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Genetic Biomarkers Applied to Environmental Air Quality: Ecological and Human Health Aspects

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1. Introduction

Exposure to air pollutants may cause health risks leading to acute respiratory infections, cancer, chronic respiratory and cardiovascular diseases. In 2008, IARC/WHO (International Agency for Research on Cancer/World Health Organization) estimated 12.4 million new cases and 7.6 million deaths from cancer worldwide (WHO, 2009). The highest rate of occurrence was of lung cancer (1.52 million new cases), which was the main cause of death of 1.31 million people. For South and Central America and the Caribbean, in 2008, about one million new cases of cancer and 589 thousand deaths were estimated.

In Brazil, estimates for 2010, also valid for 2011, indicate 489,270 new cases of cancer, and lung cancer was the third most frequent in males (18/100,000) and the fifth in females (10/100,000). However, the rates of occurrence per region for malignant neoplasms of the trachea, bronchi and lungs indicate major variations in the different Brazilian states. For 2010, the highest rates were estimated for Rio Grande do Sul both for males (48.33/100,000) and for females (21.43/100,000) These estimates increased compared to 2003 (46.90/100,000, in males and 16.80/100,000 in females), in this state (Brasil, 2003, 2009).

Although the major causes for the increases of cancer incidence include lifestyle, personal habits and diet, the role of environmental changes caused by the industrial activities favoring the release of chemicals into the environment, polluting air, water, soil and potentially contaminating the food should be considered (Brasil, 2009; WHO, 2009; Yu, 2001). The habit of smoking is the most important risk factor for the development of lung cancer. Compared to non-smokers, smokers are about 20 to 30 times more at risk of developing lung cancer. Generally the rates of occurrence in a given country reflect its cigarette use.

Epidemiological studies indicate that other major risk factors for lung cancer are repeat lung infections, a history of tuberculosis and exposure to certain chemicals mainly found in the occupational environment, such as asbestos, arsenic, beryllium, radon, a radioactive gas, nickel, cadmium and vinyl chloride. In addition there is air pollution, especially exposure to

polycyclic aromatic hydrocarbons (PAHs) and diesel oil smoke, from motor vehicles and industry, and to fine and ultrafine airborne particles. The size and composition of these particles determine their behavior in the respiratory system and the time of residence in the environment (Brasil, 2009; Pagano et al., 1996; Pope III et al., 1995, 2002).

The effects of hazardous substances on the population are expressed after a long period of exposure, making it difficult to take preventive action which will allow reverting or reducing risks. In this context, sensitive methodologies, such as biomarkers, allow defining early biological effects after exposure to environmental toxins (Van der Oost et al., 2003).

Genotoxicity biomarkers have been considered sensitive to the early diagnosis of hazardous compounds, their dispersion routes and potential risks to organisms from different ecosystems including damage to human health. In addition, these assays allow evaluating the effect of interactions between the substances present in the atmospheric compartment. This gives particular characteristics to the mixtures formed (Claxton et al., 2004). The distribution of these mixtures in the environment depends on their physical and chemical properties. Their dispersion and deposition may contaminate soils, rivers, dust and plants and affect different organisms, populations and communities, even far from the original sources. Grantz et al. (2003) highlight its effects on vigor, competitive viability and reproductive fitness at the individual level, with subsequent impacts on the ecosystem structure, function and biodiversity.

The presence of mutagenic compounds in organic extracts of airborne particles was reported for the first time in 1975, and since then research worldwide has sought to quantify, characterize and indicate sources of emission, besides the risk of exposure to air pollutants. Most of these studies investigating mutagenic and carcinogenic properties in urban air reported positive responses (Claxton et al., 2004; Claxton & Woodwall Jr., 2007). Claxton et al. (2004) reviewed the literature for studies that used the *Salmonella*/microsome assay and its variations in urban air samples. This assay represents majority of ambient air mutagenesis literature (Claxton & Woodwall Jr., 2007), it is an useful assay to compare genotoxicity among different locations and conditions (Claxton et al., 2004). The *Salmonella* assay is useful to evaluate complex mixtures influenced by different and diffuse sources, such as the atmospheric compartment. Besides, the associated responses of different strains and the presence of the mammal metabolization fraction *in vitro* (S9 fraction) obtained in this assay, indicate the classes of compound found in the samples.

FEPAM (Fundação Estadual de Proteção Ambiental, state of Rio Grande do Sul, RS, Brasil) has been developing a research program on mutagenic activity in air samples in urban and industrial areas of Rio Grande do Sul, Brazil using the *Salmonella*/microsome assay as a specific marker of the presence of these compounds adsorbed to the particulate matter in the air and its carcinogenic potential. This assay evaluates molecular mutagenic damage, and these changes result in specific genetic markers that allow measuring the presence of agents aggressive to DNA by means of the effect. The international validation and constant improvement include this biomarker in the set of tests that define the potential of genotoxic carcinogenic organic compounds (Brambilla & Martelli, 2009; Claxton et al., 2004; Kado et al., 1986; Maron & Ames 1983).

Because of the complex system that forms the airborne particulate matter and inter-individual variability in exposure, no safe threshold or no main component of these air pollutants can be established below which adverse effects do not occur (WHO, 2006). Studies associating emissions sources, mutagenic activity and chemical characterization of airborne particulate matter help to understand the effects of pollutants. Also, they can

provide better assessment for regulatory agencies to establish more restrictive, safer concentrations of the main biologically active components.

This chapter will emphasize the use of genetic damage biomarkers as early indicators for mainly organic atmospheric pollution and highlight their sensitivity and applicability for areas under different anthropic influences; to compare the mutagenic responses with the particulate matter concentration, a parameter used in different air quality standards; and finally discuss the biological effects observed in assays using samples of different particulate matter size (total suspended particles, TSP, and particles less than 10µm, PM10 and less than 2.5 µm).

2. Airborne particulate matter

Particulate matter (PM) is a complex heterogeneous mixture of extremely small particles and liquid droplets having diverse chemical and physical characteristics. PM is made up of a number of components of solid and liquid particles of organic and inorganic substances suspended in the air, including acids (such as nitrates and sulfates), organic chemicals, metals, and soil or dust particles. The potential of particles to cause health problems varies with size and other physical characteristics, chemical composition and sources.

