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DISSERTAÇÃO DE MESTRADO

MUTAGENICIDADE DE SOLOS COMO ESTRATÉGIA NA AVALIAÇÃO DE RISCOS DE ÁREA CONTAMINADA

ROBERTA DE SOUZA POHREN

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Mutagenicidade de solos como estratégia na avaliação de riscos de área contaminada

Roberta de Souza Pohren

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Orientador: Prof. Dra.Vera Mª Ferrão Vargas

Comissão Examinadora

Prof. Dra. Clarice Torres de Lemos
Prof. Dra. Kelly Cristina Tagliari Brito
Prof. Dr. Paulo Luiz de Oliveira

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RESUMO

O solo é um dos compartimentos mais atingidos pelo acúmulo de poluentes de origem antrópica, pois atua como depósito de poluentes e como fonte para outros ambientes de interface. Este estudo investigou sítio contaminado com resíduos da indústria de preservativos de madeira como fonte de compostos mutagênicos; definiu rotas e abrangência na dispersão desses contaminantes pela remobilização de partículas e deposição atmosférica, associando riscos ambientais e à saúde. Foram selecionados locais de amostragem para solos e poeira domiciliar a distâncias gradativas a partir de SI (*pool* de solo da área industrial), SR150 (150m), SR500 e SR1700; para poeira foi coletado um *pool* em área de risco, DR385 (385m) e em DR1700. Foi avaliada a atividade mutagênica através do ensaio *Salmonella*/microssoma, método de microssuspensão, em linhagens que detectam erro no quadro de leitura (TA98 e TA97a), substituição de pares de bases do DNA (TA100), em presença e ausência de metabolização hepática de ratos (*S9mix*) e YGs 1041, 1042 e 1024 para a definição de nitroderivados. Foram preparados extratos ácidos, visando definir os efeitos de compostos inorgânicos como metais pesados e de extratos orgânicos para avaliar principalmente hidrocarbonetos policíclicos aromáticos (HPAs) e nitroderivados. Os extratos foram testados também quanto à concentração dos 16 HPAs considerados poluentes prioritários pela USEPA, quanto a metais pesados de interesse e quanto ao contaminante pentaclorofenol (PCP) como marcador específico da atividade do sítio industrial. Através das análises de metais pesados foi detectado um gradiente de respostas, onde tanto a concentração de metais totais quanto a fração biodisponível de Cr e Cu apresentaram valores mais altos para SI em relação aos solos de entorno; para As, apenas na avaliação da concentração total do elemento, SI foi superior. Em relação aos efeitos mutagênicos decorrentes da mistura de compostos inorgânicos as respostas

não permitiram uma gradação entre as diferentes distâncias. O solo fonte SI mostrou mutagênese nas diferentes linhagens, em especial em TA97a na ausência de S9*mix*. As amostras de entorno apresentaram potência mutagênica em pelo menos duas cepas, mas apenas uma consonante com SI. O solo SR500, mostrou mutagênese diferenciada e resposta expressiva na linhagem TA98 com S9 *mix*. No entanto, o local SR1700 mostrou ausência de influência a partir da área contaminada caracterizando uma área de referência. Nos extratos orgânicos, as respostas de mutagênese mostram um padrão de contaminação nas áreas de influência semelhante ao apresentado por SI, parecendo indicar a mobilidade de compostos orgânicos a partir da fonte para as áreas de entorno. Houve um predomínio de compostos de ação indireta, sendo os valores de SI entre 107 a 1455 rev/g de solo. Nos locais de entorno, observou-se padrão similar em SR150; já em SR500 valores elevados de mutagênese de ação direta foram evidenciados em TA97a; SR1700, embora apresentando mutagênese do tipo erro no quadro de leitura em presença de S9 *mix*, mostrou um decréscimo de efeitos. Os testes com as linhagens YG indicaram que compostos nitrados têm ação significativa na mutagênese direta encontrada, com exceção de SR500. Foi detectada ainda a presença de hidroxiamino-compostos em todas as amostras de solos através da linhagem YG1024. Na investigação da poeira residencial de entorno foram observadas respostas mutagênicas nas diferentes cepas testadas em DR385, mono e dinitroarenos do tipo substituição de pares de bases (YG1042) e hidroxiamino-compostos (YG1024); em DR1700 não foi observada resposta positiva. Concentrações de HPAs potencialmente carcinogênicos se estendem no solo da área interna até o do local de referencia, bem como na poeira domiciliar da área de risco indicando gradiente de concentrações e efeitos. A concentração de PCP no *pool* de poeira (DR385) foi (0,491 mg/Kg), similiar a observada em SI, (0,431 mg/Kg), definindo uma rota efetiva de dispersão a partir da área industrial para regiões do

entorno. Ficou evidenciada a possibilidade de dispersão de mutágenos da área contaminada para regiões de entorno, sendo possível detectar gradientes de distância, favorecendo estimativas de risco. O estudo mostrou que é fundamental avaliar a extensão da contaminação a partir de fontes de solo impactado, visando delimitar qualquer medida de remediação dos ambientes atingidos e evitar danos potenciais ao equilíbrio ecológico e à saúde humana.

Palavras-chave: mutagenicidade, solos, poeira, dispersão, HPAs, pentaclorofenol.

ABSTRACT

Soil is one of the compartments most affected by the accumulation of anthropic pollutants, since it acts as a deposit for pollutants and as a source for other interface environments. This study investigated a site contaminated with residues of the wood preservative industry as a source of mutagenic compounds; it defined the routes and area covered by dispersion of these contaminants through the remobilization of particles and atmospheric deposition, associating environmental and human health risks. Sampling sites were selected for soils and dusts at gradually increasing distances from SI (*pool* of soil from the industrial area), SR150 (150m), SR500 and SR1700; a pool was of residential dust was collected in the area of risk, DR385 (385m) and at DR1700. Mutagenic activity was evaluated by the *Salmonella*/microsome assay, microsuspension method, in strains that detect frameshift error (TA98 and TA97a), DNA base pair substitution (TA100), in the presence and absence of hepatic metabolization of rats (*S9 mix*) and YGs 1041, 1042 and 1024 to sough nitroderivates. Acid extracts were prepared to define the effects of inorganic compounds such as heavy metals, and organic extracts in evaluating mainly polycyclic aromatic hydrocarbons (PAHs) and nitroderivates. The extracts were tested also for the concentration of the 16 PAHs considered priority pollutants by USEPA, for heavy metals of interest and for contaminant pentachlorophenol (PCP) as a specific marker of the industrial site activity. A gradient of responses was detected through analyses of heavy metals , where both the concentration of heavy metals and the bioavailable fraction of Cr and Cu presented higher values for SI compared to the surrounding soils; for As, SI was superior only to evaluate the total concentration of the element. As to the mutagenic effects of the mixture of inorganic compounds, the responses did not allow a grading between the

different distances. The source soil SI presented mutagenesis in the different strains, especially in TA97a in the absence of S9*mix*. The samples from the surrounding area presented mutagenic potency in at least two strains, but only one in accordance with SI. Soil SR500 showed differentiated mutagenesis and an expressive response in the TA98 strain with S9 *mix*. However, site SR1700 showed no influence from the contaminated area, characterizing an area of reference. In the organic extracts, the mutagenesis responses showed a pattern of contamination of the areas of influence similar to that presented by SI, and appear to indicate the mobility of organic compounds from the source to the surrounding areas. Indirect action compounds predominated, and the values of SI were from 107 to 1455 rev/g soil. At the surrounding sites, a similar pattern was observed in SR150; on the other hand, in SR500 high values of direct action mutagenesis were evidenced in TA97a; SR1700, although presenting mutagenesis in frameshift strains in the presence of S9 *mix*, showed diminished effects. Tests with YGs strains indicate that the nitrated compounds exert a significant action on the direct mutagenesis found, except for SR500. Further, hydroxyamine-compounds were detected in all soils samples through strain YG1024. Investigating residential dust from the surrounding area, mutagenic responses were observed in the different strains tested in DR385, mono and dinitroarenes of the pair substitution mutation type (YG1042) and hydroxyamine-compounds (YG1024); no positive response was observed in DR1700. Potentially carcinogenic PAHs concentrations are on the soil from the internal area until the site of reference, and also in the residential dust of the risk area, indicating a gradient of concentrations and effects. PCP concentration in the dust pool (DR385) was (0.491 mg/Kg), similar to that observed in SI, (0.431 mg/Kg), defining an effective dispersion route from the industrial area to the surrounding regions. The possibility of mutagen dispersion from the contaminated area to the surrounding regions was shown,

and gradients of distance favoring risk estimates were detected. The study showed that it is essential to evaluate the extent of contamination from the sources of soil that have been impacted, with a view to delimiting any remedial measure for the environments affected and avoiding potential damage to the ecological balance and human health.

Key words: mutagenicity, soils, dust, dispersion, PAHs, pentachlorophenol.

LISTA DE ABREVIAÇÕES

CCA	Arseniato de cobre cromatado
CETESB	Companhia Ambiental do Estado de São Paulo
CONAMA	Conselho Nacional de Meio Ambiente
DCM	diclorometano
DMSO	dimetilsulfóxido
DR	poeria residencial
HPAs/PAHs	hidrocarbonetos policíclicos aromáticos
FEPAM	Fundação Estadual de Proteção Ambiental Henrique Luís Roessler
MeOH	metanol
MOE/EOM	material orgânico extraído
NaOH	hidróxido de sódio
PCP	pentaclorofenol
SR	solo residencial
SAZ	azida sódica
S9	fração de metabolização exógena
USEPA	Agência de Protenção Ambiental dos Estados Unidos
2AF	2-aminofluoreno
2NF	2-nitrofluoreno
4NQO	4-oxidonitroquinolina
XRF	espectrometria de fluorescência de raios-X

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SUMÁRIO

1. Introdução

As inúmeras práticas agrícolas e industriais têm contribuído para a modificação de todos os compartimentos ambientais. Entre estes, o solo, considerado muitas vezes como depósito de resíduos, tem recebido toneladas de contaminantes direta e indiretamente [1], que alteram sua composição e tornam-se indevidamente um reservatório de poluentes [2]. Desta forma, o solo constitui-se numa fonte potencial de contaminação para outros compartimentos [3].

O solo é uma interface ecológica muito dinâmica, que está constantemente realizando trocas com a atmosfera, a hidrosfera e a biota; possui natureza complexa de acordo com sua composição física e química, aspectos geológicos e interações com o meio biótico. Estas diferentes características apresentadas pelos solos influenciam fortemente as respostas de toxicidade e a rota dos contaminantes presentes [4], podendo torná-los muito vulnerável às atividades humanas. Através desta riqueza de relações, o solo é reconhecido como importante via potencial de exposição [5] e representa um dos principais agentes em rotas de contaminação ambiental.

Muitos contaminantes tendem a ser adsorvidos ao material particulado do solo, contudo, a exposição, a partir desta matriz ambiental é considerada como uma efetiva rota de risco difícil de ser estimada: é possível observar os efeitos em trabalhadores diretamente em áreas contaminadas, além de verificar a exposição na vizinhança dos locais de risco via contaminantes atmosféricos, ou diretamente, pelas partículas do solo [4].

Até recentemente, a maioria dos estudos clássicos de avaliação e controle da qualidade ambiental eram exclusivamente baseados em parâmetros físicos e químicos. Este enfoque, embora fundamental, pois facilita medidas mitigadoras de controle e biorremediação, [6] não caracteriza de forma suficiente a contaminação do ambiente, uma vez que não considera a possível interação entre contaminantes, matriz ambiental e biota [7,8]. No ambiente, o efeito a ser avaliado é uma decorrência da possível interação entre as inúmeras substâncias presentes e não da ação de um único composto [9, 10, 11]. A análise das substâncias individualmente pode ser considerada uma estratégia limitada, pois além dos custos envolvidos, a maioria dos contaminantes nos solos é

desconhecida ou, está presente em concentrações muito baixas, mas que podem ser acumuladas pelos organismos, produzindo danos. Desta forma, torna-se essencial avaliar o impacto destes estressores múltiplos de uma forma mais abrangente.

Uma ferramenta importante são os bioensaios, que fornecem informações adicionais sobre riscos ecológicos, permitindo uma integração dos efeitos das concentrações biodisponíveis [2], ou seja, o efeito da mistura como um todo. Entre estes ensaios, estão os que permitem avaliar o risco potencial destas misturas complexas para genotoxicidade [4, 11, 12, 13, 14, 15, 16].

Os efeitos genotóxicos são causados por compostos capazes de reagir com a molécula de DNA, causando alterações. Caso estas não possam ser reparadas pelos mecanismos existentes, a lesão se mantém e se estabelece uma mutação. As mutações são fontes da variabilidade genética de uma população e, fundamentais para manutenção das espécies. No entanto, podem causar danos tanto aos indivíduos quanto a seus descendentes - dependendo da quantidade, do tipo e do local onde ocorrem - além de alterar o balanço dos ecossistemas [17]. O acúmulo de mutações pode gerar alterações na integridade do ambiente resultando em modificações na reprodução, e redução de populações naturais, causando desequilíbrio em ecossistemas. A detecção de danos genotóxicos torna-se, portanto, um alerta quanto a essas conseqüências ecológicas.

Os compostos mutagênicos distribuem-se nos compartimentos ambientais, tendendo a se acumular no solo e, potencialmente, gerar efeitos genotóxicos aos organismos expostos. Embora questões ligadas à produtividade e aspectos agronômicos do solo sempre tenham sido de grande interesse, muito investigadas e padronizadas, o conceito de proteção dos solos foi o último a ser abordado nas políticas ambientais. Isto se torna evidente na observação do histórico de publicações das normas ambientais: Qualidade da Água, Resolução do Conselho Nacional de Meio Ambiente (CONAMA) nº 357 do ano de 2005, revogando a nº 20 de 1986; Qualidade do Ar, Resolução CONAMA nº 3 de 1990, e por último, a legislação referente à Qualidade do Solo, Resolução CONAMA nº 420 de 2009. Estas resoluções não contemplam critérios de referência para respostas de genotoxicidade [18, 19, 20].

A literatura internacional recomenda testes biológicos, para avaliar genotoxicidade e mutagenicidade, como indispensáveis na investigação das reações dos organismos frente às misturas complexas de contaminantes ambientais [4, 21, 22], uma vez que fornecem uma estimativa precoce dos riscos oriundos de contaminantes em amostras ambientais sem conhecimento prévio da composição química das mesmas [23]. Desta forma, é importante o desenvolvimento de metodologias que permitam a avaliação dos danos causados ao DNA, caracterizando a detecção de substâncias mutagênicas como primeiro indicador de dano em presença de poluição ambiental.

Pode-se definir um sítio contaminado como área, local ou terreno onde há comprovadamente poluição ou contaminação, causada pela introdução de quaisquer substâncias ou resíduos que nele tenham sido depositados, armazenados, enterrados ou infiltrados de forma planejada, acidental ou até mesmo natural [24]. Ainda de acordo com o Manual de Áreas Contaminadas da CETESB, a definição de áreas contaminadas deve considerar também a ocorrência de danos ou riscos aos bens a proteger (como a qualidade das águas em geral, dos solos e das águas subterrâneas, além da saúde do indivíduo e da população em geral) e não só a presença de poluentes, o que indica que as avaliações devem ser bastante abrangentes.

