National Institute of
Integrated Analysis of Environmental Risk
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National Institute of Integrated Analysis of Environmental Risk

TECHNICAL-SCIENTIFIC REPORT

YEAR 2009

Invitation to bid: MCT/CNPq no. 015/2008 – National Institutes of Science and Technology

CNPq proceeding number - 573813-2008-6 – duration: 1/2/2009 to 30/1/2012

FAPESP proceeding number: 2008/57717-6 – duration: 1/3/2009 to 28/2/2014

Coordinator: Paulo Hilário Nascimento Saldiva

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1. INITIAL CONSIDERATIONS

The approval of INCT INAIRA occurred on 27/11/2008, with the signature of the granting document on 6/1/2009 and initial release of CNPq funds regarding year 2009 on 25/02/2009.

In the beginning of 2009, INAIRA coordinators established criteria and ways of organizing the release of funds, the organization of work groups according to themes, definition of coordinators and responsible persons for the groups, and finally the goals for 2009, together with the researchers. The plan of goals was sent to CNPq as requested.

In the administrative part, a complex system of spreadsheets and instruction manuals was created for the researchers to teach them how to proceed when purchasing equipments and reagents. The organization of the administrative part is properly controlled, and we have total control of expenditures, with their justifications and pertinent documentations.

The creation of the website (www.inaira.org) was an auxiliary mechanism in the organization of INAIRA, especially for the coordinators. The possibility of all researchers using the website was largely divulged by INAIRA coordination, and the response of researchers has been diverse. We believe it is a good divulgation vehicle, and we are receiving e-mails from the general public, mass media professionals.

The work groups located in centers with lesser support from local Research Support Foundations (FAPs) received most of the funds coming from CNPq in 2009. Some of these groups have not yet showed quantitative results, and are in the phase of installing laboratories. FAPESP fund was initially used in the importation of more expensive devices in São Paulo, because this agency has a well organized and efficient bureaucratic support for importation. Many of the researchers are still receiving and installing large devices in their laboratories. For some groups, such as the Aging and Cardiovascular (clinical branch) group, there were not enough funds in 2009, and such groups shall be provided with funds in 2010.

Thematic work groups were formed, and coordinators were appointed for each of them, through the meeting of the Steering Committee. Groups are working with intense collaboration, as observed in item 3.

Two important exposure systems that will be used by various INCT-INAIRA groups were fully put into operation, generating the first scientific results: The concentrator of environmental particles (CAP) and the generator of Diesel/Biodiesel particles.

The concentrator of particles was purchased through previous financings from FAPESP

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(Proc. 2002/09804-0 – Pulmonary and cardiovascular changes induced by inhalation of concentrated particulate matter from the atmosphere of São Paulo). With a very complex engineering, some years were necessary to fully complete its installation. The engineer responsible for its installation was Dr. Paulo Afonso de André, in collaboration with Dr. Petros Koutrakis and Dr. Stephen Rudnick, researchers from the Harvard School of Public Health, Boston, USA.

The assemblage of CPA is a highly complex task, imposing some very extreme difficulties that made us fear for the success of the project. Fortunately, the obstacles were overcome and the system started operation in March 2009, after which the experiments reported from page 65 on of this report were carried out.

Since the beginning of the project, the strategy of developing the CPA on a movable base was outlined. The positive side of this proposal is the possibility of approaching various pollution sources, carrying the CPA to the neighborhood of avenues, industrial areas and regions affected by the burning of biomass, thus expanding the possibility of studies developed with such type of equipment. In this project, for instance, the initial experiments were developed at USP campus in Butantã, in the yard located between ICB-I and ICB-IV. Such site represents a background scenery of São Paulo, perhaps the situation most representative of the average exposure of São Paulo residents.

Since January 2010, the concentrator is installed at the yard of FMUSP, and the first studies related to INAIRA are already in progress, as we can observe in item Collaborations among INAIRA members.