The particles are identified generally according to their aerodynamic diameter because these determine transport and removal processes in the air and deposition sites and clearance pathways within the respiratory tract. PM has been classified as either TSP (total suspended particulate matter smaller than 100 micrometers in diameter) or PM10 (particles with an aerodynamic larger than 2.5 micrometers and smaller than 10 micrometers in diameter) or PM2.5 (aerodynamic diameter smaller than 2.5 µm) and ultrafine particles, those smaller than 0.1 µm (100 nm).

The inhalable coarse particles (PM10), such as those found near roadways and dusty industries, are the particles that generally pass through the throat and nose and enter the lungs. Once inhaled, these particles can affect the heart and lungs and cause serious health effects. Fine particles (PM2.5), such as those found in smoke and haze, can be directly emitted from sources such as forest fires, or formed from gases from power plants, industries and automobiles. PM2.5 particles are dangerous since, when inhaled, they may reach the peripheral regions of the bronchioles, and interfere with gas exchange inside the lungs.

International and regulating agencies establish limits or recommendations for the different particles. The World Health Organization Air Quality Guidelines (WHO, 2006) recommend more restrictive values for PM10 (50 µg/m^3, 24 hour mean and 20 µg/m^3 annual mean) and PM2.5 (25 µg/m^3, 24 hour mean and 10 µg/m^3 annual mean). Other publications present staggered targets for particle reduction (Official Journal the European Union, 2008), or defined threshhold values (USEPA, 2008). In Brazil, total suspended particulate (TSP) (240-150 µg/m^3 24h mean and 80-60 µg/m^3 annual mean) and particulate matter less than 10 µm in diameter (PM10) (150 µg/m^3 24 h mean and 50 µg/m^3 annual mean) are regulated by the National Council of the Environment (Brasil, 1990).

3. *Salmonella*/microsome assay

The basic mechanism of the test is to determine changes in the DNA molecule, caused by reverse mutations for prototrophy, using *S. typhimurium* mutants to assess the mutagenic

activity and the carcinogenic potential of chemicals. The indicator strains present different mutations in the histidine *operon*, selected to detect substances that are able to generate base pair substitution or frameshift mutation.

Strains derived from the classical TA98 and TA100 were developed bearing a deficiency in a classical nitroreductase (NR strains) or in an *O*-acetyltransferase (1.8-DNP6 strains) (Rosenkranz et al., 1980; Rosenkranz, 1996), enabling the diagnosis of the presence of mononitro and dinitroarenes, respectively. Watanabe et al. (1989, 1990) developed a series of YGs strains with similar properties – YG1021 (pYG216), YG1024 (pYG219), YG1026 (pYG216), YG1029 (pYG219) – but presenting high enzymatic production. The genes of classical nitroreductase (pYG216) and of *O*-acetyltransferase (pYG219) are inserted in the plasmids, conferring high enzymatic activity and greater sensitivity for nitrocompounds, such as nitroarenes or dinitroarenes, hydroxyamine-compounds and aromatic amines, respectively (Watanabe et al., 1989, 1990).

The assay may be performed by various procedures, the most widely used being plate-incorporation, pre-incubation and microsuspension, which presents a 5 to 10 times higher sensitivity than the traditional Ames test (Kado et al., 1983; Maron & Ames, 1983; Mortelmans & Zeiger, 2000). The test is performed in the presence and absence of an *in vitro* system of metabolic activation, the most commonly used being the microsomal fraction – S9. The S9 was composed of a homogenate from cells of the livers of *Sprague-Dawley* rats pre-treated with a polychlorinated biphenyl mixture (Aroclor 1254) to induce an increase in the P-450 enzymes.

4. Study cases in urban and industrial areas

Our research group in Rio Grande do Sul (RS), Brazil, performed studies to assess the mutagenic activity of air samples in regions under different anthropic influences. Several sites were investigated, ranging from regions with urban predominance such as Porto Alegre, the capital of RS, an area in Triunfo, RS, under the influence of a petrochemical industry, and another where oil industry contaminants predominate, located in an urban residential area in Esteio, RS (Figure 1).

Total airborne particulate matter (TSP, particles <100 μm) samples were collected on Fiberglass filters (AP40-810, 20 cm × 25 cm Millipore) using high-volume sampler (General Metal Works Inc.). Airborne particulate matter less than 10 μm (PM10) and less than 2.5 μm (PM2.5) samples were collected on Teflon® filters (TX40HI20WW, 254 mm × 203 mm) using a high-volume collector (AVG MP10, 1200/CCV) for 24 hours. The filters were weighed and stabilized before and after sampling (45% humidity) to calculate the TSP, PM10 or PM2.5 values expressed in units of μg/m³ of sampled air.

Half of each filter was used for the extraction of the organic compounds, pooled or extracted separately according to the study objectives. Each pooled or simple sample was submitted to extraction by sonication with dichloromethane (DCM, CASRN. 75-09-2) (Vargas et al., 1998). Dichloromethane extracts the moderately polar compounds and is the most representative fraction of mutagenic activity (De Martinis et al., 1999) extracting a wide range of compounds from airborne particulate matter. DCM is preferred when the investigator did not want to extract non-organic components that would be toxic to the bacteria used in the assay (Claxton et al., 2004; Marvin & Hewitt, 2007). The percentage of extractable organic matter (EOM%) was calculated, and the mass obtained was compared to half the volume of air sampled (EOM in μg/m³), since half the filters were used. Prior to

bioassay performance, the organic extract was dried with gaseous nitrogen and resuspended in dimethyl sulfoxide (DMSO, CASRN. 67-68-5).

Fig. 1. Location of studied sites in the Porto Alegre metropolitan area, Rio Grande do Sul, Brazil.