O número de áreas contaminadas no mundo todo requer atenção especial das políticas públicas. Estima-se que os EUA tenham 45.516 locais contaminados; na Europa, mesmo com os países possuindo uma extensão territorial muito menor, caso da Alemanha, o número de áreas impactadas é considerável: 202.880 áreas [4]. No Brasil, não há estimativas quanto ao número de áreas contaminadas devido à falta de estruturas de fiscalização e registro delas, exceto no estado de São Paulo, onde o órgão ambiental (CETESB) relaciona, desde 2002, uma lista de áreas contaminadas. No último levantamento, em novembro de 2009 houve a identificação de 2.904 áreas, número que tende a ser ampliado conforme avanços na rede de investigação sobre o tema [25].

De acordo com o tipo de contaminação existente no local considerado, pode haver uma distribuição principal dos poluentes nos diferentes compartimentos do ambiente, como solo, sedimentos, rochas, e águas subterrâneas, mas também, de uma forma geral, concentrados em paredes, pisos e estruturas de construções. Estes

contaminantes podem ainda ser transportados a partir destes meios, propagando-se por diferentes vias, como, por exemplo, o ar, as águas subterrâneas e superficiais, e o próprio solo, determinando riscos ou impactos negativos sobre os bens a proteger, localizados na área ou em seus arredores [24].

O interesse pela incorporação de bioensaios na avaliação de riscos de locais contaminados vem crescendo, e é fundamental para embasar as decisões de gestão e controle das inúmeras áreas contaminadas existentes. Em relação à genotoxicidade, a escassez de estudos em solos no Brasil indica a necessidade de ampliação da pesquisa e seleção de biomarcadores para definir a presença de mutágenos e riscos potenciais à integridade dos ecossistemas. Os biomarcadores podem ser considerados medidas que refletem a interação entre respostas biológicas e agentes tóxicos do ambiente, indicadas por modificações celulares ou bioquímicas [26].

Muitos ambientes próximos às áreas industriais são atingidos pelos contaminantes através da ação dos ventos, da chuva ou do assoreamento do solo [27]. Este pode ser via de contaminação direta de diversos compostos orgânicos e metais pesados, atuando como fonte de contaminantes para a atmosfera, por volatilização ou ressuspensão de partículas contaminadas [3], através dos ventos. Da mesma forma, ele é receptor da contaminação adsorvida em material particulado atmosférico. Assim, é essencial para os estudos de rotas de contaminantes a investigação do potencial genotóxico associado aos solos.

Nesse contexto, os ensaios que medem mutagenicidade, como o *Salmonella*/microssoma ou Teste de Ames [28] apresentam biomarcadores eficazes que permitem definir, pelo efeito, a presença precoce de diversos agentes perigosos em diferentes compartimentos ambientais, inferindo suas principais rotas de dispersão no ambiente [23, 29]. O ensaio *Salmonella/*microssoma é um eficiente marcador de genotoxicidade que fornece biomarcadores moleculares para genotoxicidade capazes de indicar a presença de efeitos potenciais de contaminantes, sendo uma alternativa promissora, dentre os indicadores de qualidade do solo, a ser utilizado para avaliações de risco ecológico.

Este ensaio tem aplicabilidade reconhecida internacionalmente na detecção de substâncias com potencial genotóxico em diferentes tipos de amostras: água, efluentes, ar, sedimento [4, 22, 23, 28, 30], sendo que, na revisão da literatura, de White & Claxton [4], o ensaio com *Salmonella* foi recomendado para a avaliação de genotoxicidade de solos impactados por diferentes contribuições antrópicas.

Muitos ensaios de mutagenicidade, entre eles o *Salmonella*/microssoma, têm sido amplamente utilizados para avaliar riscos de contaminação do solo por certos agentes químicos, enfatizando-se neles compostos orgânicos e metais pesados. Entretanto, a abordagem de estudos de mutagenicidade em solos contaminados é recente. De acordo com a revisão feita por Claxton et al. [31], há relativamente poucas publicações associadas à mutagênese de solos em nível mundial.

Alguns dos trabalhos publicados visam avaliar a atividade mutagênica de extratos orgânicos de solos em áreas metropolitanas [32] e residenciais, onde foram encontradas respostas mutagênicas referentes à contribuição de nitrocompostos [33, 34, 35, 36] e em solos sob influência automotiva [35]. Já, em outro estudo, campos agricultu ráveis mostraram mutagenicidade após irrigação com efluentes domésticos e industriais [37]. Em um trabalho de Monarca et al. [1], observou-se resposta positiva decorrente da contaminação por metais pesados e hidrocarbonetos policíclicos aromáticos em solos. Alguns estudos, na Alemanha, indicam respostas de mutagenicidade em função de contaminantes como trinitrotolueno e hexógeno [38] e também atividade mutagênica em função da influência automotiva, em que foi verificado que a maior fonte dos mutágenos do solo era a atmosfera [39]. Na França, diferentes tipos de solos expostos à poluição difusa também mostraram atividade mutagênica [40].

No Brasil, há poucos estudos referentes à investigação de mutagenicidade em solos contaminados: um trabalho com extratos orgânicos de solos sob influência automotiva, no litoral do Rio Grande do Sul [41]; um estudo quanto à presença de compostos orgânicos e metais pesados e seu potencial mutagênico de solos sob a influência de rejeitos de carvão de Silva-Júnior & Vargas [29]; monografias realizadas por Souza [42] sobre mutagenicidade em extratos ácidos de solos, Meyer [43], tratando da genotoxicidade em solos de possíveis locais de referência e Pohren [44], referindo-se

ao potencial genotóxico de solos contaminados com compostos orgânicos e metais pesados avaliados pelo Teste de Ames e pelo ensaio de *Allium cepa*.

Na área de interesse deste estudo, numa antiga usina de preservação de madeira desativada, foram utilizados diferentes produtos químicos a fim de tornar a madeira mais resistente ao ataque e desenvolvimento de microorganismos. Inicialmente foi utilizada solução de pentaclorofenol em óleo e/ou óleo de creosoto. A partir de 1982, a empresa utilizou como preservante de madeira creosoto e hidrossal CCA - arseniato de cobre cromatado e desde 1998 até 2005, passou a empregar exclusivamente o hidrossal no tratamento da madeira. Estes compostos com características químicas altamente tóxicas caracterizam os riscos potenciais existentes na área de estudo.

Os compostos orgânicos com ação tóxica geralmente são de natureza antropogênica e persistentes no ambiente, destacando-se entre eles os hidrocarbonetos policíclicos aromáticos, compostos heterocíclicos e aminas aromáticas [4]. O pentaclorofenol é o principal pesticida usado como preservativo de madeira no mundo todo, sendo um composto persistente e cumulativo, considerado pela USEPA como poluente prioritário desde 1977 [45], e de uso proibido no Brasil [46]. Possui degradação lenta e é hidrofóbico, por isso acumula e é persistente nos ambientes. Entretanto, tem mobilidade ambiental devido à alta volatilidade, apresentando potencial para propagação da contaminação ambiental através da liberação, no ambiente, de dioxinas e seus derivados [47].

O contaminante orgânico creosoto, de uso proibido desde 1998, é um óleo que tem 85% da sua composição formada por HPAs [48, 49, 50], que possuem baixa biodegradabilidade, baixa solubilidade em água, possível efeito prejudicial à biota e carcinogenicidade em humanos [51, 52, 53, 54]. Embora proibido, o número de áreas contaminadas por este produto é significativo, estimando-se que nos EUA mais de 700 locais estejam impactados [55].

Alguns HPAs são selecionados pela USEPA como prioritários, pois estão entre os poluentes mais perigosos devido a sua recalcitrância no ambiente, sua mutagenicidade e sua possível carcinogenicidade [56]. Entretanto, tem sido reconhecido que há outros poluentes da classe de compostos policíclicos aromáticos que contribuem

significativamente para a toxicidade dos ambientes contaminados. Os HPAs contêm dois ou mais anéis aromáticos fundidos, com estrutura formada apenas por carbono e hidrogênio. Compostos da classe dos HPAs que apresentam pelo menos um grupo $–NO_2$ são conhecidos como HPAs nitrados ou nitroarenos (Nitro-HPAs) e reconhecidos indutores mutagênicos, capazes de acarretar prejuízos aos ecossistemas e à saúde humana [57, 58].

Apesar de certos organismos possuírem capacidade de degradar HPAs com até cinco anéis, os nitro-HPAs são degradados muito lentamente, persistindo em solos e sedimentos. A recalcitrância dos nitro-HPAs de alto peso molecular é, em parte, devida a forte adsorção à matéria orgânica do solo, baixa solubilidade, tamanho da molécula e característica polar do grupo nitro.

Dentre os compostos inorgânicos, os metais pesados como Arsênio (As), Cobre (Cu) e Cromo (Cr), resultantes da utilização de hidrossal CCA (arseniato de cobre cromatado) são elementos traço com fortes características de toxicidade associadas [59]. Estes compostos diferenciam-se da maioria dos orgânicos tóxicos, por serem de difícil degradação, podendo acumular-se no ambiente onde manifestam sua toxicidade [4]. A maioria dos metais pesados é solúvel em água e pode ser facilmente absorvida por plantas ou tecido animal. Após a absorção no organismo, tende a se combinar com biomoléculas (como proteínas e ácidos nucléicos), prejudicando suas funções [60], e alterando o material genético [61].

A região de estudo localiza-se no distrito de Barreto, município de Triunfo – RS, em uma área industrial à margem do rio Taquari, em sua planície de inundação. A usina operou de 1960 a 2005, e atualmente encontra-se desativada, possuindo um passivo ambiental identificado em estudo de impacto ambiental na FEPAM, abrangendo uma população periférica sob risco. Em avaliações iniciais, efetuadas pelo empreendedor, foi constatada a presença de metais (As, Cu e Cr) e compostos orgânicos, predominando HPAs, pentaclorofenol, dioxinas e furanos [62].

Levando em consideração estudos já realizados por Grupo de Pesquisa da FEPAM dentro do projeto “Estratégias ecotoxicológicas para caracterizar áreas contaminadas como medida de risco à saúde populacional, MCT - CNPq/ CT -

SAÚDE", foram selecionadas as possíveis áreas sob influência do sítio contaminado. Nestes estudos prévios, foi sugerida uma rota de migração de metais e de HPAs através do vento, com deposição de poeira em pontos fora do sítio industrial, indicando possíveis rotas de migração por veiculação atmosférica através da poeira, que segue a segunda direção preferencial dos ventos, a noroeste, e possível influência do micro-clima na área [62].

O presente estudo procurou definir amostras de solos da área interna da indústria, como fonte de contaminação genotóxica para áreas de entorno. Investigou a rota de dispersão dos contaminantes mutagênicos no ambiente do solo em áreas de influência e em distâncias gradativas. A coleta das amostras de solo foi realizada em locais estratégicos, de acordo com a possível dispersão de contaminantes, visando a definir a abrangência da contaminação e os riscos ambientais e à saúde humana existentes. Estes locais investigados foram solos de ambientes urbanos e rurais em residências no entorno do sítio contaminado que recebem as maiores concentrações de contaminantes, a partir da fonte considerada. O estudo buscou também relacionar respostas genotóxicas, com critérios físicos e químicos dos solos e tipologia dos estressores para evidenciar as áreas críticas e possíveis áreas de referência para o estudo. Ainda foram selecionadas amostras de poeiras domiciliares que servem como indicativo do histórico de deposição atmosférica ao longo do tempo, podendo caracterizar influências da área de contaminação próxima.

Portanto, foram selecionados locais de amostragem em áreas de risco influenciadas pela remobilização de partículas e deposição atmosférica a partir de área contaminada e possível local de referência para permitir a estimativa dos riscos à saúde humana e ao meio ambiente através das respostas genotóxicas.

A hipótese de trabalho foi: a avaliação da atividade mutagênica em solos impactados marca a presença de contaminantes e permite verificar a dispersão de compostos mutagênicos em áreas de risco de entorno selecionadas em diferentes distâncias a partir da principal fonte de contaminação do estudo.

2 Objetivos

Geral

Caracterizar o solo como uma fonte de dispersão de agentes mutagênicos para áreas de entorno em sítios contaminados, através de biomarcadores de genotoxicidade, critérios físicos e químicos dos solos e tipologia dos estressores presentes.

Específicos

i) caracterizar o potencial mutagênico de solos em área contaminada através do ensaio *Salmonella*/microssoma como marcador de mutagenicidade;

ii) selecionar locais de amostragem em solos de áreas de referência e de risco influenciados pela remobilização de contaminantes a partir da área contaminada;

iii) estimar impactos da contaminação em matrizes ambientais de área de influência do sítio contaminado definidas a partir de rotas preferenciais de dispersão de contaminantes do solo;

iv) relacionar classes químicas de contaminantes e nível de efeitos mutagênicos em linhagens bacterianas específicas;

v) definir os agentes químicos estressores e relacionar presença e concentração de contaminantes, nível de efeitos mutagênicos e características dos solos.

3 Artigo Científico

O presente trabalho resultou na preparação de um artigo científico que relata a investigação da mobilidade de contaminantes mutagênicos a partir de solos impactados visando estimar rotas potenciais de dispersão e riscos associados através de biomarcadores de genotoxicidade e parâmetros físicos e químicos: “Mutagenicidade de solos como estratégia na avaliação de riscos de área contaminada”.

3.1 SOIL MUTAGENICITY AS A STRATEGY TO EVALUATE RISKS IN A CONTAMINATED AREA

Roberta de Souza Pohren[a,b], Jocelita Aparecida Vaz Rocha[a], Karen Alan Leal[a] and 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.

*****Programa de Pesquisas Ambientais, Fundação Estadual de Proteção Ambiental Henrique Luís Roessler (FEPAM), Avenida Salvador França, 1707 CEP: 90690-000, Porto Alegre, RS, Brazil. Tel.: +55 51 33346765; fax: +55 51 33346765. ecorisco@fepam.rs.gov.br

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ABSTRACT

Soil can be a storage place and source of pollutants for interfacial environments. This study looked at a site contaminated with wood preservatives as a source of mutagens, defined routes and extent of the dispersion of these contaminants by particle remobilization and atmospheric deposition. Soil sampling sites were chosen at gradually increasing distances (150, 500 and 1700m) from SI (industrial area *pool*) and residential dust (*pool* in an area at risk at 385m and at 1700m). Mutagenesis was evaluated in the *Salmonella*/microsome assay, TA98, TA97a and TA100 strains with and without *S9 mix,* YGs strains 1041, 1042 and 1024 for nitrocompounds. Acid extracts were analyzed to define the effects of metals and organics for polycyclic aromatic hydrocarbons (PAHs) and nitroderivates, besides concentrations of these compounds and pentachlorophenol (PCP). Metal concentrations showed a gradient of responses with As, Cr and Cu (total metal) or Cr and Cu (fraction available) higher for SI. However, mutagenic effects of the mixtures did not show this grading. Site SR1700, without a response, was characterized as a reference. In organic extracts, the mutagenesis responses showed the mobility of these compounds from the source. In the surrounding area, a smaller pattern similar to SI was observed at SR150, and at the other sites elevated values of direct mutagenesis at SR500 and diminished effects at SR1700. Tests with YG strains indicated that nitrated compounds have a significant effect on the direct mutagenesis found, except SR500. The investigation of residential dust in the surrounding area enabled confirmation of the particle resuspension route and atmospheric deposition, showing responses in mutagenicity biomarkers, PAHs concentrations and PCP dosage similar to SI. The study showed that it is essential to evaluate the extent of contamination from the soil to delimit remedial measures and avoid damage to the ecological balance and to human health.

