the environment. In this study, sieving excluded only about 11% (Site 1) and 2% (Site 2) of the particles that constitute the original soils. However, even these small changes resulted in change in the mutagenic profile of the samples.

4.1. Acid extractions of soil at different pHs simulating rainfall events

This study used two acid extractions with different hydrogenionic potentials to leach the samples of Total Soil and the Soil <0.5 mm. The leaching experiments allow studying pollutant mobility, helping evaluate their possible environmental effects (Pueyo et al., 2003). Since rain leaches the soils, the pHs of the extractor solutions used were chosen to simulate the rainfall conditions observed in the particular area of study, 5.3 being the background value of the rain and 3.6 the value of the more acid rains recorded in the city of Rio Grande (Mirlean et al., 2000). This background value is similar to the pH of rain in equilibrium with carbonic gas, 5.6 (USEPA, 1990), as well as to the pH of the soils studied (5.6-5.9).

Comparing the two acid extracts used, the one with the lower pH resulted in more toxicity and less frequent, but higher mutagenicity. However, the low percentages of cell survival appear to have interfered in the detection of the mutagenic potential of these extracts, since the only two positive responses occurred in the absence of toxicity. The extraction of pH 5.3 improved the detection of the mutagenic activity of the soils evaluated. In these extracts there were great differences in the mutagenicity of Total Soil and of Soil <0.05, although the fractions have a very similar grain size. In this way an increase of direct mutagenics and loss of indirect ones in the Soil <0.5 was observed at Site 1 and an inverse relation at Site 2.

Generally a larger number of revertants/g dry soil is noted at Site 2 (urban-residential). The soil at this site is characterized by slightly higher percentages of organic matter, organic carbon and cation exchange capacity. The cation exchange capacity is directly related to the soil capacity to adsorb heavy metals. The higher the value of the CEC, the more exchange sites in soil minerals will be available for metal retention (Iwegbue et al., 2009). These authors also underscore that the presence of organic matter contributed to cation retention in the soil. The chemical analysis of these samples showed the higher presence of metals at Site 2. These characteristics may be related to the higher mutagenic potential found in these samples.

Few soil mutagenicity studies have been performed in Brazil, and previous evaluations performed by our research group used extractor solutions for pH 5.9, according to the properties of the soils evaluated. The mutagenic potencies obtained in the acid extracts at pH 5.3 (Total Soil) in the present study were similar to those observed in soil belonging to a bottom ash deposit of a coal-firing thermopower plants (Silva-Júnior e Vargas, 2009). However, compared with industrial soils contaminated with wood preservative (Pohren, 2011) only the direct mutagenicity of the urban-residential area (Site 2) was higher. These results show the high mutagenic potential of these urban soils compared to industrial areas. The presence of mutagenic compounds in urban environments has been shown in studies of different environmental matrices (Claxton e Woodall, 2007; Courty et al., 2008, Lemos et al., 2009; Traversi et al., 2009; Watanabe et al., 2008; White e Rasmussen 1998), showing the high polluting potential of anthropic activities in urban areas.

In polluted environments, rain water is a significant route of contamination, due to the removal of contaminants present in soil and air. Interaction with the atmospheric contaminants, especially sulphur dioxides, and nitrogen oxides, may cause rain acidification depending on the level of pollution, however, even rain in a non polluted atmosphere is slightly acid (USEPA, 1990). Acidification of the environment influences solubility, speciation, environmental mobility, transfer in the food chain and toxicity of several metals (Scheuhammer, 1991). Various damaging effects resulting from acid rain events have already been described in the environment and in the biota. However, few studies report the relationship between pH and environmental genotoxicity (Silva-Júnior et al., 2009; Singh & Agrawal, 2008).

Although the mutagenicity observed is associated with the ensemble of chemical substances present in the samples, the pH of the acid extracts fosters a greater availability of metals that can be associated with the effects found. The chemical analysis of the samples showed a greater variety of metals, as well as higher concentrations of these metals in the acid extracts at pH 3.6. This is expected, since leaching of the metals increases as pH decreases (Baba et al., 2008). This increase may be related to the greater toxicity found in this treatment.

The acid extracts obtained at both pHs for Total Soils and Soils <0.5 presented similar concentrations for the metals quantified. This similarity is expected, since in the sieved soils a small grain size percentage of the total samples was excluded. However, a greater frequency

of toxicity (62.5%) was observed in the soils <0.5. This effect may be associated with the change in the chemical species of the metals present, which occurred during the sieving process.