The different fractions were tested for mutagenicity using the microsuspension method with *Salmonella typhimurium* strains TA98–S9 and TA98+S9 to detect frameshift mutagens; TA98NR and TA98/1,8-DNP6 (TSP samples) or the YG1021 or YG1024 (PM10 and PM2.5 samples) to detect the presence of mononitro, hydroxyamine-compounds and aromatic amines (Kado et al., 1986; Maron & Ames, 1983; Rosenkranz, 1996; Rosenkranz et al., 1980; Watanabe et al., 1989, 1990). The assay response was considered a significant effect when the number of his^+ revertants per plate observed was double that of the spontaneous yields observed in the negative control, a significant ANOVA ($p \leq 0.05$) accompanied by a significant dose-response curve ($p \leq 0.05$). The significance of linear regressions from the dose–response curves was evaluated by SALANAL software (Salmonella Assay Analysis, version 1.0, Integrated Laboratory Systems of Research Triangle Institute, RTP, North

Caroline, USA) choosing the linear or Bernstein model (Bernstein et al., 1982). The positive responses were expressed as the number of revertants based on volume of air sampled (rev/m³).

4.1 Mutagenic activity of particulate matter in the urban area of Porto Alegre

Airborne particulate matter was investigated in the city of Porto Alegre (1.420,000 million inhabitants), capital of the RS state, in southern Brazil, 110 km from the coast, at an altitude of approximately 10m (Figure 1). Studies in different areas of the city, airborne size fractions and periods were performed by our research group (Ducatti & Vargas, 2003; Vargas et al., 1998; Vargas, 2003). The area located in the Botanical Gardens District (30°03'12"S, 51°10'29"W) about 1 km from an avenue with traffic ranging from 72,000 to 120,000 vehicles/day during the studied periods, far from large industrial areas was selected to evaluate the mutagenicity of an urban area. This area has unpublished studies performed during 2006 and 2010.

The studies reported were performed during three different periods using different particulate matter sizes: TSP (first period), Summer (December) of 1994 (Vargas et al., 1998), Spring (October) and Summer (December) of 1997 (Ducatti & Vargas, 2003; Vargas, 2003); PM10 (second period), Spring (October/November) and Summer (December) of 2006; PM2.5 (third period), Winter (August) and Spring (September/October) of 2010 (Table 1).

The first studies evaluated TSP (Ducatti & Vargas, 2003; Vargas et al., 1998; Vargas, 2003), and negative results were observed in most of the samples (Figure 2). The concentration of TSP particles agreed with what was expected for the area according to the institutional monitoring of air quality standards and below the legally established limit for Brazil (ranging from 17 to 60 µg/m³ for October and December, respectively). Mutagenic frameshift responses were observed in December/1997 (4.23 rev/m³), the highest TSP value, previously characterized as negative for mutagenicity.

Particle fraction (Study year)	Period of sampling	Particle concentration (µg/m³)	Mean Particle concentration (µg/m³)
TSP (1994)	December	41	Single filter sample
TSP (1997)	October	17	Single filter sample
	December	60	Single filter sample
PM10 (2006)	October	15	15
	November	24; 37	30.5
	December	76; 14	45
PM2.5 (2010)	August	11; 61; 34	35.3
	September	5; 14; 12; 16	11.7
	October	41; 6; 22	23

Table 1. Urban area: particle fraction, period of sampling and particle concentration.

In second period for PM10, all samples showed positive mutagenic responses and a gradient was observed between the lower and higher values of PM10 concentration (15 to 76 µg/m^3) and mutagenic activity. The concentration value of PM10 in December surpassed the limit recommended by WHO (WHO, 2006). The lowest and the highest values were observed in October (TA98-S9, 1.33 ± 0.30 rev/m^3; TA98+S9, 0.91 ± 0.14 rev/m^3) and December samples (TA98-S9, 17.95 ± 1.37 rev/m^3; TA98+S9, 10.36 ± 0.52 rev/m^3), respectively (Figure 2). Mutagenic activity decreased in the presence of S9 mix, and this response indicated that the predominant compounds present in the organic particulate matter were direct-acting mutagens. All samples showed increased responses for YGs strains, especially YG1021, indicating that nitroarenes predominated in these samples (Coronas, 2008).

In third period for PM2.5, all the samples showed positive responses for TA98, the highest being obtained without S9. The highest frameshift mutagenicity values expressed in revertants/m^3 were found in August pool samples (Figure 2). In one of the filters of this and October pool, the concentration value of PM2.5 surpassed the limit recommended by WHO (WHO, 2006), reaching the value of 61 and 41 µg/m^3, respectively (Table 1). On the other hand, the lowest value observed in the study was 5 µg/m^3, in one of the September pool filters. The elevation of the mutagenic responses shown by the YG strains indicated the participation of nitro-PAHs in direct mutagenic responses, as well as the presence of hydroxylamine compounds detected by the YG1024 strain (unpublished data).
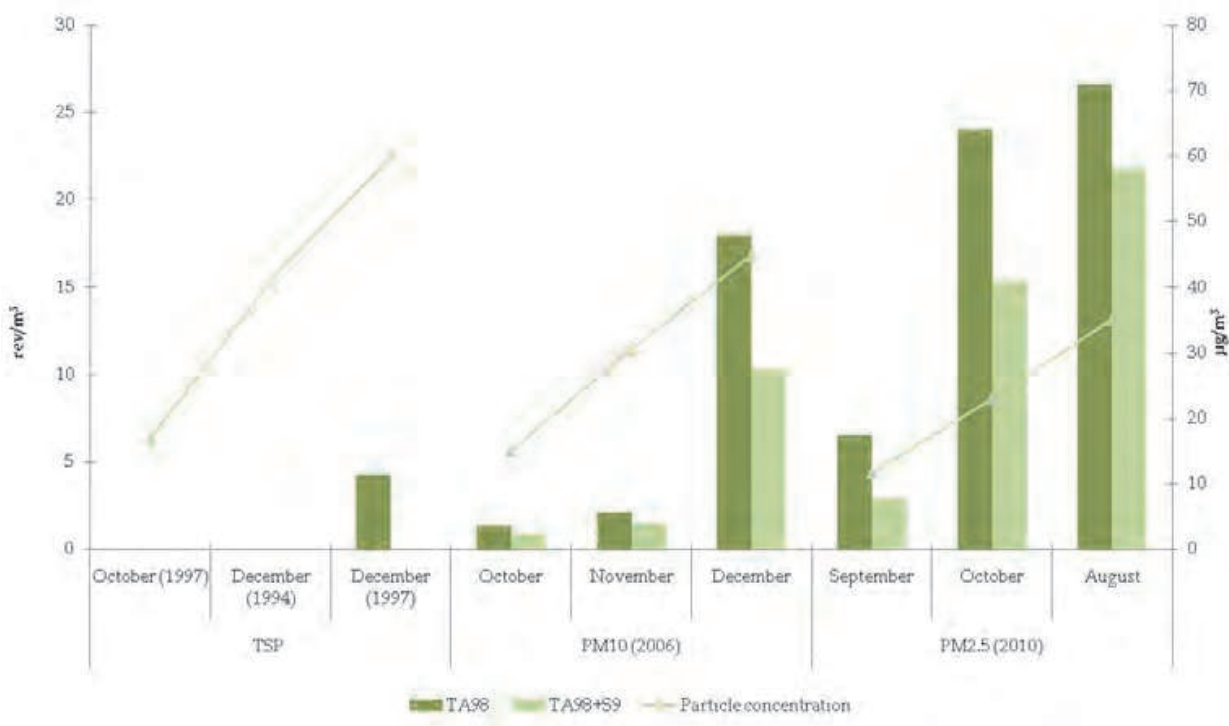