Key-words: mutagenicity, soils, dust, dispersion, PAHs, metals, pentachlorophenol.

1 Introduction

Contaminated areas are an increasing concern worldwide. In many of these impacted sites, there is a mixture of different pollutants, leading to additional problems in evaluating the extent and risks of contamination. Due to possible influence on the ecosystem, and potential health damage, it is necessary to investigate associated environmental risks, but this is difficult to do in complex environmental matrices such as soil. The chemical properties of the soils, amounts and types of xenobiotics, determine the toxicity and persistence of pollutants in the environment, as well as their effects. Tools such as biological tests are useful to integrate the effects of all bioavailable contaminants and their interactions in the ecosystems [1, 2, 3].

Industrial activities such as the preservation of wood poles result in thousands of contaminated soil sites in several countries. At these sites there are mainly contaminants such as polycyclic aromatic hydrocarbons (PAHs), dioxins and metals, such as arsenic, copper and chromium [4]. Among the PAHs, 16 are considered priority pollutants according to USEPA. Eight of them are considered potentially carcinogenic: benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene e naphthalene [5]. Although the other compounds cannot be classified as carcinogenic to humans, they can modulate the biological responses, i.e., increase or diminish the carcinogenic PAHs responses, i.e.,: acenaphthene, acenaphthylene, anthracene, benzo[g,h,i]perylene, fluoranthene, fluorene, phenanthrene and pyrene [6, 7].

In the region investigated in this study, there is a mixture of these different classes of organic and inorganic chemical compounds resulting from the industrial activity of wood preservation. Among the contaminants of interest with known mutagenic action [5] are PAHs – indicating the use of creosote in the area,

pentachlorophenol (PCP) which is a precursor of dioxins and heavy metals, such as Arsenic, Copper and Chromium, originating from the hydrosalt CCA [8].

Due to the disposal of persistent compounds with a mutagenic potential, the soil matrix forms a complex mixture. The variety of contaminants received and their possible interrelationships may be hazardous to biota and to the other interfacial environmental matrices. Biomarkers are appropriate tools for the evaluation of risks in contaminated areas, since they allow estimating the potential of complex mixtures regarding toxicity and genotoxicity [1, 2, 9]. These assays evaluate possible synergistic or antagonistic effects of the contaminants, broadening the investigation performed by physical and chemical parameters commonly used in evaluating complex mixtures. Among these assays, the *Salmonella*/microsome test is effective to evaluate genetic damage produced by environmental mutagens, and it is internationally acknowledged and used [10, 11, 12, 13]. It allows defining the early presence of contaminants in environmental compartments by their effect, indicating their main dispersion routes in the environment [14, 15] and is an essential tool to evaluate the environmental quality of contaminated sites.

The evaluation of soil mutagenicity using the *Salmonella*/microsome assay may provide significant information in procedures to characterize environmental risk in contaminated areas. This occurs in the investigation of risks involving mixtures such as PAHs. The detection of their combined effects presents a special challenge since there is no compound that determines ecotoxicity alone [16]. Contaminants can be transported from the different environments, propagating by pathways such as air, soil itself, groundwater and surface water [17].

Thus, pollutants can be distributed from the main source of contaminants, in this case the industrial site soils, to the surrounding soils, influenced by the remobilization

of particles and atmospheric deposition. Hence, the study investigated the mutagenic potential of soils in a contaminated area, identifying possible routes to the surrounding soil at gradually increasing distances from the site. It associated the study of house dust samples as a historical indication of this deposition, seeking to characterize the influence of the existing contamination area. In addition to the *Salmonella*/microsome assay as a marker of mutagenicity, physical and chemical parameters were evaluated in the soil and dust matrices, and dosages of the main chemical groups potentially present.

2 Material and Methods

2.1 Area of Study

The area of study is located in the municipality of Triunfo (Rio Grande do Sul, RS, Brazil), close to the confluence of the Taquari and Jacui rivers, in the northeast of the state of Rio Grande do Sul, on the left bank of Taquari river (Figure 1). The relief of this area in the Taquari flood plain does not vary much. Local geology is characterized by sedimentary rocks, fine to very fine sandstones with intercalations of laminates (argilites and shales) [17].

The soil at the site is contaminated due to the industrial activity of a wood preservative plant which operated from 1960 to 2005. The chemicals used to treat the wood were, initially, a pentachlorophenol solution in oil and/or creosote oil, from 1982 onwards, the alternate use of creosote and hydrosalt CCA (Chromated Copper Arsenate) and, since 1998, the use of hydrosalt exclusively. The area is located on the outskirts of an urban area, with predominantly rural characteristics and a limited residential area. The plant is no longer active and an environmental problems has already been identified as the result of the use of preservatives, with the presence of metals (As, Cu and Cr) and

organic compounds, predominantly PAHs, as creosote degradation products, besides pentachlorophenol, a dioxin precursor in the area [17].

2.2 Sampling Sites

The sampling sites were selected according to a study previously performed by the research group, in which it was found that, metals and PAHs migrate from the soil of the contaminated area, through wind, with dust deposition at points outside the industrial site [17].

Thus, soil sampling was performed at strategic sites according to the possible dispersion of these contaminants of interest, looking at their potential dispersion route. The samples investigated were soils from the industrial site and from urban and rural environments in the houses around the contaminated site, located in the second preferential wind direction, sampled at 2009-2010. Residential dust samples were also collected and they are a historical witness helping delimit the area of influence of the source of contamination.

The sampling points selected were: *(i)* SI, industrial soil, a soil pool representing the contamination of the internal area of the company (S 29º 52' 17.13", W 51º 43' 7.26"; S 29º 52' 17.27", W 51º 43' 8.8"; S 29º 52' 17.94", W 51º 42' 56.32") and (*ii* to *v*) soil samplings in areas close to the plant, progressively distant from the main source of contamination, SR, residential soils. Residential soils were: *(ii)* SR150 at approximately 150m (S 29° 52' 23.7", W 51° 43' 4.94"), *(iii)* SR500, at approximately 500m (S 29°52'26.39", W 51°42'48.70"), *(iv)* SR1700, sampling at a site of possible reference soil 1700 m from the area (S 29° 52' 40.6", W 51° 42' 3.7"), *(v)* DR385, a pool of dust collected in houses located in the risk area at approximately 385m from the site (S29°52'24.20", W51°42'51.84"; S29° 52'24.53" , W51° 42' 53.17"; S29° 52'22.62",

W51° 42' 56.77"; S29° 52'23.27", W51° 42' 57.13"; S29°52'22.35", W51°42'57.52"; S29° 52'22.76", W51° 42' 58.54") and *(vi)* DR1700, a pool of dust collected at an area away from this site, to be considered a reference dust (S 29º 52' 40.61", W 51º 42' 3.7") according to Figure 1.

The granulometric characterization of soil samples had a simple classification, where, among the soils sampled, SR150 has a higher percentage of clay, followed by SR1700, SI and SR500. In the composition of silts, soils are in the following order: SR150>SR500>SR1700>SRSI. As to the percentage of organic matter (OM) and organic carbon (OC) in these soils, it is observed that SR500 is outstanding with higher values than the other points. It has a greater than double value of OM and OC (2.8 and 1.3% respectively) compared to the collection point most distant from the area, SR1700 (1.1 and 0.51% respectively).

2.3 Sample collection

The samples were collected according to the recommendations of the *Health and Environmental Guidelines for Selected Timber Treatment Chemicals,* New Zealand [18] and USEPA [19], and all the points were marked by GPS.

Approximately 1 Kg of samples were collected for soil sampling at a depth of 0 to 20 cm, consisting of three sub-samples, homogenized to obtain a single sample for each of the collection points – and the excess plant residue was removed. The samplings were performed after a minimum period of seven days without rain. The samples were collected using stainless steel spatulas. Then they were dried at ambient temperature for up to 48 hours, selected in a sieve (2 mm) and placed at 4ºC in dark glass flasks protected from light until they were used for the tests [20].

Dust samples were collected in the attics of six residences distributed throughout the area under the influence of the dispersion from the wood preservation plant, forming a pool influenced by the contaminated area. The reference dust was collected at a single home away from the area of influence of the industrial site. The sampling was performed using the sweeping technique [21, 22], where the material deposited under the roof of the houses was collected through the trapdoor, removing the material deposited within arm's reach. Brushes from natural bristles were used to collect the dusts which were stored on glass plates, kept at 4ºC and protected from light until they were used in the tests.

2.4 Sample Preparation

The soil samples collected were used to prepare acid and organic extracts for later analysis. On the other hand, only organic extraction was performed for the dust samples.

2.4.1 Acid Extract of Soils

This extract was prepared according to the Norma Brasileira NBR 10005 (Brazilian Standard) [23], adapted according to experience in the laboratory. The samples were solubilized in an acetic acid solution (CASRN. 64-19-7) and sodium hydroxide (CASRN. 1310-73-2) (soil: solvent, 1:2, g/mL, acid solution pH 4.91 - similar to the pH of the soils at the site, shaken in a table shaker for 24 hours, and later centrifuged at 13,000 x g, for 15 minutes at 4 °C, filtered in a Millipore membrane with 0.45µm porosity, divided into aliquots: one of them stored for up to 24 hours at 4ºC, to evaluate mutagenicity using the *Salmonella*/microsome assay and the other aliquot processed for the analysis of metals.

2.4.2 Organic Extract of Soils

These extracts were obtained according to the USEPA method (EPA *Method* 3550C. *Ultrasonic Extraction,* 2007) [24]. The soil sample was homogenized for 15 minutes with a stainless steel spatula, and in 15 g of this sample the solvents, pesticide grade dichloromethane (DCM, CASRN. 75-09-2) and methanol (MeOH, CASRN. 67-56-1) were added at a proportion of 2 (DCM): 1 (MeOH) and extracted in ultrasound (THORNTON – 1800W power) for 10 minutes in two cycles. The liquid was filtered in a chromatographic column of sodium sulfate and celite and concentrated in a rotary evaporator 40°C. The extract was stored in a graduated tube, from which a 1 mL aliquot was removed to determine the extracted organic matter (EOM). This final extract was divided into an aliquot for analysis of the organic compounds and another for the mutagenicity assay.

2.4.3 Organic extract of Dust

The domestic dust samples were extracted using the DCM solvent at a proportion of 2 (dust) : 3(solvent), in ultrasound (THORNTON - power 1800 W) in a 10-minute water bath (stage repeated three times), at a controlled temperature of 30ºC, followed by simple filtration with a Millipore membrane, 0.5µm porosity, washing the sample with the same solvent. The filtered volume was concentrated in a rotary evaporator at 40°C, to obtain the final extract. The extract was stored in a graduated tube, from which a 1 mL aliquot was removed to determine the extracted organic matter (EOM). This final extract was divided into an aliquot to analyze the organic compounds and the other for mutagenicity assay.

2.5 Evaluation of Mutagenic Activity

The *Salmonella*/microsome test by microsuspension method, Kado Test [25, 14], a modification of the assay [10], which allows analyzing small amounts of sample, was used to evaluate mutagenicity. In the soil samples this assay was performed in organic and acid extracts. On the other hand, for the dust samples, the *Salmonella*/microsome test was performed in organic extracts.

The assays were performed in duplicate, including negative controls (nutrient medium- 100µl/plate, and the solvent used in the assay: 100 µl/plate of acid solution for inorganic extract and 5µl DMSO/plate for organic extract) and positive according to the strain and treatment used: (4-oxide-nitroquinoline: 4NQO, 0.5µg/plate, CASRN. 56-57-5 *Sigma Chemical Company*; sodium azide: SAZ, 0.5µg/plate, CASRN. 26628-22-8 *Sigma Chemical Company*; 2-nitrofluorene: 2NF, 0.15µg/plate, CASRN. 607-57-8 Merck do Brasil; and 2-aminofluorene: 2AF, 1µg/plate, CASRN. 153-78-6 *Sigma Chemical Company*).

Mutagenesis was evaluated for soil samples at six concentrations, which were the following for acid extracts: 25, 50, 75, 100, 150 and 200 mg of dry soil equivalent; for the organic extracts, the concentrations tested were: 10, 20, 40, 80, 120 and 160 mg of dry soil equivalent; on the other hand the dust samples were tested at different concentrations: 2, 4, 8, 12, 20, 40mg of dry dust equivalent, and the a reference dust was tested only at the first four concentrations. All the tests were performed in the presence and absence of a P450 *in vitro* metabolism exogenous system of mammals, *S9 mix* [10]. The assays with the YGs strains were performed only in the absence of *S9 mix*. In the test aliquots of the bacterial strain (growth of $1X10^{10}$ cell/mL) of the different concentrations of soil extract and *S9 m*ix or 0.1 M phosphate buffer were incubated for 90 min at 37°C. This mixture was solidified with a soft agar solution

containing traces of histidine and biotin in Petri plates, incubated for 72 hours at 37ºC [25].

The *Salmonella typhimurium* strains, TA98 and TA97a, which detect frameshift mutagens were used, TA97a being described in the literature as most sensitive to heavy metals [26] and PAHs [10]; TA100 which detects mutagenics by base pair substitution [10]. Strains with a high production of nitroreductase and O-acetyltransferase were also used in the organic extracts. They are specific for the diagnosis of nitrocompounds: YG 1041 (derived from TA98) and YG 1042 (derived from TA100) sensitive to mono/dinitroarenes, and YG 1024 (TA98 derivative) sensitive to dinitroarenes and aryl-hydroxy-amino compounds [27, 28, 29].

The cell survival assay was performed in parallel to the mutagenicity test. In this assay, the survival rate was expressed as a percentage of colonies grown in a complete medium at the different concentrations of the sample in relation to the number of colonies formed in the control plate. Samples with less than 60% cell survival in at least one of the dosages compared to the negative control were considered cytotoxic [30].

Total soil extract blanks were also made as a negative control. The same methodologies were used, without a sample, with the same procedures and same solvents used for sample extraction. Strain TA100 of S.*typhimurium* was used to evaluate blank mutagenicity and did not show any mutagenic activity. The blanks were tested with the amount of extract corresponding to the maximum concentration of equivalent sample per plate utilized.

2.5.1 Analysis of the Mutagenicity Assay Results

Mutagenic activity was expressed by the number of revertants per dry gram of soil equivalent (rev/g dry soil equivalent) or revertants per mg of dust (rev/mg dust),

calculated by the linear portion of the dose-response curve. The number of revertants per plate was analyzed using the statistical program SALANAL (Salmonella Assay Analysis, version 1.0 of Research Triangle Institute, RTP, North Carolina, USA) selecting the linear or Bernstein models. The sample was considered mutagenic when statistical significance was observed in the regression analysis ($p \leq 0.05$) and in ANOVA ($p \leq 0.05$) [11, 30, 31].