Among the metals detected in chemical analysis, cadmium, chromium and iron presented a positive mutagenicity in the *Salmonella* assay (Bennicelli et al., 1983; Brusick et al., 1976; Mandel e Ryser, 1984; Marzin e Phi, 1985). Zinc, copper, nickel and aluminium were reported as non mutagenic in this assay (ATSDR, 2005; Marzin e Phi, 1985), but presented clastogenic potential (ATSDR, 2004; 2005a; 2005b 2008). Some metals alone are weakly or marginally mutagenic, and may become important secondary sources of genetic damage, when they act synergistically with other substances. An example of this is cadmium, which can increase the mutagenicity of nitrosamines synergistically, at 30 times higher levels than would be expected from the additive effect of these substances (Mandel and Ryser, 1984).

No lead, arsenic and vanadium were found in the extracts evaluated, while cadmium and chromium occurred at small concentrations only in the extract of pH 3.6. The presence of total metallic contaminants has already been described previously for the surface soils in the region (FEPAM, 2010). Hg, Ni, Cr, Cu, Zn and Pb were detected in areas close to the oil refinery. In this way, the absence of lead in the acid extracts in this study could be explained by the predominantly insoluble characteristics of this metal.

Considering all the extracts evaluated, the highest concentrations were found for zinc, iron and aluminium. Although anthropic sources may enrich the content of these metals, they are abundant soil constituents and are common at high concentrations (Silva-Junior e Vargas, 2009).

A previous study performed by our research group attempted to identify possible areas of reference for soil mutagenicity in the state of Rio Grande do Sul (Meyer, 2008). In this context a sandy soil area located in a conservation unit was identified. The extraction by acid leaching (pH 4.9) of the soil at this site resulted in negative responses for mutagenicity and toxicity, besides the detection of only four out of nine metallic elements (Zn = 0.2; Fe = 0.06; Mn = 0.5 and Al = 0.4 mg/kg soil). In this study, the values of these metals are two to twelve times higher, suggesting an anthropic contribution. The aluminium was an exception and

occurred at a concentration similar to that of the area of reference, which may be attributed to its natural origin.

Other comparisons of the metal contents found in the city of Rio Grande can be made with studies performed in the same state, using a similar methodology. Only the total extracts of pH 5.3 were considered for this purpose. The highest copper and nickel values occurred at Site 1, presenting a higher concentration than in industrial soils contaminated by wood preservatives, and the adjacent residential soils (Pohren, 2011). Similar (Cu) and higher contents (Ni) were identified than soils of coal ash deposits and from an adjacent site subject to atmospheric contamination (Silva-Júnior and Vargas, 2009). Thus the nickel contents in the urban-industrial area were high, and may be associated with the oil refinery. Nickel is a natural component of oil emitted to the atmosphere during the combustion process (Bradl, 2005). Together with vanadium and iron, nickel is one of the most abundant metals present in oil (Caumette et al., 2009). Besides, this metal was considered an efficient marker of the impact of atmospheric emissions caused by the oil refinery activity in the city of Rio Grande (Garcia et al., 2010). The same authors observed copper enrichment around this refinery, suggesting that it originates from port activities.

The zinc and iron contents were higher at Site 2, more elevated than those found in the soils in the studies cited previously (Silva-Júnior e Vargas, 2009; Pohren, 2011), except for the values found for iron at the site adjacent to the coal ash deposit. Further, cadmium was found exclusively in the pH 3.6 extracts at this site. Among the anthropic activities mentioned as possible anthropogenic sources of zinc, the metallurgical and mechanical branch is present in areas, as well as the fertilizer plants and vehicle exhaust. The fertilizer plants are also mentioned as possible emitters, among other metals, of cadmium and irons. The latter is also a natural component of oil, and can be emitted during oil combustion (Bradl, 2005; Caumette et al., 2009; FEPAM, 2010).