Fig. 2. Mutagenic activity of organic airborne particulate matter extracts evaluated by the *Salmonella*/microsome assay for TA98 strain (revertants/m^3) in Porto Alegre urban area ordered by TSP, PM10 or PM2.5 mean concentrations (µg/m^3). −S9, +S9, absence, presence of S9 mix fraction. TSP samples were not evaluated in the presence of S9.

4.2 Mutagenic activity of particulate matter in industrial areas
4.2.1 Petrochemical area
An area under the influence of a petrochemical complex situated in the town of Triunfo, RS, in southern Brazil (Figure 1), was studied at different times for mutagenicity of organic compounds adsorbed in TSP and PM10 (Coronas et al., 2008; Vargas, 2003). This station is 6.1 km away from the main smokestack of the central raw material production area of this petrochemical district (benzene, toluene, xylene, ethene, propane and butadiene), located in the main atmospheric dispersion quadrant of this complex and 1.4 km from a federal highway (29°49′35″S, 51°24′56″W). This area (14,600 ha) is 30 km upstream from Porto Alegre, in a mixed rural, urban and industrial area.

The study was performed in two periods (TSP and PM10) subdivided into eleven samplings. The first period for TSP during the winter (July and August) and spring (November) months of 1995 and three pools during different stations (January to April; May to August; September to December) of 2000; and the second for particles less than 10 μm (PM10) during summer (February), fall (April), winter (June, July, August) and spring (November) months of 2005. The particles concentration for both TSP and PM10 were within Brazilian air quality standards, although the sampling of the November/2005 pool presented values above those recommended by WHO (WHO, 2006) in almost all filters samples (Table 2).

Particle fraction (Study year)	Period of sampling	Particle concentration (μg/m³)	Mean Particle concentration (μg/m³)
TSP (1995)	July - August	52; 41; 86	59.7
	November	56; 92	74
TSP (2000)	January - April	31; 27; 38; 51	36.8
	May - August	13; 14; 57; 17	25.3
	September - December	8; 38; 31; 38	28.8
PM10 (2005)	February	11; 34; 25; 24	23.5
	April	28; 19; 31	26
	June	46; 44; 10; 9; 37	29.2
	July	27; 26	26.5
	August	28; 20; 41; 10; 23	24.4
	November	35; 111; 88; 71; 58	72.6

Table 2. Petrochemical area: particle fraction, period of sampling and particle concentration.

All samples evaluated were positives for frameshift mutagens, and the results were generally higher in PM10 than TSP. The highest mutagenic activity was detected for TSP in the 1995 samplings and for PM10 in the July/2005 monthly pool (Figure 3). Mutagenicity for organic extracts of TSP and PM10 samples in rev/m³, showed no relationship with the largest TSP and PM10 magnitudes (μg/m³), thus mutagenic activity

was not directly related to particle concentration. Hence, the increase of this mutagenic induction is a consequence of the specific danger classes of compounds adsorbed in the airborne particulate matter. Figure 3 shows the data in growing order of concentration values of TSP and PM10 in µg/m³.