2.6 Chemical Determinations in the Soil Samples

The organic extracts of soil were obtained using the EPA 3550C method – extraction by ultrasound [24], and the PAHs were analyzed by gas chromatography coupled to the mass spectrometer (GC/MS SHIMADZU model QP5050A Quadrupole system by SIM - single ion monitoration). The main PAHs species were investigated, focusing on 16 species classified as priority pollutants: Acenaphthene, Acenaphthene, Anthracene, Benzo(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthrene, Benzo(g,h,i)perylene, Indeno(1,2,3-cd)pyrene, Benzo(k)fluoranthrene, Chrysene, Dibenzo(a,h)anthracene, Phenanthrene, Fluoranthrene, Fluorene, Naphthalene, Pyrene. On the other hand, in the acid extracts, metals of interest were investigated (fraction available) by inductively coupled plasma optical emission spectrometry (ICP-OES/PERKIN ELMER/OPTIMA 7300 DV) at the Soils Laboratory of the Federal University of Rio Grande do Sul.

The raw soil samples were characterized as to total metal content by X-Ray fluorescence spectrometry (XRF), (Philips, model PW 2404, Netherlands) at the Geochemistry Laboratory of UNICAMP, in Campinas, São Paulo, Brazil and as to the contaminant pentachlorophenol (PCP) by GC/MS after organic extraction at Laboratorio Bioagri Ambiental in Piracicaba, São Paulo, Brazil.

2.7 Chemical determinations in the Dust Sample

The dust samples were submitted to extraction for PAHs quantification by Gas Chromatography with Mass Spectrometry, according to the EPA 8270-d method at ALAC Laboratory (ALAC - Análises Laboratoriais Assessoria e Comércio, RS, Brazil) in, and of the contaminant pentachlorophenol (PCP) by GC/MS after organic extraction at Laboratório Bioagri Ambiental.

2.8 Statistical Comparative Analysis

Descriptive analyses of data were performed using graphs to compare the effects observed for each mutagenicity biomarker in SI, and the possible dispersion of these effects at the surrounding points, 150, 500 and 1700 meters away. The levels of the main stressors detected in SI and surrounding sites were also compared: PAHs for organic extracts and heavy metals for acid extracts. These analyses sought to find whether the profile of effect and SI stressors is repeated similarly at the different distances analyzed

3. Results

3.1 Evaluation of mutagenic activity

The mutagenic activity of soils in the area studied was evaluated by the *Salmonella*/microsome assay, using different strains in the presence and absence of the hepatic metabolism fraction in mammals (S9 mix). Acid extracts were studied to define the effects of inorganic compounds such as heavy metals, and organic ones emphasizing the detection of PAHs and nitroderivate activities.

3.1.1 Mutagenicity in Acid Extracts of Soils

The acid extracts of soils (Table 1) presented significant mutagenic responses in the different strains, and the internal soil of the industrial area, SI, presented the greatest sum of effects of the different strains, except for base pair substitution mutagens depending on the presence of S9 *mix*. As to the samples located at gradually increasing distances from the site, SR150 sample, presented mutagenic activity only in the base pair substitution strain, TA100, both in the presence and absence of exogenous metabolism. The SR500 soil showed a differentiated mutagenesis with a high response in frameshift TA98 strain in assays with S9 *mix* and less intensity in TA97a and TA100 in the absence and presence of S9 *mix* respectively; soil SR1700 did not present a mutagenic response. Evaluating cell survival, almost all samples were cytotoxic at a concentration of 100mg dry soil equivalent, in the presence of *S9 mix*. However, the assays in the absence of *S9 mix* showed cytotoxicity only for SR1700 at a concentration of 75mg dry soil equivalent.

The sum of mutagenicity responses obtained in the acid extracts (Figure 2) emphasizes the different sensitivities observed in the assays. There is a clear presence of base pair substitution mutagens depending on *S9 mix* at sites SR150 and 500, but absent in SI.

3.1.2 Mutagenicity in Organic Extracts of Soils

The mutagenesis responses for the organic extracts (Table 2) showed a predominance of compound with indirect action. In the soil of the contaminated area (SI), the mutagenicity values ranged from 107 to 1455 revertants/g dry soil equivalent. In the surrounding sites sampled, a pattern similar to SI was found at SR150 with a variation of 33 to 1285 rev/g dry soil equivalent; on the other hand, at SR500 high values of direct action mutagenesis were shown in TA97a; the samples of the possible reference site – SR1700, although presenting mutagenesis in frameshift strains in the

presence of S9 *mix*, showed decreased total effects. Most of the samples presented cytotoxicity at the concentration of 160 mg and 120 mg/plate tested both in the presence and in the absence of S9 *mix*.

The information on EOM showed that the soil of the internal area resulted in a higher amount of extracted material (2170 µg/mL) than in the other points where soils were sampled. The values of the samples from the surrounding areas (SR150 = 530 µg/mL; SR500 = 740µg/mL; SR1700 = 570µg/mL) were similar to each other.

Considering the sum of effects detected by the different strains tested (Figure 3), it can be seen that the mutagenesis of organic extracts of soils allowed the visualization of a profile similar to SI in the soils surrounding the contaminated area at SR150, although with the absence of a response in TA97a in the presence of S9 mix; SR500 presented differences in the global spectrum detected, with the presence of direct action frameshift compounds, detected by strain TA97a and the sum of higher effects than those observed in SI; SR1700 presented a lower intensity mutagenic response.

Observing specifically the effects of the strains tested with *S9 mix*, it is noted (Figure 4) that the pattern detected at SI is expressed to a greater or lesser extent in the surrounding soils, damages of the DNA frameshift error type predominating. They may be associated with the PAHs, identified in the chemical analyses with a pattern in accordance with the biological responses.

Observing the effects with the different strains in the absence of metabolism (Figure 5), a similar and not very expressive pattern is noted in the samples, except at SR500 which presents high mutagenic activity in the TA97a strain, indicating the presence of differentiated compound groups; and SR1700 where no base pair substitution mutagens occur.

Evaluating the sum of effects resulting from organic and inorganic compounds detected by the different strains used (Figure 6) the magnitudes of responses obtained at the different sites are clear. Thus, the high response observed in direct assays of the TA97a strain at SI (3933±0.736 rev/g dry soil equivalent) showed the presence of inorganic compounds (Table 1) and at SR500 (3637±0.467 rev/g dry soil equivalent) the presence of organic compounds (Table 2). There is an outstanding difference found at SR1700, with mutagenic activity that is only organic and much lower than the internal soil of the contaminated area. As to SR150 the outstanding difference is the absence of responses to TA97a.

In the evaluation of the presence of nitrocompounds in the mutagenic responses, the use of strains YG1041 and YG1042 showed that this class of compounds contributed significantly to the existing damage potencies (Figure 7.a), except at SR500. At this site it was clear that the damages detected were not mainly the result of nitroderivates (Figure 7.b). The presence of hydroxyamino-compounds was also found in all soil samples through strain YG1024 (SI, 543±0,04; SR500, 474±0,048; SR150, 274±0,026 and SR1700, 274±0,032 rev/g dry soil equivalent).

For all samples, the mutagenic potency was higher in the strains sensitive to nitroarenes (Figure 7.b), than in their respective ancestral strains. The SI soil presented the mutagenic activity of 3726±0,335 rev/g dry soil equivalent in strain YG1041 compared to their parental TA 98 (107±0,04 rev/g dry soil equivalent); similarly, but with a higher magnitude, it was observed compared to strain YG1042 and the derivative TA100 (4519±0,278 rev/g dry soil equivalent), and this pattern was similar in SR150 (3849±0,233 rev/g dry soil equivalent) and SR1700 (4572±0,917 rev/g dry soil equivalent), although of a different nature, with emphasis on YG1041 and YG1042,

respectively. Site SR500 continued to present differentiated values: 469±0,082 rev/g dry soil equivalent in strain YG1041.

3.1.3 Mutagenesis in Organic Extracts of Dust

As a second route to estimate the dispersion of contaminants from soil resuspension, it was decided to investigate the dust accumulated in the attics of houses located in the surroundings of the contaminated site (Table 3). In the sample pool of six houses located approximately 385 m from the site, which compose sample DR385, high positive responses were observed in the different strains.

The information on EOM showed high values for the residential dust samples, especially in the area of risk (DR385 = 6330 µg/mL; DR1700 = 1290 µg/mL).
Analysis of the nitrosensitive strains showed the presence of mono and dinitroarenes, only of the pair substitution mutation (YG1042) type. The presence of hydroxylamine-compounds was also found through strain YG1024.

In the sample of dust collected at a distance of 1700 from the area, DR1700 (reference area) no positive response was found for mutagenicity (Table 3).

3.2 Chemical Evaluations

3.2.1 Evaluations of Metals in Soils

The raw soil samples were analyzed for the total content of metals of interest, As, Cu and Cr (Table 4). Comparing them to guiding values for soils established in CONAMA Resolution 420 [32] (Table 5), it could be noted that As, in industrial soil, SI, presented a higher value than the limit of investigation in an agricultural area (35 mg/kg); also in SI, the Cr concentration was above the prevention value (75 mg/kg). According to Dutch norm [33] (Table 5), the levels for these metals are above (As and Cu) or similar (Cr) to reference values. In the soils of the surrounding area the results

were below the reference values for prevention. It is perceived that SR1700 presents lower values for the metals evaluated, compared to the other surrounding soils. The results for mercury (Hg) were within the limit foreseen in the legislation, of 0.5 mg/Kg of soil at all points [32]. Evaluating the sum of these metals at each point sampled, it is noted that SI presents higher values (193 mg/Kg), followed by SR500 (48.9 mg/Kg), SR150 (45.5 mg/Kg) and SR1700 (39.5 mg/Kg).

Table 4 presents the analysis of the bioavailable fraction of metals extracted in the acid fraction. Since this concerns only the mobile metal content in soils, it can be seen that all values were lower than those found in the analysis of total metals (Table 4). Taking the results found in SR1700 as reference, the most distant point from the contaminated area, it is noted that most of the metals show values below this soil for possible reference. Comparing the sums of metals found in the available fraction, it is seen that SI and SR150 – the closest point to the contaminated area, presented similar values (2.1 mg/Kg and 2.7 mg/Kg respectively). On the other hand, SR500 presents a higher value equal to 4.16 mg/Kg.

The metal As was lower than the limit of detection in the technique used for all samples. In the SI soil, the values of Cr and Cu are clearly higher than those of the other samples (0.032 and 0.06 mg/Kg respectively); at SR500, the concentration of Fe, Mn, Zn and Al was higher than in the other samples, including the sample of internal soil from the contaminated area. Cu in both techniques, both for total metals and for available fraction, was higher than the other samples in the surroundings at SR500 (16.0 mg/Kg and 0.02 mg/Kg respectively). Metals such as Pb and Cd presented very similar profiles in all samples.

3.2.2 Evaluations of PAHs in Organic Extracts of Soils

The results of the total PAHs concentrations (Figure 8.a) and the potentially carcinogenic (Figure 8.b) analyzed in the organic extracts show a contamination profile in the area. SI is outstanding, with high values for organic compounds, decreasing as the distance from the area increases.

The PAH concentrations found surpass the legal limits of guiding values for soils presented in mg/Kg [32] (Table 6), some of these compounds being outstanding. In SI, the concentration found for benzo(a)pyrene was the only one that surpassed the limit of investigation, and was eight times greater than the quality reference in agricultural soils; for benzo(a)anthracene, it proved 20 times higher than the limit foreseen for prevention, and in the other PAHs the latter was surpassed from two to nine times. In the surrounding soils, it was observed that some PAHs presented values from 1.2 to 5.0 times the limit of prevention, with an outstanding and continuous presence of benzo(a) pyrene surpassing the limits of prevention.

3.2.3 Evaluations of PAHs in Organic Extract of Dust

The concentrations of priority PAHs in the dust pool collected in the houses close to the contaminated site, DR385, were also measured. A concentration equal to 41,064.05µg/Kg of soil was observed, almost double the value found in the internal soil of the contaminated area (24,946.17 µg/Kg). The sum total of potentially carcinogenic PAHs is equal to 8,605.548 µg/Kg also higher than that of SI (3,436.02 µg /Kg) (Figure 9). Observing the isolated concentrations except for naphthalene and chrysene, the values of PAHs in dust were higher than those observed in soils.

3.2.4 Evaluation of PCP in Soils and Dust

The concentration of pentachlorophenol as a specific marker of the area was investigated and a concentration of 0.491 mg/Kg was found in the pool of dust collected from the homes, DR385, similar to that detected in SI: 0. 431 mg/Kg dry soil. These values were higher than the limit foreseen in the legislation concerning soils (0.16mg/Kg dry soil) [32]. In the other soils the PCP value found was below the method limit of detection.

4. Discussion

The study sought to create work strategies to investigate contaminated soil sites as sources of toxic and genotoxic compounds, routes and extent of dispersion of these contaminants through the soil, and to define the existing environmental and human health risks.

The different characteristics presented by the complex soil matrix may directly influence the toxicity responses and the route of the contaminants found [34, 35], rendering them vulnerable to the contributions of environmental contamination. The heterogeneity of the spatial distribution of soil characteristics and contaminant concentrations should be considered [16] in the criteria used to select sampling points and to interpret the results found. Differences in the type of soil affect the composition of the mutagenic constituents, and may influence the bioavailability of contaminants. Considering the characteristics of the soils in the region studied, a large part presents a potential for contaminant concentration on the surface, favoring dispersion from the soil itself [8].

The study investigated the dispersion route of the mutagenic contaminants in the urban environment through samples of soils and residential dust in areas under the influence of a deactivated wood preservative plant with known environmental problems. Thus, sampling sites were chosen, gradually more distant from the source, possibly

influenced by the remobilization and deposition of atmospheric particles. The residential dust survey performed in surrounding areas looked for an indication of atmospheric deposition over time.

In order to estimate the industrial area soil as a source of mutagenic contaminants for areas surrounding the plant, the work strategy related the genotoxic responses obtained, with physical and chemical criteria of the soils including the typology of main stressors, showing the critical surrounding areas and possible sites of reference.

The company operated for over 40 years, producing and disposing of large volumes of residues and inputs of the process used within its area. The residues generated are potential environmental hazards. In this study the atmospheric route of pollutant dispersion from the area of interest is investigated.

In order to evaluate the different soil samples, the *Salmonella*/microsome assay was used as a biomarker of mutagenicity acknowledged by the international community to diagnose this environmental matrix [34]. The soil samples, constituted by a mixture of different classes of chemical substances, were separated for analysis in acid and organic extracts. In acid extraction priority was given to evaluating effects caused by heavy metals based on contamination in the area investigated due to the use of hydrosalt CCA ($CrO_3.CuO.As_2O_5$). This product presents known carcinogenic characteristics according to the *International Agency for Research on Cancer* (IARC) [5], and is formed by the association of several salts in proportions varying according to type.

The comparison of raw soil samples with the guiding values for soils established in CONAMA Resolution 420 [32], indicated a variation in the total content of metals of interest. In SI, As was the only element that surpassed the limit value of investigation for agricultural areas, as well as the concentration of Cr that was above the prevention

value. It is important to emphasize that, comparing total amounts of metals of interest in SI, these are bellow investigation or industrial intervention levels presented by Brazilian and Dutch legislation [33] respectively (Table 5). However, for As the amount is above investigation levels for agricultural areas and Cr is above prevention values considered in National law. According to Dutch norm, the levels for these metals are above (As and Cu) or similar (Cr) to reference values.