Another important device that will be used in experimental studies is the generator of biodiesel/diesel particles. Such device will be used in various experimental and *in vitro* studies of INCT INAIRA. The device was assembled during 2009 (see report of Dr. Ana Julia Lichtenfels, Development thematic group and Cardiovascular Thematic group report). The device was assembled at USP Laboratory of Atmospheric Pollution, where the two already mentioned studies took place. However, despite the installation of the exhaustion system in the laboratory, there were problems with the fuel burning smell in other laboratories. Thus, the device will be transferred to the FMUSP yard, and the design of the container to shelter it is already being executed.

The groups involving clinicians are in a more initial phase than those experimental groups, which was already expected. The approvals from the ethics committees are more time-consuming,

and in various studies there is the need of importing higher complexity devices for the measurement of clinical outcomes. The assemblage of the environmental pollution exposure system for humans is also very time-consuming.

In terms of scientific production, in 2009, thirty three (33) scientific articles, indexed in the PubMed by INAIRA members, were published, with themes involving air/environmental pollution and health. The abstracts of those articles can be visualized in the *website*, Scientific Publications section.

2. STEERING COMMITTEE – CONDUCTED MEETINGS AND DECISIONS;

Two meetings of the Steering Committee were conducted.

In the first one, decision was made about how study groups would be grouped, the coordinators of each field, the construction of the website and the elaboration of the video requested by CNPq. The redistribution of funds in relation to the original project was also discussed again, considering that with the variations of the dollar exchange rate and the budget finally provided by CNPq, not all the researchers could be provided with all their requests.

The research groups were grouped according to similar themes, with a main coordinator and responsible researchers for each subproject. Therefore, nine thematic groups were formed: Epidemiology, Biomonitoring, Development, Inflammation, Cardiopulmonary, Carciogenesis, Endocrinal, Aging and Education.

The projects related to each Thematic group can be seen in our website www.inaira.org.

In this meeting, the way of elaborating the plan of goals was decided, which was then forwarded to CNPq in the beginning of the year as requested. Ever since, small meetings with group coordinators and steering committee members are taking place on a routine basis, for the discussion of research protocols and intergroup collaborations.

The second meeting took place on March 31, 2010, with the presentation of the first results obtained in the concentrator of particles and in the motor-generator of biodiesel burning. In this meeting, the priorities for 2010 were discussed, and the results of each group were also presented.

3. ACTIVITIES OF COOPERATION AMONG PARTICIPANT GROUPS OF THE INCT;

We observed, along this year, that there was interaction among thematic groups. For

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instance, there is a great partnership between the Biomonitoring and Environmental Education groups, which have intricate biomonitoring projects with plants and environmental education in schools of the interior of the country. The Biomonitoring thematic group has been doing a very adequate work of interaction among its various members in the country.

Many of the groups dealing with animal experiments will need to make their exposures at the concentrator of environmental particles or at the generator of diesel/biodiesel particles which are next to the FMUSP Pollution Laboratory. As an example, the Fiocruz group from Bahia sent animals with metabolic disorder to São Paulo to receive urban particulates in the concentrator of particles in March 2010.

The *in vitro* studies of the Inflammation group will need particles that will be collected by groups in the field in the epidemiology studies, for instance, or even in the concentrator of particles, which will be used by these groups.

Coordinators, however, recognize that it is highly necessary to increase the scientific exchange with groups outside São Paulo. We believe that, with the scientific progress of projects, INAIRA can contribute for this better interaction in the form of results presentation seminars, participation in theses defense, etc.

4. Cooperation Activities between INCTs and other institutions (companies, NGOs, governmental institutions, etc.);

INAIRA, through its members, has been significantly contributing for the public debate about the adverse effects of different types of air pollution on the human health. In 2009, INAIRA members contributed with the printed media or via web at least in 64 occasions, see website inaira.org, News in the Media section.