4.2. Extractions of organic compounds and possible soil-atmosphere relations

The soils at both sites evaluated in this study presented organic contaminants with direct and indirect action which induced higher responses at the site with urban-industrial characteristics (Sitel 1). The quantities of extracted organic matter were higher in the soils <0.5, compared to Total Soils at both sites. Thus, a greater effect would be expected in fraction <0.5 mm, but the direct and indirect mutagenic responses alternated between the soil

fractions at Site 1, while at Site 2 both responses occurred only in Total Soil. The results suggest a different composition of mutagenic substances associated with the two soil fractions studies. Hence, handling the samples to exclude the coarse sands, as done in the Soils <0.5, may have modified the active synergistic, additive or antagonistic effects, and also the loss of volatile substances.

In a broad review of soil mutagenicity, White and Claxton (2004), described the origin of contaminated soils at: (i) industrial sites located within industrial facilities; (ii) urban/suburban sites, located in the cities, in places that do not directly receive industrial wastes; (iii) rural and agricultural sites, located outside the urban and suburban area, including remote park-like settings. The geometric means of revertants cited in this review for the *Salmonella* assay (strains TA98 and TA100) were compared by analysis of variance, showing a significant relation between site category and mutagenic potential. As to the categories proposed by White and Claxton (2004), the soils analyzed in this study presented mutagenic potentials that enabled classifying Site 1 as urban and Site 2 as rural.

Another form of classification was used by Endo et al. (2004), based on the mutagenic potency of 544 samples of soil from Japan. According to these authors, the soils can be classified as four mutagenic potentials: low (up to 100 revertants/g); moderate (100-1000 revertants/g); high (1000-10000 revertants/g) and extreme (more than 10000 revertants/g). Following this criterion, the samples in this study present low (Site 2) and moderate mutagenicity (Site 1). It should be underscored that this classification was based exclusively on soils in Japan, and may not be representative of other regions. Further, values around 100 revertants/g of soil, such as those seen at Site 2, were considered by some authors as natural background levels of soil mutagenicity (White e Claxton, 2004).

When present in the soil, the PAHs are generally retained in the surface layers. PAHS are indirect mutagenics, and require metabolic activation to become genotoxic (Pereira-Netto et al., 2000). Thus, the responses of the organic extracts in the *Salmonella*/microsome asay, in the presence of the S9 fraction, can be attributed to the presence of these compounds. In this study, surface soils were evaluated, and indirect mutagenesis was found at both sites, occurring in different fractions. Studies have shown that the PAHs content in soil contributes 17-25% of the mutagenicity found in strain TA98+S9 (Courty et al., 2008; White and Claxton, 2004).

The presence of mutagenic nitrocompounds in the soils sampled was investigated using strains YG1021 and YG1024. Mutagenicity for mononitroderivates was detected only at Site 2 (Total Soil), while lower potentials were observed for dinitroarenes at both places. These results are in contrast with studies in Japanese soils which show a close relation between the direct mutagenicity in strain TA98 and the concentration of dinitropyrenes in the soil (White e Claxton, 2004). The nitroarenes were identified as principal mutagenic constituents in surface soils in Kyoto, with contributions of up to 22% in the mutagenic response observed in the extracts (Watanabe et al., 2008). The cytotoxicity observed in all extracts in the absence of metabolization may have made it difficult to detect the direct mutagenic potential, both in the parental strain (TA98) and in its derivates (YGs). Wesp et al. (2000), also found it difficult to evaluate the mutagenicity of soils exposed to automobile exhaust due to the toxicity obtained in the absence of fraction S9.

As observed in the organic soil extracts, a greater mutagenic potential was observed at Site 1 for atmospheric particulate matter, and the direct action compounds predominate in both matrices. Indirect mutagens observed in smaller proportions in PM2.5 were also smaller in the soil, considering that their occurrence was limited to fraction <0.5 mm. Thus, sieving appears to have concentrated these substances, possibly less volatile PAHs. The same appeared to have occurred with the dinitrocompounds present only in this fraction. Studies with atmospheric particulate matter showed the preponderance of dinitroderivates in industrial areas (Coronas et al., 2009; Pereira et al., 2010). However, the responses of PM2.5 in this period were higher for nitroarenes possibly associating themselves with the urban contribution (Ducatti e Vargas, 2003; Kaffer, 2011; Vargas et al., 1998; 2003). At this site also, the only higher value than the maximum concentration of PM2.5 recommended by the WHO occurred, suggesting poorer environmental quality.