Most of the samples presented higher responses in assays in the absence of S9 mix metabolization. Studies with nitrosensitive strains performed by Coronas et al. (2008), indicate a significant contribution of nitro and amino derivatives of PAHs to the total mutagenicity of suspended particulates (Apel et al., 2010). In fifty percent of the cases, the nitrosensitive strains were more sensitive for mononitrocompounds or for dinitrocompounds.
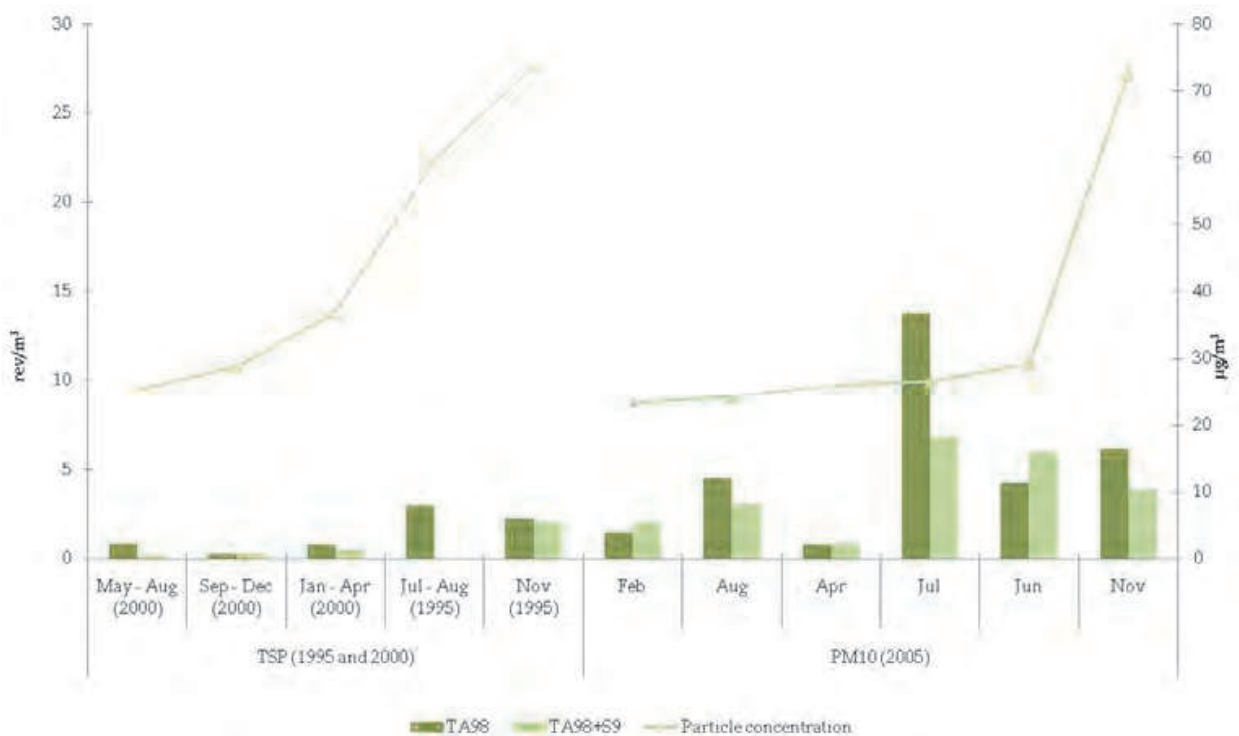
Fig. 3. Mutagenic activity of airborne samples evaluated by the *Salmonella*/microsome assay for TA98 strain (revertants/m³) in Triunfo in an area under the influence of a petrochemical complex ordered by TSP, or PM10 mean concentrations (g/m³). −S9, +S9, absence, presence of S9 mix fraction.

4.2.2 Oil refinery area

An urban/residential area (29°51′29″S; 51°09′25″W) under the influence of an oil refinery plant was assessed for mutagenicity in airborne particulate matter (Coronas et al., 2009). The refinery is located in the city of Canoas (324,000 inhabitants), close to the region that borders on the neighbour municipality, Esteio (Figure 1). In this area PM10 samples were collected weekly from October to December 2006. The collector was placed in the town of Esteio (80,700 inhabitants), Rio Grande do Sul state, in southern Brazil. The dominant winds there are from the Southeast, and therefore Esteio is most influenced by the plant, and the PM10 collector was installed at a site predisposed to particle deposition. Two automatic stations from the Rio Grande do Sul Environmental Protection State Foundation (FEPAM) measure

PM10, SO_2, NO_X, O_3, CO and meteorological parameters continuously. PM10 organic extracts were also quantified for 16 United States Environmental Protection Agency (EPA) priority polycyclic aromatic hydrocarbons: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]flouranthene, benzo[a]pyrene, dibenzo(ah)anthracene, benzo[ghi]perylene, and indeno(1,2,3-cd)pyrene.

During the period studied the concentration of PM10 particles ranged from 9 to 62 µg/m³ (Table 3), and the latter concentration was the only one that surpassed the values recommended by WHO (WHO, 2006). All samples showed positive responses for mutagenesis for TA98 strain (Figure 4).

Particle fraction (Study year)	Period of sampling	Particle concentration (µg/m³)	Mean Particle concentration (µg/m³)
PM10 (2006)	October 1	9; 23	16.0
	October 2	62; 27	44.5
	November 1	19; 46	42.0
	November 2	14; 20	17.0
	November - December	15; 37	26.0
	December	47; 40	43.5

Table 3. Oil Refinary area: particle fraction, period of sampling and particle concentration.
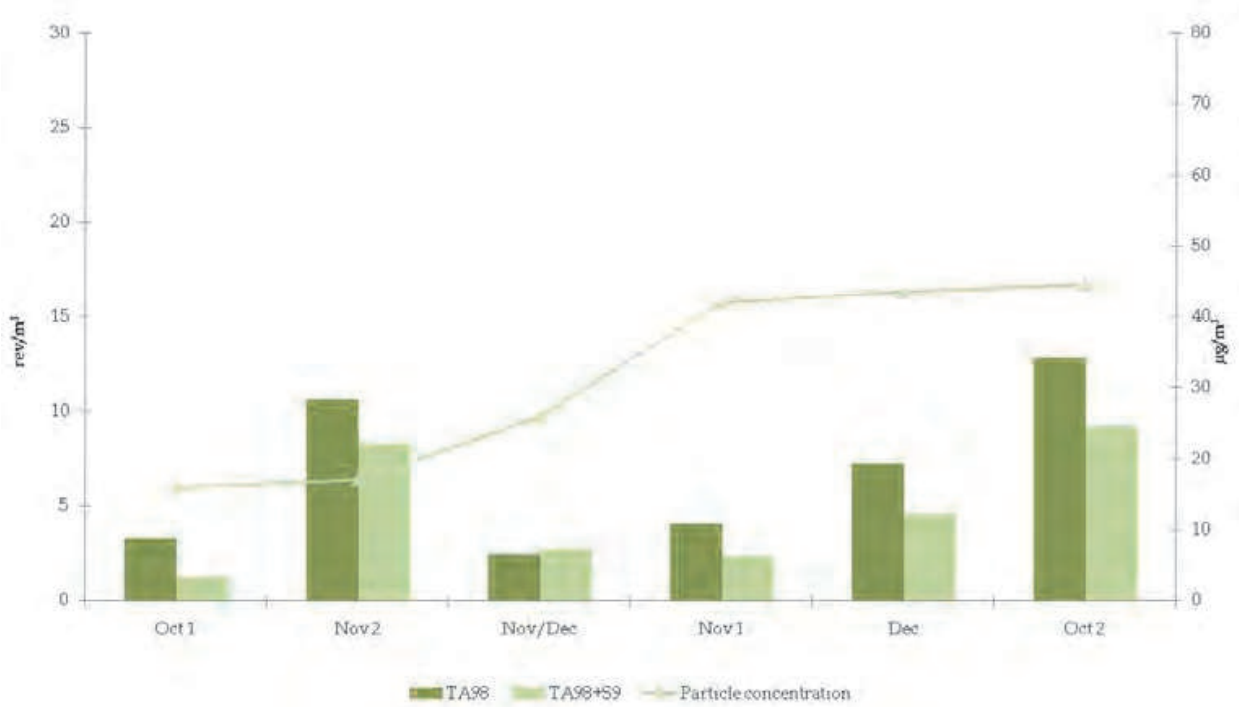

Fig. 4. Mutagenic activity of airborne samples evaluated by the *Salmonella/*microsome assay for TA98 strain in an urban area under the influence of oil refinery plant ordered by PM10 mean concentrations (g/m³). −S9, +S9, absence, presence of S9 mix fraction.