In the international sphere, INAIRA coordinator has acted as consultant and expert in important occasions. Professor Paulo Saldiva participated, in September 2009, in the meeting of the World Medical Association in Copenhagen for the elaboration of the document *Health and Climate Change,* which would be presented in the COP-15 in that same year. In addition, Professor Saldiva is from the panel of experts of the document *Air Quality and Health*, for the document *Critical Earth Observation Priorities* of the *Group on Earth Observation (GEO). GEO* is a volunteer partnership of governments and international organizations. It coordinates a structure in which partners can coordinate new projects, define strategies and request investments, see the website

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www.earthobservations.org.

Professor Paulo Saldiva has acted as partner and consultant of the **Secretariat of Green and of Environment** in some occasions throughout 2009. In August 2009, the Secretariat of Green and of Environment of the city of São Paulo carried out an event to discuss the global warming issue, at municipal level: the "I Municipal Meeting on Climate Changes - São Paulo in Direction to Copenhagen". The objective of the event was to debate with São Paulo city politicians issues related to global warming, what are the effects of climate change on human health, and what are the necessary measures to comply with the Municipal Law on Climate Changes, which was sanctioned in June of this year, and that establishes as goal for 2012, the reduction of 30% of greenhouse gas emissions in the city. For such a purpose, the meeting had presentations of three specialists on the matter: the environmentalist Fábio Feldmann, the professor of USP Medical School, Paulo Saldiva, and also the Secretary of Green and Environment of São Paulo, Eduardo Jorge. Then, representatives from the Cades – Municipal Council of Environment and Sustainable Development, and from the Regional Councils of Environment and Culture of Peace, among other institutions, took part in the open debate about the topic.

Professor Saldiva has been an active participant of the ***Nossa São Paulo*** (Our São Paulo) movement. The executive-secretariat of the movement is formalized in the legal figure of the Instituto São Paulo Sustentável (ISPS), non-profit organization that received the OSCIP accreditation (Civil Society Organization of Public Interest). In 2009, two important activities are highlighted. The manifest of the World without Car day, where Professor Saldiva gave the lecture “The Impact of Pollution on Public Health”, in the theater of São Paulo University Medical School (Avenida Doutor Arnaldo, 455). The main theme was the effect of high sulfur contents diesel and the consequences of the noncompliance of CONAMA resolution 315/2002, which provided for the commercialization of a cleaner fuel starting on January 2009.

In addition, Professor Saldiva has actively participated in the “Public Act for a cleaner Diesel”, in opposition to the non-reduction of sulfur contents in the diesel within the established period of time by Petrobrás.

Professor Saldiva is also participating and supporting the formation of the new **NGO "Instituto Saúde e Sustentabilidade"** that is dedicated to study the interaction between human health and environment. The NGO wants to show the problems of urban sustainability, call the attention to its effects on health, and that only environment doctors, responsible for public

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decisions, can save it. For such purpose, a movie was idealized, with the city of SP playing the role of an ill patient. The movie was exhibited during the lecture of Professor Saldiva in the Conference of Environment Doctors carried out in São Paulo, on November 28, 2009. The video can be seen at "http://diamundialsemcarro.ning.com/video/instituto-saude-e".

At last, in February 2010, Professor Saldiva was invited by Dr. Francisco Graziano Neto to take part in the **Interinstitutional Work Group of the State Secretariat of Environment**, with the assignment of revising the air quality standards and to enhance the integrated issue of air quality in the State of São Paulo.

Other INCT-INAIRA groups have been establishing partnerships with public entities such as prefectures, municipal secretariats and non-governmental organizations, as well as with schools. The list of partners with which the thematic groups Biomonitoring and Education of INAIRA formed partnerships is presented as follows:

1. House, Environment and Health, Municipal Prefecture of Guaratinguetá, SP (educational space);

2. Cônego José Bento Technical School, Environment Secretariat of Jacareí, SP;

3. University of Taubaté (UNITAU - Municipal Institution)

4. Secretariats of Environment and Education of São José dos Campos, SP;

5. Secretariat of Education of São Jose dos Campos, SP

6. Vale do Paraíba University (UNIVAP), in São José dos Campos, SP;

7. Salesians University Center of São Paulo (UNISAL), Lorena, SP;

8. Secretariat of Environment of Jacareí, SP;

9. Municipal Prefecture of Mirassol D'Oeste, MT;

10. Municipal Prefecture of Vila Rica, MT;

11. Secretariat of Health of Vitória Municipality, ES;

12. Ashoka (www.ashoka.org)

13. Rede Rema Atlântico (www.remaatlantico.org/)

14. Instituto Eco Desenvolvimento (http://www.ecod.org.br)

15. Grupo Ambientalista da Bahia - GAMBA (www.gamba.org.br)

16. Instituto Baleia Jubarte (www.baleiajubarte.com.br/)

17. Grupo de Recomposição Ambiental – GERMEN (http://ospiti.peacelink.it/zumbi/org/germen/index.html)

18. Hugo Sarmento School – Coração Roxo Project

5. NATIONAL AND INTERNATIONAL EVENTS: PRESENTATION OF PAPERS, ORGANIZATION OF COURSES, SEMINARS, LECTURES AND ROUND TABLE DISCUSSIONS;

INCT has financially contributed for the participation of twenty researchers in National and International Congresses. The main events organized by INAIRA members or in which researchers will take part are listed in Scientific Events. Some participants put their testimonies about the Congresses in the website (inaira.org), as observed in the item Reports and Testimonies. The number of events is already quantified in the table of indicators. Here, we present the titles of main events and works in which INAIRA researchers took part along the year.

Conferences

1. Conference Environment Doctors – 28.11.2009 – Venue: Theater of the São Paulo Medical Association - Av. Brigadeiro Luiz Antonio, 278, 8o. andar
2. International Workshop on Air Quality Criteria for the protection of human and aquatic organisms health -- 16 to 20.11.2009 - Venue: Quality Riviera Hotel in Jundiaí
3. IX National SBMCTA Congress – 11 to 14/11/2009 – Venue: UFOP Conventions Centers, located at Rua Diogo de Vasconcelos, no. 328, Bairro Pilar, in the city of Ouro Preto, Minas Gerais.
4. I Extension Course on Atmospheric Pollution and Health - - 09 to 13/11/2009 – Venue: Post-Graduation Amphitheater - Address: Rua Sarmento Leite, 245 - Porto Alegre, RS
5. IV Meeting in Fundamental Aspects of DNA Repair and Mutagenesis - - 08 to 10/11/2009 – Venue: Federal University of Minas Gerais (UFMG), located Av. Antônio Carlos, 6627, Pampulha, in the city of Belo Horizonte, Minas Gerais.
6. 47th Brazilian Congress of Medical Education - COBEM -- 17 to 20.10.2009 - Venue: at ExpoCuritiba, in Curitiba, the 47th Brazilian Congress of Medical Education – Room: Parque Tingui Auditorium
7. I International Symposium of the Cardiopulmonary and Metabolic Rehabilitation center of the German Hospital Oswaldo Cruz -- 16.10.2009 - Venue: German Hospital Oswaldo Cruz
8. XVI Brazilian Congress of Toxicology – 10 to 14.10.2009 – Venue: RHODES EVENTOS - Rua Tomé de Souza, 67 - Seg. Andar - Savassi - Belo Horizonte/MG
9. Unemat Climate Changes is partner in the conduction of the III Meeting of the Pro-

Environmental Group in Pontes and Lacerda -- 26.09 to 11.10.2009 - Venue: headquarters of the Lions Clube