The urban-residential site presented a greater concordance between mutagenic responses in the soil samples and particulate matter. The mutagenic profile, similar in both matrices, showed similar contributions of direct and indirect action mutagenics, as well as a greater contribution of nitroarenes. Further, cytotoxicity was recorded in the absence of metabolization, in both soil fractions and in August for PM2.5. The loss of mutagenic activity in the samples of Soil <0.5, in all treatments, suggests the predominance of volatile compounds lost while preparing the sample.

All the PM2.5 samples presented a mutagenic potential, but the same did not occur with the soil samples. The sensitivity of the nitrosensitive strains YG1021 and YG1024 was higher in the samples of atmospheric particulate indicating the existence of a mixture of mono and dinitroarenes. The pattern found, with greater contributions from nitroarenes, is similar to that observed in a previous study at the same site (Lemos et al., 2011) and in urban areas, as cited previously.

The mutagenicity of PM2.5 in this study was superior to that found in a previous evaluation of this particulate at the same sites, during spring and summer (Lemos et al., 2011). The same study also showed worse quality of the air at the urban-industrial site. Higher mutagenesis was also observed during the winter in a study with total suspended particles (TSP) in the city of Rio Grande (Pereira et al., 2008).

5. CONCLUSIONS

The study showed that distinct biological responses are observed when analyzing samples of Total Soils with minimum handling and samples sieved to exclude coarse fractions. The determining factors of these changes require further investigation. However, the absence of a general tendency in these responses suggests that in the *Salmonella*/microsome assay the use of Total Soils is more appropriate, because it allows evaluating soil under conditions closer to the original ones.

Relations between the mutagenicity of organic compounds from the soil and atmospheric particulates, in this study, enabled a clearer characterization of anthropic influence at both sites. However, the complexity of these environmental matrices, as well as that of the pollutants made it difficult to explain the possible exchanges between air and soil.

Soil extraction at pH 3.6 provided a greater variety and concentration of metals, and also higher toxicity. However, extraction at pH 5.3 enabled a better identification of the presence of mutagenic compounds in surface soils. Therefore, the leached soil extracts made available compounds with toxic and mutagenic effect, with potencies varying according to the pH of the solution used. Thus, rainfall in the region can act as a dispersion route for contaminants, as it washes soils in the urban area. Acid rains are an additional risk for the availability of more toxic substances. It should be underscored that the acid solutions used in this study only mimic the conditions of pHs observed in rains in the area of study. It is

suggested that further studies use acid solutions with different chemical compositions, as well as rainwater itself as an extractor solution, aiming to increase knowledge on the effects of these rains on environmental genotoxicity.

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4. CONSIDERAÇÕES FINAIS

A introdução crescente de variados poluentes em matrizes ambientais de áreas urbanas causa prejuízos ao ecossistema e dificulta as medidas de monitoramento e controle ambiental.

A Fundação Estadual de Proteção Ambiental Henrique Luís Roessler (FEPAM), dentro de seu Programa de Pesquisas Ambientais, vem realizando estudos em áreas de interesse sócio-ambiental quanto à poluição oriunda de atividades industriais e urbanas. O presente estudo integra o projeto EcoRisco Saúde, onde a cidade de Rio Grande foi escolhida como área-alvo para investigação de contaminantes dispersos.

A cidade de Rio Grande possui um histórico de degradação ambiental, com um crescimento desordenado que levou ao posicionamento muito próximo das zonas portuária, industrial e urbana. Os lotes residenciais formam um adensamento urbano junto à área industrial, sendo habitados principalmente pelas populações de baixa renda. Esta realidade gera preocupação quanto à mistura de emissões que interagem nestas áreas.

O presente estudo detectou a presença de substâncias genotóxicas em diferentes frações de material particulado atmosférico e solos, além da presença de contaminantes orgânicos e inorgânicos. Foram estudados dois locais, sendo um de característica urbano-industrial e outro urbano-residencial. O primeiro apresentou, em geral, pior qualidade em relação ao segundo, salientando o risco envolvendo populações que residem próximas a zonas industriais. Entretanto, deve-se considerar a contribuição das emissões urbanas, cujo potencial genotóxico também pode ser evidenciado.