Some toxic materials in soil are already in the toxic form, or are potentially able to resolubilize in water, others are firmly connected to the soil particles and would be unlikely to resolubilize. In this way, the extraction of soil samples with a weakly acid solution was an appropriate method to investigate the bioavailable soluble fractions of metals. It was defined that extraction would be performed in a pH similar to that of the soils in the region of study, pH 4.91, more effective than using aqueous extracts [9, 20, 36].

In the evaluations of mutagenesis performed in acid extracts, no clear pattern of transfer to the environment was seen, but the differences in assay sensitivity were clear. The source soil SI showed mutagenic activity in all strains, underlining the results obtained in an assay with TA97a in the absence of S9*mix*. The negative responses observed in SR1700 allowed considering it as a reference for the area of study as to inorganic compounds. This result may reflect the existence of a contamination gradient of these compounds beginning in the area considered the source, since this point is located further away, 1700m from the contaminated area. The other samples of the surrounding soil presented mutagenic potency in at least two different strains, but only one that is in accordance with SI. Soil SR500, showed an expressive result of 3,856±0.458rev/g dry soil equivalent (Table 1) in strain TA98 with S9, indicating a differentiation in terms of effect in relation to the other samples. Another difference

detected is sensitivity to base pair substitution mutagens in assays in the presence of S9 *mix* in the surrounding soils, SR150 and SR500, absent in SI.

In the evaluation of metals of the available fraction of acid extracts (Table 4), considering the concentrations observed at site SR1700, negative for mutagenesis, as a reference for the area, considerable variation was observed in the concentration of some metals in the other soils collected. Some of the metals present in the area, which are most representative of environmental concern and risk, thanks to their easy solubilization and mobilization, by order of priority, are: chromium (Cr), copper (Cu), nickel (Ni), zinc (Zn) and aluminium (Al) [37]. The SI soil presented as enriched for chromium, copper and nickel compared to the other points studied, characterizing the possible influence of these metals in the mutagenesis responses found.

Cu is an essential element, but necessary to organisms in small amounts. Although there is no determination of carcinogenicity for copper according to IARC [5], at concentrations above the limits required by an organism, it can trigger toxic responses, with a potential for bioaccumulation [38]. Chromium in soil is present mainly in the insoluble form, with low mobility. The anaerobic decomposition of organic matter in soil can increase chromium (III) mobilization through the formation of soluble compounds, especially if the pH is low. Chromium (IV), soluble and non-adsorbed, and the soluble complexes of chromium (III) can be leached from the soil. However, under oxidizing conditions, chromium (IV) may be present, and it is a relatively soluble form, mobile and toxic to living organisms [39, 40, 41]. Furthermore, for SI sample, except for the TA98 strain, the addition of S9 mix decreased or blocked the mutagenicity detected (Table 1). These results agree with several authors [41, 42, 43] who found a high decrease in the mutagenicity of chromium VI when microsomal or cytosolic fractions of rat liver were present, probably due to the capacity of these

enzymes to reduce Cr(VI) to Cr(III). The element As was detected in the analysis of total metals, although not in the available fraction. Its toxicity also depends on the chemical form and species under which it is found in the environment. Inorganic species under the form of As(III) are more mobile than As(V), and the presence of iron and manganese oxide also has an influence, and may diminish the mobility and availability of As in the soil. Mercury was analyzed based on the fact that this element is known to be relevant for environmental contamination studies. However the values found did not show a differentiation, and were within the limit established by law [32]. Although manganese appears at a high concentration, including the reference area, it is not related to the mutagenic activity detected by the *Salmonella*/microsome assay [44].

In sample SR500, zinc, iron and aluminium showed higher concentrations; in SR150, zinc and aluminium are outstanding, and may be associated to the mutagenic potential found for TA100 in the presence of the metabolism system. As described in the literature [45] the element iron is associated with damages when it is in the presence of a metabolism system, and aluminium also appears as responsible for mutagenicity considering strain TA98 + S9*mix* [46] according to the high value of damage presented by this strain in SR500.

The results suggest that the metals in the area could possibly lead to mutagenic effects, reinforcing concern about their deposition in soils and the resulting environmental problems. Although a gradient is visible, based on the SI and reference area, for the concentration of bioavailable metals, the effects detected in the mixture of inorganic acid fraction analysed by the different mutagenic damage biomarkers used, showed no clear pattern of transfer between SI and the surrounding soils.

Mobility of heavy metals is highly influenced by the pH of samples, particle size distribution, carbon content present in the soil and other physical and chemical variables

[47]. According to the organic matter and carbon content result presented for SR500 in relation to the other sampling points, its higher organic portion content could be associated with the differentiated response considering some strains: about 85% of this organic matter consists of humic material [34]. These substances are complex molecules that could be associated with the greater decomposition of pollutants and the possible generation of decomposition products, which could result in a more mutagenic profile for this soil.

Divalent metals, which are toxic, have low mobility in soils with more clay and silt, that could be associated with the lower SR150 responses which present the highest contents of these fine fractions of soil. The clay particles have a negative load and can retain positively charged ions such as zinc, aluminium, arsenic, nickel and cadmium.

Analyzing the mutagenicity of the organic extracts a predominance of frameshift mutagens was seen depending on metabolism, except for SR500, with the specific presence of direct action compounds detected in strain TA97a (Table 2). Base pair substitution mutagens detected through TA100 plus *S9 mix* were also present in a smaller potency, except for site SR1700. Organic compounds such as PAHs require an exogenous metabolism fraction to show their effects [29, 48, 49]. Some of these compounds, especially the potentially carcinogenic ones [5], were found in the area, and may be associated with the responses obtained in the presence of a metabolism fraction. This evidence appears to indicate the true contribution in the surrounding area from compounds that possibly originated at the contaminated site. The mutagenic activity found at SR150, SR500, and even at the reference site SR1700, although with lower values, emphasized the presence of dependent mutagens of *S9 mix*, such as a study in other area near of the region [15].

The chemical constitution of creosote, the main product responsible for PAHs contamination in the area studied, may vary depending on the source where this oil was obtained. Basically, about 90% of its composition consists of PAHs, the main ones being: 21% phenanthrene, 10% fluorene, 10% fluoranthene, 9% pyrene, 3% naphthalene, 2% anthracene, 3% chrysene, 10% acenaphthalene, 8.5% dibenzofuran, 4% methylanthracenes, 3% methylfluorenes, 3% methylphenanthrenes, 2% dimethylnaphthalenes, 2% carbazole, 2% benzofluorenes, 1,2% 2-methylnaphthalene, 0,9% 1-methylnaphthalene, 0,8% biphenyl [50]. Among these, all first seven compounds cited appeared at variable concentrations in the different soil samples collected.

Comparing the concentration of total PAHs (Figure 8.a) found in the soils, it is noted that SI is outstanding for its high concentration. On the other hand, in the surrounding soils there appears to be a grading of these organic contaminants in terms of carcinogenic PAHs, since the area collected at a greater distance from the contaminated site showed a lower value for the concentration of the compounds evaluated. Through the responses for PAHs with a carcinogenic potential (Figure 8.b), a profile of contamination by this class of compounds is seen, and the samples collected in the area surrounding the contaminated site present a gradient for some contaminants found in the area: benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene and benzo[k]fluoranthene. Among the 16 priority PAHs of EPA, only eight are considered mutagenic, and they require the presence of an activation mixture for their metabolites to show an effect [29, 51, 52]. However, the presence of the other PAHs may explain the cytotoxic responses observed (Table 2).

Some soil samples with mutagenic results have a low concentration of PAHs. This behavior may be explained by strong interactions between the soil matrix and this

group of compounds, which may result in low concentrations of PAHs in the extracts obtained. PAHs will sorb strongly to soils with typical organic matter contents. Only a small fraction of the less hydrophobic PAHs is likely to partition into soil-water and be transported by leaching [35]. Anyway, it can be inferred that measuring only the PAHs content is not an explanatory parameter for soil mutagenicity and possible associated risks [52].

It should be stressed that, although the concentration of carcinogenic PAHs is expected to be responsible for the mutagenic effects, the total PAHs can modulate the mutagenicity response [53], extending the effects. Several authors have demonstrated that many non-mutagenic substances (e.g. anthracene, naphthalene, polychlorinated dibenzo dioxin congeners) can modulate (e.g. enhance or diminish) the effects of mutagens such as BaP [6]. Looking individually at which PAHs with a carcinogenic potential are present in the samples, it is noted (Figure 8.b) that all are above the limit of prevention of the Brazilian Resolution [32], Criteria and Guiding Values for quality of soil (Table 6). However, high molecular weight PAHS (with five or more aromatic rings) were found, which have recalcitrant properties and mutagenic effects [54]. The volatility of these compounds diminishes as the molecular weight increases, and the half lives of these higher molecular weight compounds are relatively long and indicate that they degrade slowly [49]. This is the case of BaP, a mutagenic compound present in all soil samples in this study, which can remain in the soil from 270 days to over 5 years [55], classified in Group 1 as *Carcinogenic to humans* [5].

There is a similar pattern between the PAHs found in the SI soil sample and those collected in the surrounding area, although at low concentrations, and SR500 presents exactly the same group of carcinogenic compounds as SI. In soils SR150 and SR1700, which are similar to each other, the only ones not detected were

indeno(123cd)pyrene and dibenzo(ah)anthracene. Considering that SC500 is located in a direction that is close to and favorable to the deposition of contaminated particles by resuspension [56] and transport by winds from the area considered the source, a possible dispersion route to the surrounding area is identified. BbF(benzo(b)fluoranthene), BkF (benzo(k)fluoranthene), and BaP (benzo(a)pyrene) compounds predominate. These compounds are recognized as important mutagens in soils [29, 51].

As to the mutagenicity responses of biomarkers, in SI a predominant potency is noted for mutagens detected in strain TA97a, in the presence of S9mix, an also important response in SR500. This biomarker for mutagens is described in the literature as more sensitive than the responses in TA98 and TA100 for many PAHs, such as pyrene and perylene [57]. In this study, pyrene is present at a higher concentration in SI than in the surrounding soils. Compounds such as pyrene, although not considered carcinogenic, can be mutagenic through metabolic conversion to pyrenoquinones; like other quinones they can be generated from different PAHs on being metabolized. Once deposited on the soil the compounds will be subject to various partitioning, degradation and transport processes that may directly influence the mutagenicity responses.

Wilcke et al. [58], emphasize that generally PAHs concentrations in soils diminish exponentially as the distance from the source of contamination increases. In previous studies it was observed that the distance over which the PAHs are transported diminishes as their molecular weight increases, since the proportion of particulate matter that undergoes faster atmospheric deposition increases. However, the lighter PAHs can be distributed over long distances. In the present study, even at 1700m from

the contaminated area it was possible to identify the presence of PAHs like benzo(a)anthracene, benzo(a)pyrene and naphthalene.

The contaminated sites may be influenced by several compounds, such as heavy metals, PAHs, PAHs derivatives and heterocyclic aromatic compounds from the use of several products in the wood preservation processes. However, in the environment, the possibility of other anthropic influences besides the source investigated should be considered. In this study, proximity to a coal thermopower plant located 12 km from site SR1700, in the preferential wind direction in the region, may influence the results observed. White & Claxton [34], in a review of literature on soil mutagenesis, acknowledge that it is difficult to accurately calculate the mutagenic risks associated with contaminated soils, or houses close to the contaminated sites, and the problem of measuring effects and sources generating the impact is clearly seen.

Looking only at the effects of the strains tested, without adding the metabolism fraction (Figure 5), a difference at point SR500 compared to the other soils could be seen, with the presence of frameshift mutagens with direct action and sensitive to strain TA97a, suggesting that possibly there are other compounds causing damage at this site. The differences in mutagenic compounds at site SR500 are also clearly seen when analyzing the presence of chemical compounds with nitrated substitutes detected through specific nitrosensitive strains (Figure 7.a). High mutagenesis values can be seen in all the other samples, due to the presence of this class of compounds, corroborating the hypothesis that other compounds are causing the damage observed in this sampling area. Other differences were observed, such as the absence of responses to strain TA97a at SR150.

Biological conversion and decomposition of chemicals including mutagens by bacteria may also affect the differences in mutagenic constituents in these soils [29].

Some compounds like oxygenated PAHs (OPAHs) are emitted together with PAHs (primary sources) or are post-emission degradation products (secondary sources) of PAH conversion by photo oxidation, chemical oxidation and microbial transformation processes (metabolites) [58]. Thus, these OPAHs could be associated with the differentiated response found in SR500, since this soil presented the highest organic matter contents, and could be signaling processes of biotransformation by microorganisms in this soil. Since biotransformation without biodegradation may increase the toxicity associated with the organic pollutants via humification or polymerization mechanisms, the mutagenic activity could be altered. [59].

Concerning the analysis of the mutagenic effect of nitrocompounds, the SI site is outstanding in the detection of nitroarenes, indicating that the internal area of the contaminated site (SI) is contaminated by this class of compounds, similarly in SR150 and SR1700, highlighting YG1041 (detects frameshift mutagens) and YG1042 (detects base pair substitution mutagens), respectively. Considering damage caused by direct action compounds in relation to the strains that detect nitrocompounds, it can be verified that there is an indication of the action of nitro/dinitroarenes [60, 61] in the different soils. Also outstanding is the presence of arylhydroxyamino-compounds in all samples detected via strain YG1024, highly sensitive to this class of compounds, without S9*mix* [29]. These results indicate that YG1041 and YG1042, which have high levels of the nitroreductase and acetyltransferase enzymes, needed for the intracellular metabolic activation of nitroarenes and/or aromatic amines, are essential biomarkers to identify possible risks associated with contaminated soils. This information extends the visualization of the mutagenic effects.

The PAHs oxidation products include a wide range of compounds, some of them nitro-derivatives (NHPAs). Since both the formation, occurrence and chemical analysis

of nitro-PAHs is recently reported, there are few studies about it. With respect to their toxic effects, although environmental levels are lower than PAHs, these compounds are considered stronger mutagens than their parent PAHs, because of their direct mutagenic potency [62, 63] whereas PAHs first require enzymatic activation

The mutagenic potential responses of organic extracts appear to indicate a pattern of contamination in the areas of influence similar to those presented by the soil industrial, appearing to indicate the mobility of organic compounds from the source to the surrounding areas. Based on the organic extracts it can also be verified which nitrated compounds have a significant effect on the mutagenic activity found. The presence of stressors and responses of bioindicators compatible with the influence of SI itself could be found at the site of reference, 1700 meters from the area.