For most of the samples, the mutagenic activity decreased in the presence of S9 mix, except for Nov/Dec which increased slightly. This response indicated that the predominant compounds present in the organic particulate matter were direct-acting mutagens. All samples showed increased response for mutagenic activity for the YGs nitrosensitive strains (Coronas et al., 2009), indicating the presence of nitrocompounds in the evaluated sites, with preponderance of results involving the YG1024 strain. The PAHs content is shown in figure 5. Sample Oct 2 showed the highest PAHs concentrations and also the highest mutagenicity response. Indeno(1,2,3-cd)pyrene and Benzo(g,h,i)perylene showed higher concentrations than the other PAHs in all samples. Eight of the 16 PAHs analyzed are classified by The International Agency for Research on Cancer (IARC, 2010) as group 1 (carcinogenic to humans: benzo[a]pyrene), group 2A (probably carcinogenic to humans: dibenzo(ah)anthracene) or group 2B (possibly carcinogenic to humans: naphthalene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]flouranthene, indeno(1,2,3-cd)pyrene). In these samples the carcinogenic PAHs represented more than 66% of the total PAHs concentration (Figure 6).

All samples showed an increased response for mutagenic activity for the YGs strains, indicating the presence of nitrocompounds in the evaluated site (Coronas et al., 2009). Strain YG1024 produced higher values of revertants/µg of EOM in five of the six samples, while at site 2, in an urban area, strain YG1021 showed higher values in most of the samples.

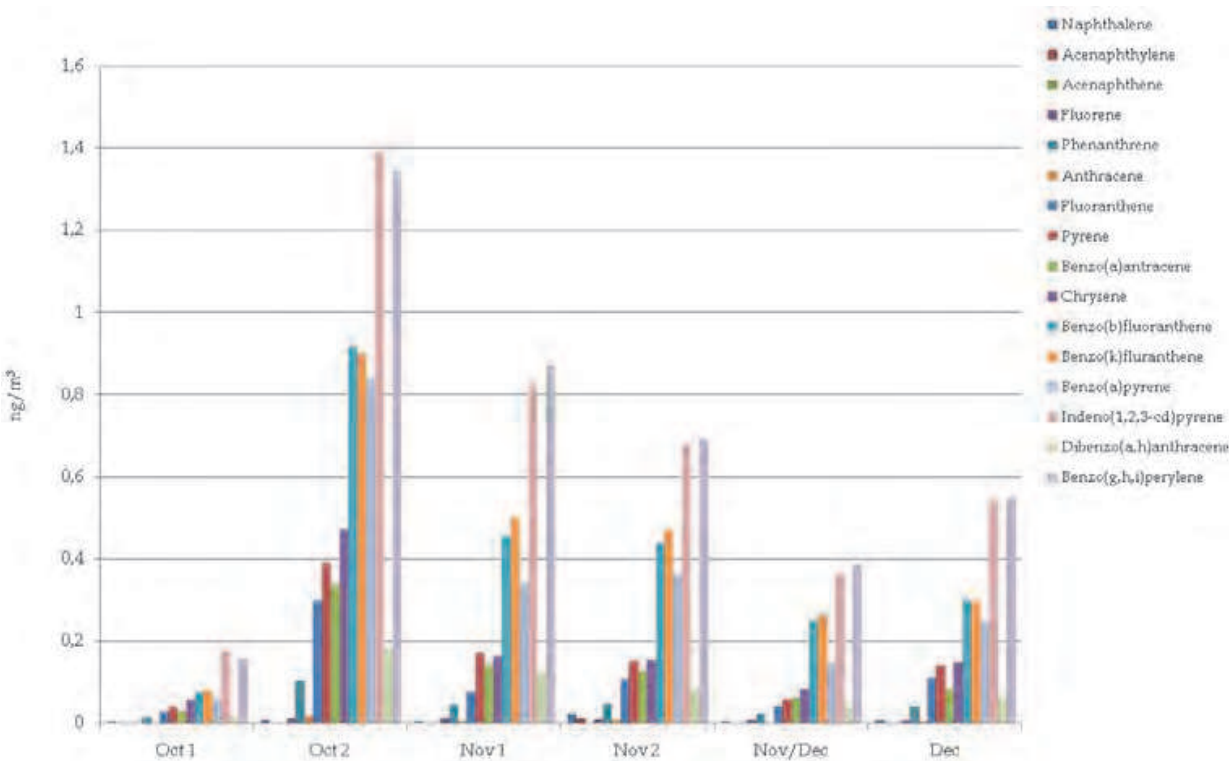Fig. 5. PAH concentrations (ng/m³) in PM10 samples from an urban area under the influence of oil refinery plant.