O emprego do ensaio *Salmonella*/microssoma se mostrou adequado na identificação de efeitos genotóxicos como indicador precoce de contaminação ambiental a partir de amostras de material particulado atmosférico e solo. A avaliação integrando o uso de diferentes linhagens e da fração de metabolização hepática auxiliou na compreensão das classes de compostos presentes na área. A utilização das linhagens sensíveis a compostos orgânicos nitroderivados (YG1021 e YG1024) permitiu detectar o potencial mutagênico de algumas amostras, incluindo situações em que foram observadas respostas negativas na linhagem básica (TA98). Dessa forma, é importante que linhagens específicas a compostos químicos de interesse na área investigada sejam acrescentadas nos estudos ambientais, sempre que possível.

A investigação do material particulado atmosférico, realizada através de extrações orgânicas e aquosas, evidenciou maior risco de exposição associado à fração de compostos orgânicos das partículas finas (PM2,5). O ensaio *Salmonella*/microssoma não detectou a presença de possíveis mutagênicos nas concentrações obtidas dos extratos aquosos. Entretanto, estes apresentaram citotoxicidade em 50% das amostras, enquanto que a análise química mostrou a presença de compostos metálicos potencialmente biodisponíveis. Estes resultados, aliados à existência de poucos estudos de genotoxicidade de compostos inorgânicos no material particulado, sugerem a importância da busca por métodos de preparação da amostra mais adequados, aliados a ensaios sensíveis para a avaliação do efeito mutagênico destes compostos.

A caracterização da atividade mutagênica do PM2,5 apresentada neste estudo é inédita no Brasil, contribuindo para a avaliação de ambientes impactados por ações antrópicas. A continuidade de estudos no país avaliando este particulado, em áreas com diferentes características, propiciará mais subsídios para o monitoramento e gestão ambiental.

A determinação das concentrações de partículas em suspensão é mundialmente utilizada como um dos parâmetros de avaliação da qualidade do ar. No Brasil, a legislação reguladora da concentração máxima de material particulado atmosférico data de 1990, referindo-se às partículas totais em suspensão e às partículas inaláveis grossas, inexistindo regulamentações acerca da concentração de partículas PM2,5. A determinação das concentrações de PTS e PM2,5 durante o período de estudo mostraram que a maioria dos filtros amostrados está em conformidade com os valores máximos permitidos pela legislação nacional brasileira (PTS) ou recomendados pela Organização Mundial de Saúde (PM2,5). Entretanto, todas as amostras apresentaram potencial para mutagenicidade em ao menos um tratamento. Assim sendo, é importante que os padrões de qualidade do ar adotados pela legislação nacional sejam revisados. A incorporação de parâmetros considerando PM2,5, já realizada em outros países, se faz necessária devido ao maior risco de exposição a essas partículas, que constituem a fração inalável capaz de atingir a região alveolar.

A acidificação do ambiente influencia na solubilidade, especiação, mobilidade ambiental, transferência na cadeia alimentar e toxicidade de vários metais. Vários efeitos danosos resultantes dos eventos de precipitações ácidas já foram descritos no ambiente e na biota, entretanto, poucos trabalhos relacionam o efeito do pH na genotoxicidade ambiental. A

utilização de extrações ácidas de solo, com soluções de pHs diferenciados, foram escolhidos para simular as condições das precipitações observadas na área de estudo em questão. Uma vez que a chuva proporciona a lixiviação dos solos, os resultados evidenciaram que as precipitações da região podem atuar como rota de dispersão de contaminantes, com risco adicional na disponibilização de substâncias tóxicas nos eventos de precipitação ácida.

A avaliação de solos foi realizada em sua composição granulométrica total e na fração <0,5 mm, resultando em respostas biológicas distintas, que podem ter refletido possíveis alterações das amostras durante o processo de peneiramento. Embora os fatores determinantes destas alterações necessitem maior investigação, a ausência de uma tendência geral nas respostas sugere que, no ensaio *Salmonella/*microssoma, o emprego do solo total é mais adequado, por permitir avaliação do solo em condições mais próximas às originais.

A investigação simultânea da presença de contaminantes orgânicos e inorgânicos torna-se importante, à medida que o ecossistema está exposto a uma mistura de poluentes. Neste estudo, a utilização conjunta de diferentes métodos de extração de compostos orgânicos e inorgânicos permitiu uma avaliação integradora da qualidade das matrizes ambientais complexas avaliadas. O uso de metodologias sensíveis, que detectem precocemente a existência de danos no ambiente, propicia a adoção de estratégias de prevenção para proteção do ecossistema e monitoramento ambiental, adequados às condições locais.

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6. ANEXOS

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