In their review, White & Claxton [34], present averages for revertants in organic extracts of soil, calculated from the analysis of logarithmic distribution of the results of mutagenic potency for different strains ("N" different for each case), defining geometric mean values and categorizing areas as rural, urban/suburban and industrial. In this study, analysis of variance (ANOVA) was used to investigate the relationship between mutagenic potency and the category of site use. Significant differences could be observed between the categories ($p < 0.0001$) for the assay conditions investigated. Table 7 presents a descriptive summary of the mutagenic potency of the assay for each combination of categories of sites and *Salmonella* strain. Taking into account the results obtained for mutagenesis in this study, soils SI and SR150 presented values in TA98 +*S9 mix* which are beyond those presented in the industrial category. On the other hand in strain TA98 –S9 *mix*, SI shows values above the rural category, and SR150, values below of the rural category. The other responses obtained, in strain TA100 +S9 *mix*, SI as in the range almost values in the urban-suburban, SR150 and SR500 also shows

values above the rural category. In this way, the presence of frameshift mutagens depending on S9 mix classifies the area and its surroundings (SR150), as a high risk region for international references. The presence of potentially carcinogenic PAHs is added to the definition of cause-effect. It should be pointed out that, according to other studies [34, 64, 65], most of the mutagenic activity in soils was also detected by high responses in frameshift TA98 and lower ones through base pair substitution TA100 strains in the presence of metabolic activation, just as in TA100 –S9 *mix*, there were no positive responses in any sample in this study.

The values presented by the review of White e Claxton [34] are an important reference for the study of mutagenicity in soils, but they do not present any information for responses to the TA97a or YGs strains, and only refer to assays obtained from organic extracts. In this study, biomarkers with different effects were considered in an approach that, besides organic extracts, analyzes the acid extracts of soil (Figure 6), providing better evidence concerning the seriousness of the data observed.

The chemical analysis of the main compounds in the different compartments was an important tool to characterize the mutagenic agents, allowing the evaluation of a possible dispersion of contaminants from the impacted area to the surrounding environment. The complementation of the study with an analysis of pentachrolophenol in the environmental matrices soil and dust allowed inferring a possible route of ecological and human exposure to this contaminant, selected as a specific marker for the site investigated. The responses found at a same level of concentration in soil and in DR385, referring to the pool of samples collected from six houses in the contaminant dispersion area, confirmed the resuspension of contaminated soil particles to surrounding urban regions, although PCP is less mobile and tends to remain associated with the soil particles because of its chemical properties [66, 67]. PCP is produced by

the chlorination of phenol, and its solutions consist primarily of chlorinated phenols and heavy petroleum oils. It is designated as a priority pollutant by the Environmental Protection Agency. It is corrosive, toxic and a precursor of dioxin congeners which makes the associated risks worse, since the presence of dioxins has already been detected in the soil of the region studied [8]. PCP is degraded by sunlight and by microorganisms [67], which could justify the absence observed for this compound in the samples of surrounding soils.

The evaluation of dust from house attics provided information about the local atmospheric dispersion, and serves as an indirect measure of air pollution and of the potential exposure of the region around the contaminated site. Compared to the soil samples, the responses found in the analysis of DR385 showed higher concentrations of PAHs and heavy mutagenic activity in all mutagenicity biomarkers. Due to the characteristics inherent to dust, such as its grain size composition and considerable specific area, it is a vector of air pollution, while it is transported and deposited in different places, helping understand the area under the influence of the contaminated site [4, 68].

Although the prevailing wind direction in the region of study is southeast (24.9%), and it is expected that the contaminants will be transported towards the river and less populated areas, the results show that there is influence from the northwest (13.7%), the second preferential wind direction in the region, which favors dispersion of appear to be dispersed by dust, since the responses found show the possible influence of the products used and stored in the industrial area, especially pentachlorophenol, a specific marker of the site. Even if the values are not significant for pentachlorophenol in the house soils studied 150 and 500 m from the source area, the results of residential dust at 385m, concerning the chemical stressors and mutagenicity biomarkers analyzed

show that there is a remobilization of contaminants from the soil and, by atmospheric dispersion, they accumulate in the surrounding urban area. It should be pointed out that, as in the dust collected a greater distance away, at DR1700, no positive responses for mutagenicity were observed in the strains tested.

According to other studies [29, 48, 61, 64], a variety of soils can be contaminated via atmospheric deposition with compounds such as PAHs and nitroderivates. Atmospheric deposition of contaminants on soils takes place through processes of wet and dry deposition which can occur in either of two phases. In the case of wet deposition, the compound is either dissolved in the precipitation or it is associated with atmospheric aerosols that are scavenged by the precipitation. For dry deposition, the compound is deposited on soils by atmospheric turbulence and molecular diffusion, and if it is associated with particles also by gravitational settling or impaction [35].

It should also be pointed out that there are other possible dispersion routes for contaminants from the area, which were not within the scope of this study: water transport through one of the streams towards the Taquari river, accumulation of contaminants in the sediment of this river [69] or groundwater flow [17]. Our study focused on the risk of atmospheric transport from the contaminated soil in the area.

5. Conclusions

The result of the variety of chemical compounds present in the internal area, as a function of chemicals used during plant operation may be different environmental effects, ranging from synergistic, which increase genotoxicity from the pollutants present [60] to antagonistic, where the mixture of pollutants may have a less toxic effect that when the products are used alone.

Different response patterns were observed between the source investigated and surrounding sites. It should be considered that these differences can be caused by the experimental design of the study, where a pool of soils representing the industrial process was prioritized as a source of contamination. However, other areas of diffuse contamination are present at this site, such as residues/products disposed of in buried drums. We emphasize that these diffuse differences, which are difficult to map, exert an influence with a differentiated profile of the route prioritized in this study: soil as a source of hazards from the remobilization of its contaminants in the industrial process area.

The heavy metals concentration gradient from SI was clear, but the mutagenic effects resulting from the mixture of inorganic compounds did not allow a grading between the different distances. However, site SR1700 showed no influence of inorganic compounds from the contaminated area.

As to organic extracts, the presence of carcinogenic PAHs extends from the internal area to the site of reference, forming a gradient of concentrations and mutagenic effects that appear to reflect the influence the soil of the industrial area in the surrounding regions. In general, the responses were higher in the organic extracts in the presence of S9 mix.

The evaluation of samples with nitrosensitive strains showed the presence of this class of compounds in the response pattern of most soils analyzed. The analysis of pentachlorophenol, a specific marker of the area, in soil and in residential dust, confirms that soil particles are resuspended from the contaminated area and deposited in the urban surroundings.

The *Salmonella*/microsome assay monitoring molecular damage was used as a biomarker for different molecular effects, helping to determine the impacts and define

the stressors. It is thus a precise tool to evaluate the degradation of environmental quality by identifying the presence of concentrations of pollutants that cause mutagenesis. By using mutagenicity markers and estimating the concentrations of possible stressors in association, responses were observed confirming the hypothesis that mutagens from the contaminated areas may disperse to surrounding regions. It is possible to detect distance gradients, favoring the estimation of risks. It is clearly essential to evaluate the extent of contamination from sources of impacted soil, since without correct delimitation of the impact, any remedial measure or procedure to minimize risks would not be effective [16].

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Table 1. Mutagenicity and Cytotoxicity in Acid Extracts of Soils (SI, industrial and SR, residential) in the presence (+S9) and absence (-S9) of metabolism fraction.

	TA 98		TA 97a		TA 100		Cytotoxicity	
	-S9	+ S9	-S9	+ S9	-S9	+ S9	-S9	+ S9
SI	[a]214±0.073	1025±0.040	3933±0.736	453±0.250	256±0.138	[b]NS	[c]NT	[d]T
SR150	NS	NS	NS	NS	850±0.050	528±0.110	NT	[d]T
SR500	NS	3856±0.458	154±0.063	NS	NS	549±0.219	NT	NT
SR1700	NS	NS	NS	NS	NS	NS	[e]T	[d]T

[a]number of revertants/g dry soil equivalent ± SD; [b]NS: non significant; [c]NT: non cytotoxic, T: cytotoxic sample: % cell survival less than 60% compared to the negative control in at least one of the dosages tested; Cytotoxicity at [d]100mg; [e]75mg; Concentrations tested: 25mg, 50, 75,100, 150, 200mg; Negative Control (rev/plate±SD) 100 mL of acid/plate solution. –S9 mix: TA98(38.67 ±13). TA97a (173.73±52.08). TA100 (197.89±52.82); +S9mix: TA98(39.09±9.55). TA97a (167±25.09). TA100 (224.62±32.02); Positive Control (rev/plate±SD): –S9 mix: TA98: 642.91±166.80 (4NQO – 0.5µg/plate). TA97a: 792.80±138.06 (4NQO – 0.5µg/plate). TA100: 605.38±103.68 (AZD – 5µg/plate); +S9mix: 2AF – 10µg/plate: TA98(484.50±119.59). TA97a(533.33±82.37) and TA100(425±88.80).

Table 2. Mutagenicity and Cytotoxicity in Organic Extracts of Soils (SI, industrial and SR, residential) in the presence (+S9) and absence (-S9) of metabolism fraction.

	TA 98		TA 97a		TA 100		Cytotoxicity	
	-S9	+ S9	-S9	+ S9	-S9	+ S9	-S9	+ S9
SI	[a]107±0.040	827±0.083	[b]NS	1455±0.340	NS	307±0.144	[c]T	[d]NT
SR150	33±0.017	1285±0.286	NS	NS	NS	80±0.036	[c]T	NT
SR500	NS	NS	3637±0.466	655±0.182	NS	388±0.175	[e]T	[e]T
SR1700	NS	317±0.033	NS	386±0.130	NS	NS	NT	NT

[a]number of revertants/g dry soil equivalent ± SD; [b]NS: non significant; [d]NT: non cytotoxic; T: cytotoxic sample: % cell survival less than 60% compared to the negative control in at least one of the dosages tested; Cytotoxicity at [c]160mg; [e]120mg; Concentrations tested: 10mg, 20, 40,80, 120, 160mg; Negative Control (rev/plate±SD) 5 µL DMSO/plate –S9 mix: TA98(54.40±6.88). TA97a (182.27±11.30). TA100 (169.09±21.75); +S9mix: TA98(56.25±6.77). TA97a (178.67±37.81). TA100 (192.09±22.89); Positive Control/plate±SD): –S9 mix: TA98: 642.91±166.80 (4NQO – 0.5µg/plate). TA97a: 792.80±138.06 (4NQO – 0.5µg/plate). TA100: 605.38±103.68 (AZD – 5µg/plate); +S9mix: 2AF – 10µg/plate: TA98(484.50±119.59). TA97a(533.33±82.37) and TA100(425±88.80).

Table 3. Mutagenicity and Cytotoxicity in residential dust samples (DR) in the presence (+S9) and absence (-S9) of metabolism fraction.

		DR385	DR1700
TA 98	-S9	[a]1340±0.163	[b]NS
	+S9	2534±0.203	NS
TA 100	-S9	1332±1.434	NS
	+S9	5519±1.000	NS
TA 97a	-S9	NS	-
	+S9	1316±0.132	-
YG 1041		964±0.409	-
YG 1042		4483±0.195	-
YG 1024		4402±0.323	-
Cytotoxicity	-S9	NT	[c]NT
	+S9	[d]T	NT

[a]number of revertants/mg dust equivalent; [b]NS: non significant; - : not tested; [c]NT: non cytotoxic, [d]T: Cytotoxicity in 40mg; Concentrations tested: 2, 4, 8, 12, 20, 40mg; Negative control (rev/plate±SD) 5 µL DMSO/plate –S9 mix: TA98(32.33 ±5.92). TA97a (173.33±13.65). TA100 (147.17±48.83); +S9mix: TA98(30.00±3.74). TA97a (160.67±22.28). TA100 (160.67±22.28). 113±16.09 (YG1041). 119.33±22.27 (YG1042). 91.33±8.08 (YG1024) Positive Control (rev/plate±SD): –S9 mix: TA98: 438.50±11.50 (4NQO – 0.5µg/plate). TA97a: 792.80±138.06 (4NQO – 0.5µg/plate). TA100: 589.50±72.50 (AZD – 5µg/plate); +S9mix: 2AF – 10µg/plate: TA98(364.5±62.50). TA97a(488±61).TA100 (268.38±56.68). 3561.50±292.50(YG1041). 357±8 (YG1042). 2595±511 (YG1024).

Table 4. Concentration of Total Metals in the Soils and Concentration of Metals in the Acid Extracts of Soils (SI, industrial and SR, residential)

Metal (mg/Kg)	SI	SR150	SR500	SR1700	LD
As[a]	43,0	7,0	5.9	4.8	3
Cr[a]	96,0	24.5	27,0	23.8	12
Cu[a]	54,0	14,0	16,0	10.9	3
Hg[a]	0.03	0.02	0.02	0.03	0.01
As[b]	<LD	<LD	<LD	<LD	0.04
Cr[b]	0.032	0.008	0.02	<LD	0.008
Cu[b]	0.06	0.006	0.02	0.01	0.008
Ni[b]	0.018	<LD	<LD	<LD	0.008
Fe[b]	<0.06	<0.06	0.36	<LD	0.06
Mn[b]	1.44	1.06	1.64	0.64	0.04
Zn[b]	0.18	0.46	1.02	0.62	0.06
Pb[b]	0.06	0.04	0.06	0.06	0.02
Al[b]	0.3	1.22	1.02	0.66	0.16
Cd[b]	0.006	<LD	0.004	0.004	0.004

Concentration of metal: mg/Kg dry soil.
[a] Total Metals.
[b] Available fraction obtained according to NBR10005 (mg/Kg dry soil).