DNA damage in people residing and/or working downwind from this oil refinery was investigated (Coronas et al., 2009). Samples of peripheral blood, buccal mucosa and PM10 were simultaneously collected and evaluated using the comet, micronucleus and

Salmonella/microsome assays, respectively. The subjects showed significantly increased DNA damage in lymphocytes detected by the comet assay and no difference in the frequencies of micronucleated buccal mucosa cells. These genetic lesions were not associated with the tobacco smoking habit, age and recent exposure to X-ray for diagnosis.
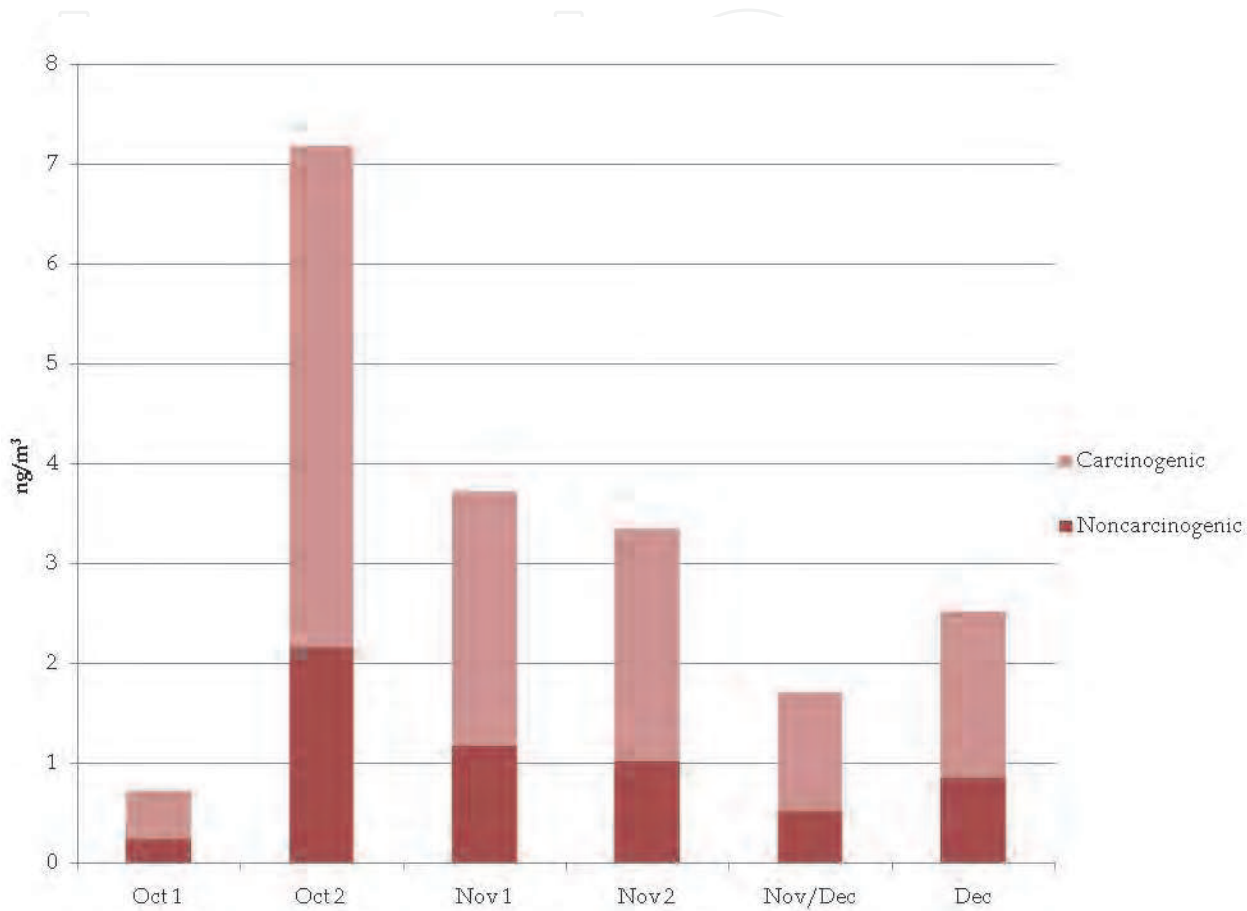

Fig. 6. Concentrations of potentially carcinogenic PAHs (ng/m³) according to IARC (2010) in PM10 samples from an urban area under the influence of oil refinery plant.

4.3 Discussion

The case studies showed the mutagenicity of TSP, PM10 or PM2.5 DCM-fraction in the urban area of Porto Alegre city and in two industrial regions. One of these areas was under the specific influence of a petrochemical industry and another was influenced by contaminants from an oil refinery, but inserted in an urban area.

The site studied in Porto Alegre city had been used as a reference for the urban area (RS, Brazil) presenting absence or low mutagenic activity in previous studies (Ducatti & Vargas, 2003; Vargas et al., 1998). These studies evaluated TSP organic extracts, which might account for the low mutagenic response, since the lowest particle fractions concentrate organic and mutagenic compounds (Hayakawa et al., 1995; Pagano et al., 1996). An increase in mutagenic induction was observed, generally, for the PM10 and PM2.5 DCM-extracts as the particle diameter diminishes. However, is necessary to

consider that traffic of motor vehicles increased during this new period of diagnosis. Other studies detected the 16 most important PAHs in PM10 at this site (Dallarosa et al., 2005). Just as the capacity to penetrate more deeply into the respiratory tract rises with the decreased diameter of the particle, increased mutagenic activity is also reported in the literature (Claxton et al., 2004).

The studies in urban area appear to indicate a trend between the concentration of TSP, PM10 and PM2.5 and intensity of the mutagenic effect observed. However, this evidence was not clearer in industrial areas. The increase of the mutagenic induction in these areas can be a consequence of the specific hazard classes of compounds adsorbed in the airborne particulate matter.

Although, most concentrations of particulate matter are in accordance with the primary and secondary standards for atmospheric particulate matter TSP and PM10 in Brazil (Brasil, 1990), the different studies showed that the *Salmonella*/microsome assay was sensitive to define areas contaminated by mutagenic compounds (Coronas et al., 2008; Coronas et al., 2009; Ducatti & Vargas, 2003; Pereira et al., 2010; Vargas, 2003).

However, a concentration of 150 $\mu g/m^3$ (Brazilian legal standards for 24 hour-mean) is related to an approximately 5% increase in daily mortality according to the World Health Organization (WHO, 2006). A more restrictive value (50 $\mu g/m^3$ 24 hour-mean) for PM10 concentration is suggested by WHO air quality guidelines to decrease adverse health effects. In the present study, three pools presented filters with values higher than this standard (WHO, 2006): one in an urban area and two pools in areas under industrial influence. For PM2.5 our results showed that one sampling suppressed WHO air quality guidelines (2.5 $\mu g/m^3$ 24 hour-mean) (WHO, 2006).