10. I WORKSHOP ON BIOMONITORING IN THE VALE DO PARAÍBA – 25/09 to 06/10/2009 - Venue: IAI-INPE Auditorium – São José dos Campos
11. I Integrated Congress on Health, 7th Academic Journey of Medicine of Dourados – 28.09.2009 – Venue: Various / Municipal Theater / University Hospital / Unit I Auditorium
12. IX Meeting of Post-Graduation in Cardiology UFF/INCOR – USP – 11.11.2009 – Venue: Aloísio de Paula Amphitheater, 2o floor front, Antonio Pedro University Hospital/Rua: Marquês de Paraná 303 - Centro - Niterói – RJ - – Professor Paulo Saldiva Lecture
13. Meeting on the Effects of the Occupational and Environmental Exposure to Aerosols - - 23 to 25/09/2009 - Venue: CETES Auditorium _ Center of Health Technology / Faculty of Medicine/UFMG, Belo Horizonte – Av. Alfredo Balena, 190 6th floor / Sta Efigênia
14. Workshop – Sustainability, Health and Citizenship - - 12.09.2009 – Venue: Medical School of São Paulo University – Av. Dr. Arnaldo, 455.
15. 7th Congress of Toxicology in Developing Countries - - 06 to 10/09/2009 – Venue: Sun City, South Africa
16. 7th International Congress of Pharmaceutical Sciences - - 06 to 09/09/2009 – Venue: Ribeirão Preto, São Paulo, Brazil
17. 1st Ibero-American Meeting on Toxicology and Environmental Health (IBAMTOX 2009) - - 06 to 08/09/2009 – Venue: Ribeirão Preto, São Paulo, Brazil
18. National Forum on Cardiovascular Diseases and the Environment – 14.08.2009 – Venue: Espaço Sociocultural - Teatro CIEE, Rua Tabapuã, 445 - Itaim Bibi - SP.
19. Symposium on Lead and Human Health – July 30 to 31 of 2009. Venue: Espaço Cultural Capela, USP Campus in Ribeirão Preto
20. I Meeting Meteorology and Health - - 30/05/2009 – Venue: Center of Weather Forecast and Climatic Studies of the National Institute of Spatial Researches (CPTEC/INPE), in Cachoeira Paulista (SP).
21. American Thoracic Society International Conference 2009 - - 15 to 20/05/2009 – Venue: San Diego, California
22. 5th World Environmental Education Congress - 10 to 14/05/2009 – Venue: Montreal, CN
23. World Congress on Public Health – April 28 to May 1 of 2009 – Istanbul - Turkey
24. International Congress on Occupational Health – ICOH 2009 – March 12 to 16 of 2009 – Cape Town – South Africa.

Meetings and Lectures: All lectures were posted in the website with a notice sent to all members of INAIRA.

1. Acute cardiovascular and inflammatory toxicity induced by the inhalation of diesel and biodiesel fuels particles. Master´s dissertation of José Mara de Brito – 19.03.2010 - Venue: Instituto do Coração – INCOR - Address: Av. Dr. Enéas de Carvalho Aguiar, 44 – 2nd floor - block I
2. Air pollution and human health: certainty and doubts" – 16.12.2009 – Venue: USP Medical School – Parasitology Amphitheater, 2nd floor, room 2303.
3. Seminar: The Sucroenergentic Sector and São Paulo State House of Representatives: Building a Positive Agenda - - 18.11.2009 - Venue: Franco Montoro Auditorium of the São Paulo State House of Representatives - Professor Paulo Saldiva Lecture

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4. TEDx São Paulo - - 14.11.2009 – Venue São Paulo – Professor Paulo Saldiva Lecture
5. I Municipal Conference of Environmental Health - - 26.09.2009 – Venue: auditorium I of UniSant'Anna, located at Rua Voluntários da Pátria, 421, block I, 6th floor, São Paulo - SP. Professor Paulo Saldiva Lecture
6. Effects of exposure to particulate matter (PM 2.5) from atmospheric pollution on the spermatogenesis of two generations of mice. - - 11.09.2009 – defense of thesis of Adriana Pires, Venue: Nina Rodrigues of Oscar Freire Institute
7. I Municipal Meeting on Climate Changes – São Paulo Bound to Copenhagen" – 29.08.2009 – Venue: MAC Auditorium. Address: Ibirapuera Park, Gate 3, Av. Pedro Álvares Cabral – São Paulo/SP. Professor Paulo Saldiva Lecture
8. Lecture on H1N1 influenza, with professor Paulo Saldiva (FM) - - 21.08.2009 – Venue: University Council Room – Address: Rua da Reitoria, 109, University City, São Paulo. Professor Paulo Saldiva Lecture
9. Class on Biomonitoring: "Micronucleus test in Tradescantia pallida" - - 08.06.2009 – Venue: Vale do Paraíba – SP. Given by Débora Já de Araujo Lobo, INAIRA member
10. Scientific/Administrative Meeting of Biomonitoring - - 22.05.2009 – Venue: Venue: Department of Pathology, FMUSP, room 1163. Biomonitoring Group.
11. Lecture: Reproductive Toxicology - - 20.05.2009 – Venue: Room 1154 – First floor - FMUSP
12. Word Carfree Day – 22.09.2009 – Venue: City of São Paulo. Professor Paulo Saldiva Lecture
13. Lecture: Biodiesel and emissions – 06.05.2009 – Venue: Room 1163 – First floor – Department of Pathology, FMUSP.
14. Lecture: ´´Animals as bioindicators of exposition or biomarkers of effects to human health´´ 28.04.2009 - Venue: Post-graduation room of the Department of Pathology, FMVZ-USP.
15. Debate "The Impact of Pollution on the Public Health" - Venue: Auditorium of USP Medical School – City: São Paulo - SP