Table 5. Guiding values for metals of interest

Metal (mg/Kg)	Netherland[1]		Conama´s Resolution[2]			
				Investigation		
	Reference	Intervention	Prevention	Agricultural	Residencial	Industrial
As	29	55	15	35	55	150
Cr	100	380	75	150	300	400
Cu	36	190	60	200	400	600

Reference to total metals: mg/Kg dry soil.
[1]Netherlands (2000); [2]CONAMA-420 (2009)

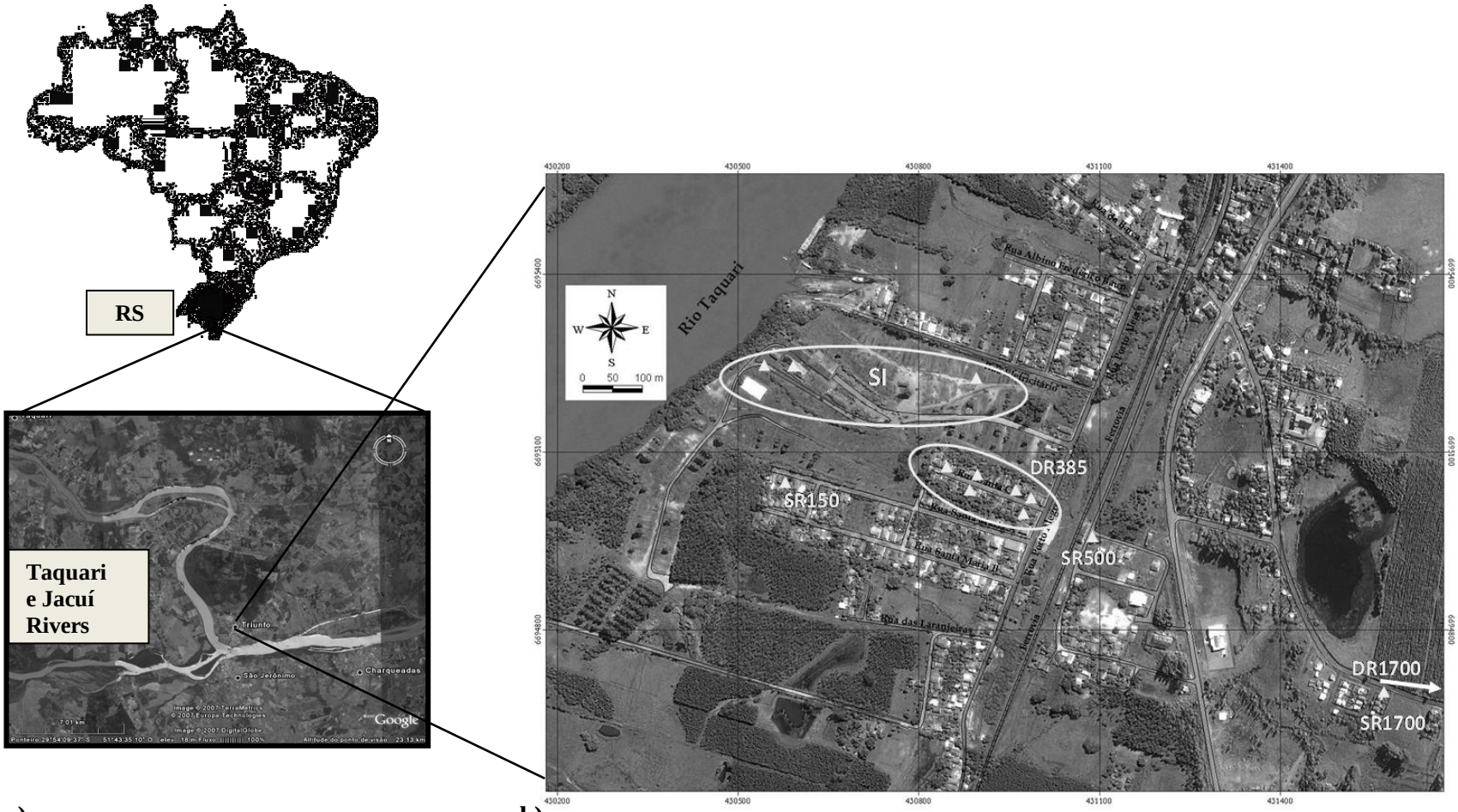
Table 6. Guiding values for potentially carcinogenic PAHs for soils – CONAMA Resolution 420

		Investigation		
PAH (mg/Kg)	Prevention	Agricultural	Residencial	Industrial
Benzo(a)antraceno	0.025	9	20	65
Benzo(k)fluoranteno	0.38	-	-	-
Benzo(a)pireno	0.052	0.4	1.5	3.5
Indeno(123cd)pireno	0.031	2	25	130
Dibenzo(ah)antraceno	0.08	0.15	0.6	1.3
Naftaleno	0.12	30	60	90
Criseno	8.1	-	-	-

Table 7. Geometric Mean of Mutagenicity in *Salmonella*

	Use of Soil		
Strain	Rural	Urban/suburban	Industrial
TA98+S9	[a]60±5	470±50	950±170
TA98-S9	57±6	430±100	770±180
TA100+S9	96±10	460±40	3180±1460
TA100-S9	120±10	260±30	130±130

[a]nº of rev/g dry soil ± SD obtained in organic extract.



a) **b)**

Figure 1. a) Area of Study – contaminated site; **b)** sampling point sites, SI: industrial soil, SR: residential soil at 150, 500 and 1700 m, DR: residential dust at 385 and 1700 m from the source area.

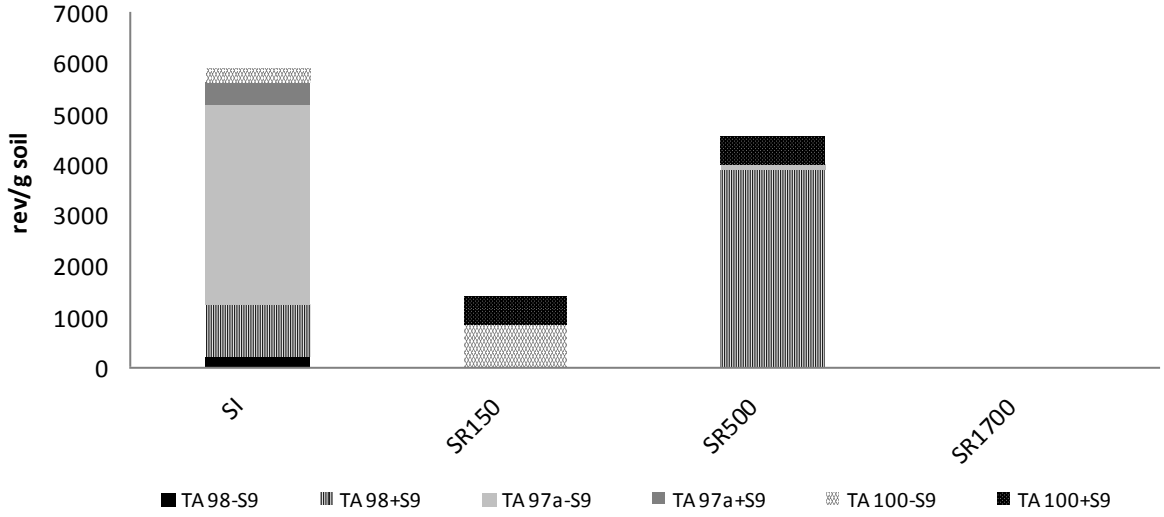


Figure 2. Mutagenic activity in revertants/g dry soil equivalent in the acid extracts of soils referring to the sum of effects of the strains that detect frameshift mutagens (TA98 and TA97a) and base pair substitution (TA100) in the presence and absence of metabolism (+S9 and –S9); SI, industrial soil; SR, residential soil.

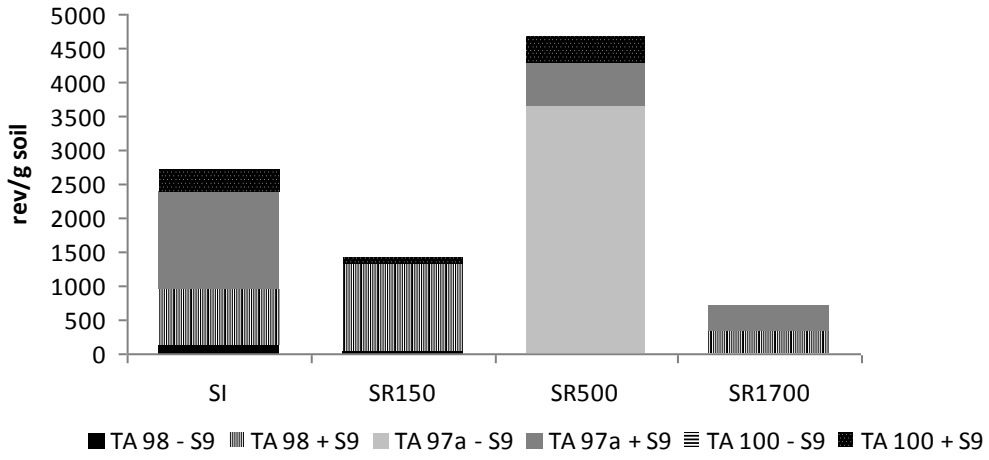


Figure 3. Mutagenic activity in revertants/g dry soil equivalent in the organic extracts referring to the sum of effects of the strains that detect frameshift mutagens (TA98 and TA97a) and base pair substitution (TA100) in the presence and absence of metabolism (+S9 and –S9); SI, industrial soil; SR, residential soil.

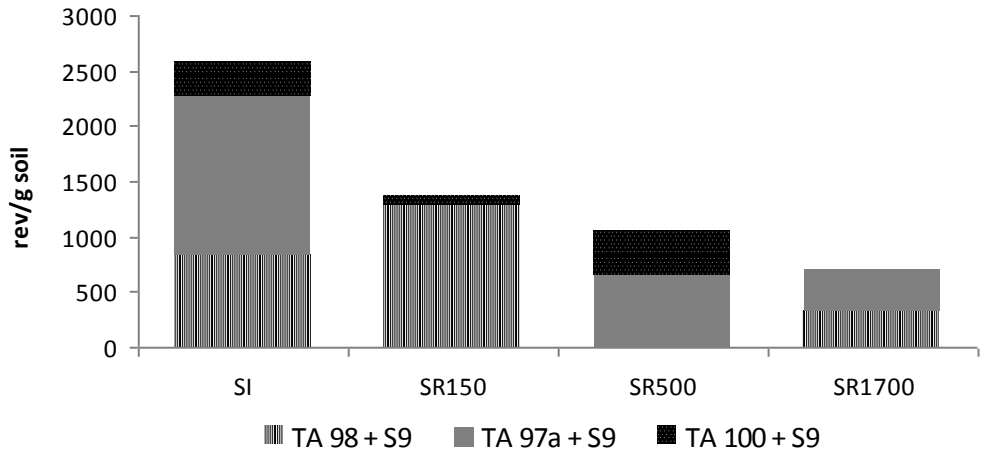


Figure 4. Mutagenic activity in revertants/g dry soil equivalent in the organic extracts of soils referring to the sum of effects of the strains that detect frameshift mutagens (TA98 and TA97a) and base-pair substitution (TA100) only in the presence of metabolism (+S9); SI, industrial soil; SR, residential soil.

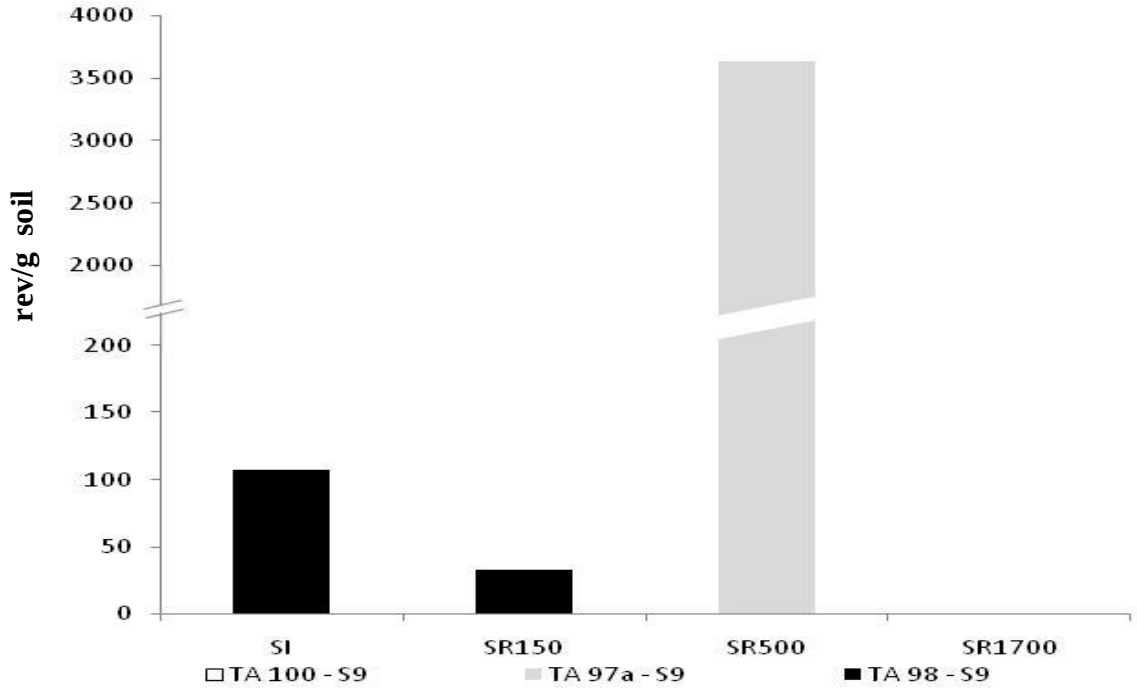


Figure 5. Mutagenic activity in revertants/g dry soil equivalent in the organic extracts referring to the sum of effects of the strains that detect frameshift mutagens (TA98 and TA97a) and base pair substitution (TA100) only in the absence of metabolism (- S9); SI, industrial soil; SR, residential soil.

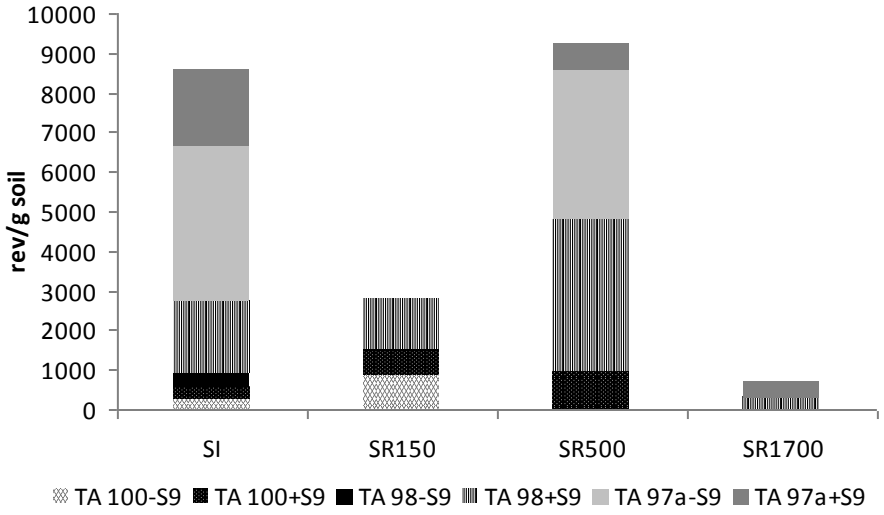


Figure 6. Mutagenic activity in revertants/g dry soil equivalent in the acid and organic extracts resulting from the sum of effects of strains TA98 and TA97a (frameshift mutagens) and TA100 (base pair substitution) in the presence and absence of metabolism (+S9 and –S9); SI, industrial soil; SR, residential soil.