Approximately half the ambient mutagenicity can be ascribed to atmospheric reaction products of two-to-four-ring PAH. However, PAHs are commonly present in airborne particles and increase mutagenic activity when S9 mix is used (Lewtas, 2007; Claxton et al., 2004), and can explain part of the mutagenic induction observed in these studies. In the area under the influence of the refinery, the presence of PAHs can account for part of the mutagenic activity with S9 mix. In these samplings, 66% of the total concentration of PAHs detected was classified by IARC (IARC, 2010) as potentially carcinogenic. A recently published study of our research group (Pereira et al., 2010) highlighted the presence of carcinogenic PAHs compounds and mutagenic activity in a city located in the main atmospheric dispersion quadrant of a petrochemical industrial complex region (22 km distant), with a long history of mutagenic activity in its atmospheric particulates and water resources (Vargas et al., 2003; Vargas et al., 2008).

Nevertheless, other compounds extracted in the base/neutral fraction (DCM) are responsible for direct mutagenicity in organic samples analyzed by *Salmonella*/microsome assay, for example nitroarenes and aromatic amines (Coronas et al., 2008; Coronas et al., 2009; DeMarini et al., 1994; Pereira et al., 2010; Vargas, 2003; Watanabe et al., 1989; Watanabe et al., 1990). Not all of the mutagenicity can be ascribed to particular compounds, but much of it is due to nitro-PAH lactones and to simpler nitro-PAH (Lewtas, 2007). This supports the importance of co-pollutants in the mutagenic activity of airborne particles. Reactive gases (NO_X, O_3) under photoactive conditions can produce mutagenic compounds from non-mutagenic organic compounds commonly present in ambient air (Claxton et al., 2004). The present comparative study appears to indicate a more significant presence of mononitro compounds in urban areas and dinitro compounds in industrial areas influenced by petrochemical compounds.

5. Conclusions

Salmonella/microsome is the most widely used assay for monitoring mutagenic compounds in airborne particulate matter. Although several mechanisms are associated with cancer due to environmental exposure, the primary event of carcinogenesis by genotoxicity is the mutagenic event. The assay allows modeling carcinogenic potency without identifying all the specific carcinogens present in the mixture, by using strains sensitive to different classes of compounds.

All these results highlight the evidence that the mutagenic activity of airborne particulate matter organic DCM-extracts detects hazardous atmospheric compounds, more closely related to the types and reactivity of compounds adsorbed in airborne particulate matter than to the particle mass. It also indicates that particulate matter concentrations alone do not present sufficiently good air quality standards to avoid damage to the environment and human health. Besides the particulate concentration, the characterization of the present hazardous compounds and the reactivity of the mixture formed by it are essential to support exposure estimates.

Reviews of literature (Claxton et al., 2004; Claxton & Woodwall Jr., 2007) discuss the importance of the results that have already been identified in the *Salmonella*/microsome assay, pointing out that their international standardization and applicability to the definition of the presence of compounds potentially carcinogenic to humans and to define the health risk assessment. Claxton & Woodwall Jr. (2007), present tests in different levels of organisms used to evaluate mutagenic compounds in the air, and emphasize the limitations of some assays regarding mammal responses, such as the metabolic pathways, exposure cycles and extrapolation from observed potency to carcinogenic potency.

Studies in this field of knowledge need to advance with additional work on the mutagenic effects of volatile and semi-volatile organic fractions, metals associated with mutagenic potency and the role of atmospheric transformation in raising the complexity of the samples adsorbed in airborne particulate matter. The chemical identification of compounds in these mixtures is complex. This emphasizes the value of associating biomarkers and chemical analysis, defining the main classes of compounds found in the sample and their dispersion, and predicting effects on ecosystem stability and human health. The integration of *in vitro* and *in vivo* responses complements the understanding of the pathways and action mechanisms of compounds and mixtures. Finally, it is necessary to intensify the efforts in the monitoring of fine particles, such as PM2.5, which concentrate a significant amount of mutagenic substances besides presenting a greater probability of deposition in the smaller conducting airways and alveoli. These studies can favor early measure to protect the environment and human health.

Biological tests like *Salmonella*/microsome assay are a sensitive tool to screen the presence of specific chemical compound classes in environmental monitoring programs to characterize areas under the influence of anthropogenic activities. It should be mentioned that there has been an advance in Brazilian environmental law, in which the state of Rio Grande do Sul included the genotoxic assays, especially *Salmonella*/microsome, to control industrial effluents (CONSEMA, 2006).

6. Acknowledgment

7. References

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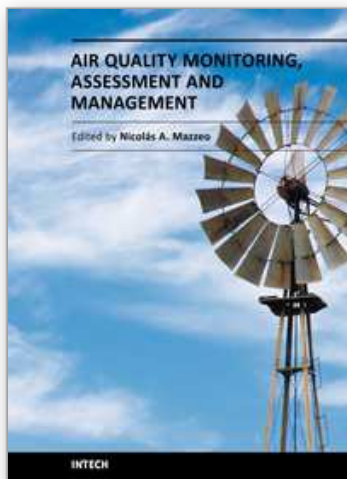
Air Quality Monitoring, Assessment and Management

Edited by Dr. Nicolas Mazzeo

Human beings need to breathe oxygen diluted in certain quantity of inert gas for living. In the atmosphere, there is a gas mixture of, mainly, oxygen and nitrogen, in appropriate proportions. However, the air also contains other gases, vapours and aerosols that humans incorporate when breathing and whose composition and concentration vary spatially. Some of these are physiologically inert. Air pollution has become a problem of major concern in the last few decades as it has caused negative effects on human health, nature and properties. This book presents the results of research studies carried out by international researchers in seventeen chapters which can be grouped into two main sections: a) air quality monitoring and b) air quality assessment and management, and serves as a source of material for all those involved in the field, whether as a student, scientific researcher, industrialist, consultant, or government agency with responsibility in this area.

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