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6. ACTIVITIES OF HUMAN RESOURCES FORMATION AND QUALIFICATION

Until March 2010, the following scholarships have been distributed:

CNPq Scholarships

Scholarship Student	Proceeding	Validity Time	Mod. Category	Status	Thematic Group
Amanda de Andrade Lanzotti	107409/2009-7	01/08/2009 to 31/01/2012	IC	Cancelled by the coordinator	Cardiopulmonary
Amanda de Flório Barizon	102775/2010-9	01/03/2010 to 31/05/2012	IC	Pending of beneficiary acceptance	Biomonitoring
Débora Alvares Leite Figueiredo	107416/2009-3	01/08/2009 to 31/01/2012	IC	Cancelled by the coordinator	Cardiopulmonary
Esthalin Moreira da Silva de Souza	112724/2009-4	01/08/2009 to 31/01/2012	IC	In force	Biomonitoring
Felipe Duarte Coelho de Sousa	104919/2009-4	01/07/2009 to 30/06/2012	IC	In force	Biomonitoring
Fernanda Nogueira Dantas de Souza	104341/2009-2	01/06/2009 to 31/05/2012	IC	In force	Education
Fernando Rodrigues da Silva	137419/2009-0	01/10/2009 to 31/05/2012	IC	In force	Epidemiological
Gabriel Nunes Campina	104340/2009-6	01/06/2009 to 31/05/2012	IC	In force	Education
Guilherme Franco Cavalheiro	139548/2009-2	01/11/2009 to 30/04/2012	IC	In force	Cardiopulmonary
Ivan Gregorio Hetem	144610/2009-4	01/12/2009 to 30/11/2012	IC	In force	Epidemiological
Jéssica Bistafa Liu	144451/2009-3	01/12/2009 to 30/11/2012	IC	In force	Endocrinal
Jéssica Silva Lima	138357/2009-9	01/10/2009 to 31/05/2012	IC	In force	Biomonitoring
Lílian Caroline Paschoal	104124/2009-1	01/05/2009 to 30/04/2012	IC	Cancelled by the coordinator	Biomonitoring
Luís Fernando Amato Lourenço	137418/2009-4	01/10/2009 to 31/05/2012	IC	In force	Epidemiological
Mariana Fadigatti Picolo	107422/2009-3	01/08/2009 to 31/01/2012	IC	In force	Epidemiological
Nilva Nunes Campina	150816/2009-0	01/06/2009 to 31/05/2010	PDJ	In force	Education
Paula Chaves Sampaio	372247/2009-1	01/08/2009 to 31/07/2011	AT-NM	In force	Cardiopulmonary
Rauda Lucia Navega Cruz Mariani	150571/2009-7	01/05/2009 to 30/04/2010	PDJ	In force	Biomonitoring
Rodrigo Souza Machado	138664/2009-9	01/11/2009 to 30/06/2012	IC	In force	Biomonitoring
Tânia Raquel da Silva Ferreira	127166/2009-2	01/08/2009 to 31/01/2012	IC	In force	Biomonitoring