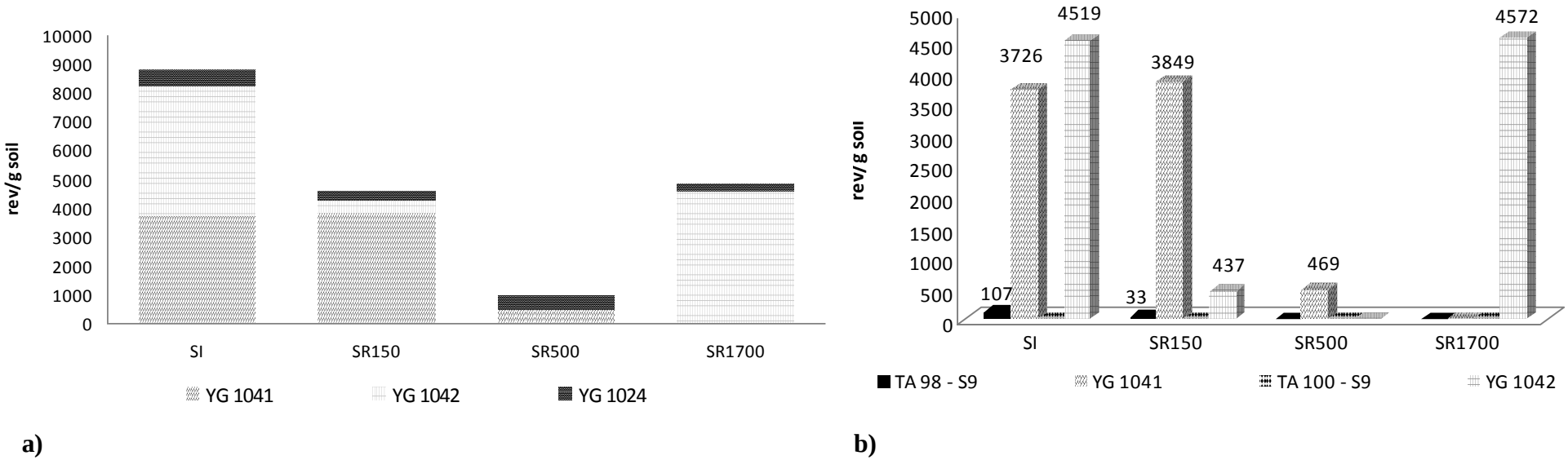
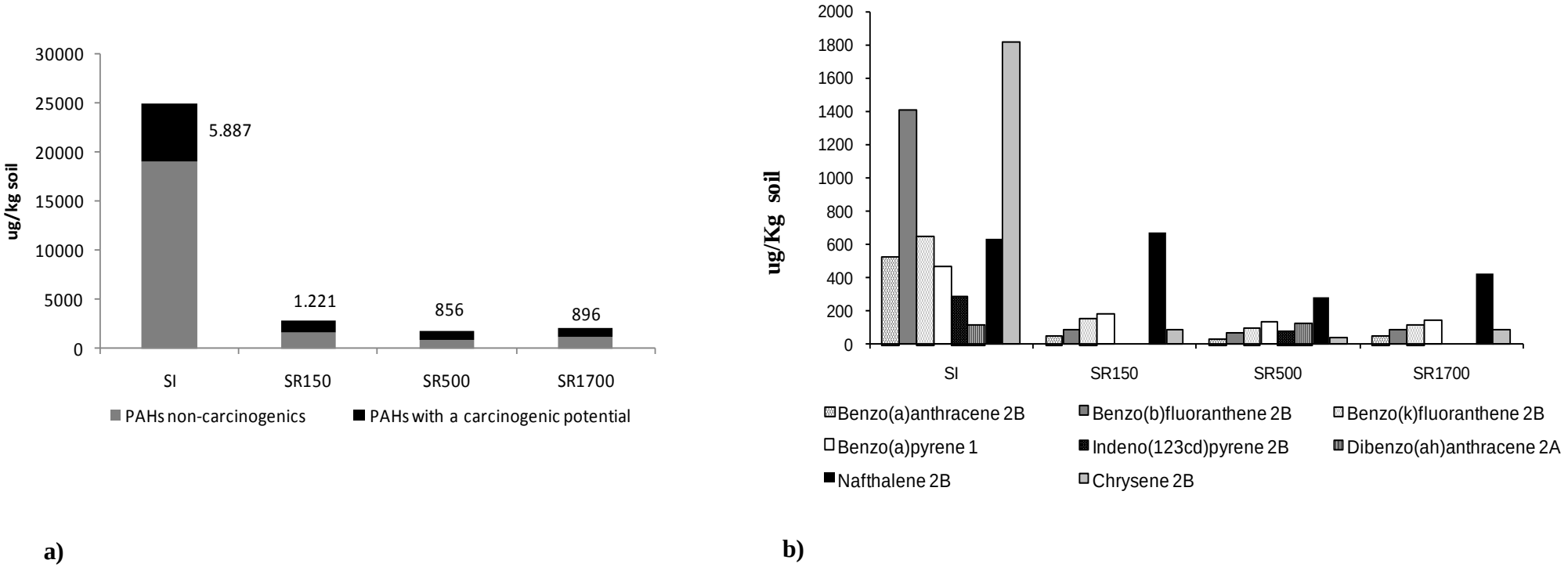


Figure 7. a) Mutagenic activity in revertants/g dry soil equivalent in the organic extracts referring to the strains sensitive to nitrocompounds; Negative Control (rev/plate±SD): 113.11±28.53 (YG1041), 200.83±19.65 (YG1042), 38±5.18 (YG1024); Positive Control (rev/plate±SD): 2NF (0.15g/plate), 515±195.07(YG1041), 689.75±154.44 (YG1042), 624.75±247.02 (YG1024); **b)** Mutagenic activity in revertants/g dry soil equivalent in the organic extracts referring to strains TA98 and TA100, without metabolism and their derivatives sensitive to nitrocompounds. SI, industrial soil; SR, residential soil.



a) **b)**

Figure 8. a) Total PAH concentrations, emphasizing in the sum the PAHs with a carcinogenic potential and non-carcinogenics evaluated in soils. The concentration values highlight the potentially carcinogenic PAH responses; **b)** Concentrations of potentially carcinogenic PAHs (Group 1: *Carcinogenic to humans*; Group 2A: *Probably carcinogenic to humans*; Group 2B *Possibly carcinogenic to humans*; Group 3 *Not classifiable as to its carcinogenicity to humans* (IARC, 2010) measured in the soil. SI, industrial soil; SR, residential soil.

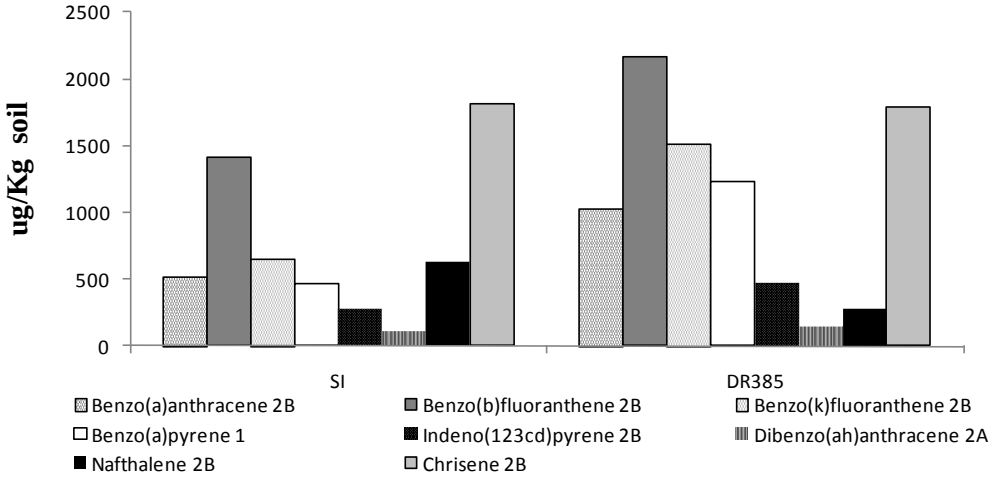


Figure 9. Potentially carcinogenic PAHs concentrations (Group 1: *Carcinogenic to humans*; Group 2A: *Probably carcinogenic to humans*; Group 2B *Possibly carcinogenic to humans*; Group 3 *Not classifiable as to its carcinogenicity to humans* (IARC, 2010) measured in the residential dust (DR) and soil industrial (SI) samples.

4 Considerações Finais

Áreas de solo contaminadas a partir do uso industrial de processos de preservação de madeira representam uma preocupação mundial quanto a alterações da qualidade ambiental e repercussões na saúde da população. As avaliações de impacto baseadas apenas em ferramentas tradicionais, como análises físicas e químicas, não traduzem adequadamente muitos dos riscos existentes.

Os estudos em amostras de solo permitem uma informação espacial e integrada da contaminação de uma região particular, sendo a verificação de possíveis danos mutagênicos, a ferramenta que pode antecipar medidas de proteção à qualidade ambiental, bem como qualificar processos de remediação de locais contaminados.

A aplicação de biomarcadores de genotoxicidade em amostras de solos não apresenta um histórico, na literatura, tão abrangente quanto às investigações em outros compartimentos, tornando-se necessário um fortalecimento nesta área de pesquisa.

O presente trabalho foi realizado no Programa de Pós Graduação em Ecologia da Universidade Federal do Rio Grande do Sul em parceria com a Fundação Estadual de Proteção Ambiental Henrique Luís Roessler - FEPAM.

O estudo buscou estratégias para investigar solos contaminados como fonte de compostos tóxicos e genotóxicos, definindo rotas e abrangência de dispersão desses contaminantes do solo para áreas de influência no entorno.

Para esta meta, foi escolhido, como região de investigação, um sítio com contaminação de solo por preservativos de madeira, em avaliação de impacto ambiental na FEPAM. A área está atualmente desativada, mas caracteriza importante passivo ambiental, em investigação quanto aos possíveis riscos que representa para sua área de influência. Em função da atividade industrial realizada durante mais de 40 anos de

operação da Usina, além das características extremamente tóxicas dos produtos utilizados nos processos produtivos nesse período – pentaclorofenol, creosoto, arseniato de cobre e cromo (CCA), o sítio pode servir de fonte de contaminação para o ambiente de entorno.

Considerando a interação entre os vários compartimentos ambientais, as características complexas dos solos e a dificuldade de modelar a dispersão de poluentes a partir de sítios contaminados, o trabalho se propôs a investigar a dispersão dos contaminantes pela via atmosférica, partindo da contaminação do solo da área de processo industrial, até possíveis locais de influência no entorno, em distâncias gradativas. Aliou-se também, ao estudo, a avaliação de poeira que se mostrou uma via preferencial de dispersão dos contaminantes para a área circundante, ampliando a caracterização dos riscos potenciais no ambiente urbano/rural próximo.

Diante das características variadas do solo, estudos nesta matriz complexa requerem abordagens diferenciadas para permitir uma correta visualização dos potenciais de risco envolvidos. Desta forma, optou-se por empregar estratégias de partição da amostra em extratos ácidos e orgânicos, associar o estudo dos estressores químicos, respeitando o histórico de sucessão dos preservantes de madeira empregados na área e associar o uso de biomarcadores para diferentes efeitos moleculares de mutagênese com análises dos principais grupos químicos. Esta estratégia permitiu delimitar as áreas de influência da contaminação a partir da fonte. Assim, de acordo com os resultados observados, a área industrial pode ser considerada como uma via de poluentes ativa para o ambiente, sendo o solo interno do sítio industrial a principal fonte de contaminação.

Embora não se tenha observado um padrão claro de transferência para o ambiente nas avaliações de mutagênese realizadas nos extratos ácidos, esta avaliação

permitiu verificar maior atividade mutagênica frente a todos os biomarcadores em SI e pelo menos dois diferentes efeitos nas amostras de solo do entorno. Destaca-se ainda resposta elevada em SR500, de natureza diversa da observada em SI, indicando a influencia de estressores diferentes. Em SR1700, foram observadas respostas biológicas negativas, permitindo considerá-lo como referência para a área de estudo quanto a compostos inorgânicos. Já através das análises de metais pesados, foi detectada uma gradação nas respostas, em que, tanto a concentração de metais totais quanto a fração biodisponivel dos elementos Cr e Cu, apresentaram valores mais altos para SI em relação aos solos de entorno; para As, apenas na avaliação da concentração total do elemento, a concentração em SI foi superior. É importante ressaltar que comparando o teor total destes metais de interesse em SI, verifica-se que, em relação ao As, o valor está acima do limite de investigação para área agrícola e para o Cr, acima dos valores de prevenção de acordo com a legislação Nacional. Em relação à norma Holandesa, os níveis destes metais estão acima (As e o Cu) ou similares (Cr) aos valores de referência.

Para os extratos orgânicos avaliados, as respostas de mutagênese mostraram um padrão de contaminação nas áreas de influência semelhante ao apresentado por SI, indicando a mobilidade de compostos orgânicos a partir da fonte para as áreas de entorno. Predominaram respostas causadas por compostos de ação indireta, mutagênicos do tipo erro no quadro de leitura. Nos locais de entorno, observou-se padrão similar em SR150; SR500 também indicou a presença de mutágenos dependentes de S9*mix*, além de valores elevados de mutagênese de ação direta, caracterizando também a presença de mutágenos de natureza diversa de SI; SR1700 mostrou um decréscimo de efeitos mutagênicos em linhagens do tipo erro no quadro de leitura, dependentes de S9 *mix*.

Como a atividade mutagênica encontrada em SR150, SR500 e mesmo SR1700 indicou a presença de mutágenos dependentes de S9*mix*, ficou evidenciada a efetiva

dispersão de compostos possivelmente originados no sítio contaminado, como os HPAs encontrados nas análises químicas de todas as amostras. Concentrações de HPAs potencialmente carcinogênicos estiveram presentes desde o solo da área industrial até o do local de referência, SR1700, bem como na poeira residencial da área de risco, DR385, indicando um gradiente de concentrações para estes compostos.

A verificação de efeitos através das linhagens nitrosensíveis indicou a presença desta classe de compostos na maioria dos solos analisados, que representa sérios riscos em função de já serem reconhecidos como potentes mutágenos. Na investigação da poeira residencial de entorno, foram observadas respostas mutagênicas nas diferentes cepas testadas, efeitos decorrentes de mono e dinitroarenos na linhagem YG1042 e de hidroxiamino-compostos (YG1024); em DR1700, não foi observada resposta positiva para mutagenicidade. A análise de pentaclorofenol, como marcador específico da área, realizada no solo e na poeira das casas permitiu a confirmação de que partículas de solo são ressuspensas a partir da área contaminada e se depositam no entorno urbano/rural.

Embora tenha se observado efeitos mutagênicos nos extratos orgânicos que possivelmente evidenciam o emprego de creosoto e PCP na área industrial, deve ser ressaltada a possibilidade de outros compostos orgânicos como dioxinas e furanos, devido ao uso do PCP, oferecerem riscos a partir da região avaliada.

O ensaio *Salmonella/*microssoma permitiu visualizar efeitos diversos através dos marcadores usados, sendo uma técnica que evidencia respostas em níveis mais baixos de organização biológica, o que favorece a identificação de danos iminentes, e caracteriza a sua eficiência e vasta utilização. De acordo com Bickham [63], embora danos causados por poluentes sejam em nível molecular, estes compostos também podem iniciar uma cascata de respostas em nível tecidual, na saúde do organismo, nas taxas de reprodução, na genética populacional e por fim, nos processos evolutivos

incluindo a extinção. Desta forma, a avaliação da sustentabilidade dos ecossistemas passa pelo estudo de contaminantes ambientais, uma vez que há efeitos emergentes que podem comprometer a diversidade genética.

As respostas obtidas, neste estudo, destacaram a amplitude de riscos inerentes a sítios de solos contaminados. Trouxeram novas informações a serem consideradas no delineamento de estratégias de remediação para esses sítios, visando à proteção do ambiente e da saúde humana e sendo fundamentais em estudos ecológicos.

Assim, considerando a região investigada, as conclusões da pesquisa mostraram que o ambiente de entorno do sítio contaminado está sendo exposto a agentes tóxicos e genotóxicos, que causam danos potenciais ao patrimônio genético da fauna e da flora atingida pelos contaminantes, bem como alteram a qualidade de vida e a saúde da população próxima. Recomenda-se que a área seja monitorada durante os processos de descontaminação e remediação necessários, para acompanhamento dos impactos gerados nas áreas de entorno.

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6 Anexos

Anexo 1. Regras da Revista

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