CAPES Scholarships

Scholarship Student	Proceeding	Validity Time	Mod. Category	Status	Thematic Group
Priscila Iovine	Awaiting	01/03/2010 to 28/02/2011	Master´s degree	In process of implementation	Biomonitoring
Maria Angélica Peres	Awaiting	01/03/2010 to 28/02/2011	Post-doctor´s degree course in the country	In process of implementation	Development
Ana Paula Garcia Martins	Awaiting	01/03/2010 to 28/02/2011	Post-doctor´s degree course in the country	In process of implementation	Epidemiology

It should be observed that there are still 19 scholarships from other sources which are still

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been distributed by the coordinators of the various thematic groups.

7. ABOUT INAIRA WEBSITE (WWW.INAIRA.ORG)

In response to a request from CNPq, we created a support website to the researchers connected to this National Institute of Integrated Environmental Risk Analysis (INAIRA). Since its creation, on February 11, 2009, the website is hosted under the servers of HostMonster, a reputed websites host that currently keeps 600,000 websites.

The website was developed by two researchers of the group, Miriam Lemos and Paulo Sérgio Panse Silveira, containing some static pages of INAIRA presentation, a set of 193 PERL programming modules, and MySQL databank support. Currently, this set has 164 registered researchers, functionally organized in 44 groups (researchers might belong to more than one group), with right to include or complement information for the use of other researchers or for divulgation to the public, depending on his/her functions in the project.

The following categorized information was so far (02/2010) included by the researchers:

- 28 advertisements of scientific events,
- 64 links for media news about the group,
- 52 abstracts of research projects,
- 104 goals, which the system controls and informs to the coordinators insofar they are attained.
- 19 group meetings and lectures given,
- 53 scientific publications of INAIRA researchers including articles and abstracts in congresses annals,
- 8 worksheets and documents, usually of internal control,
- 6 articles with instructions and tutorials for group guidance,
- 5 articles with reports and testimonies of researchers about events they took part in,

Totalizing 339 items registered in the website.

The volume of information transmitted to its users, since the website creation, has been usually over 300 Megabytes per month (Fig. 1).

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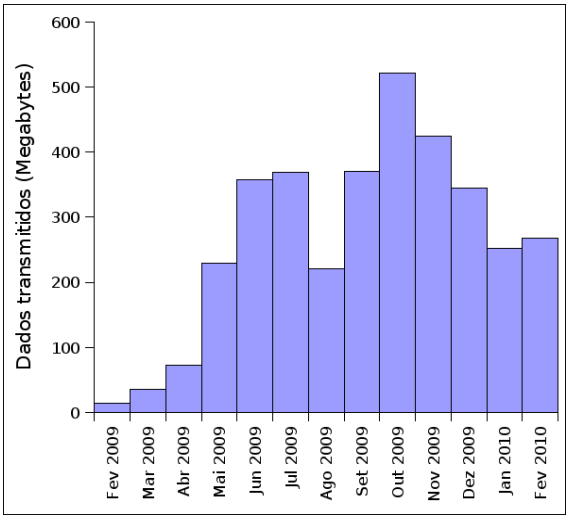

Fig. 1: Volume of data requested by INAIRA website users (download). We observed an initial growth of the demand (first 4 months), followed by oscillations of approximately 300 Mb/month.

Also, there is a homepage for each researcher, and e-mail distribution services that facilitate the communication between the website members. The organization allowed by the systems available at the website and the incentive to everyone communicate their progresses have been a facilitator for the coordination of projects in progress of this Institute.

8. MAIN TECHNICAL-SCIENTIFIC RESULTS

All thematic groups have sent a complete report that is accessible to the website reviser: www.inaira.org. Since CNPq has defined a limit of pages for the scientific report, the reviser can consult you in case of need of more information. The complete version are available only in Portuguese. To consult the full information please follow this instructions:

Access www.inaira.org

Log in using one of the following case sensitive usernames and passwords

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Select the option Project Reports at website main menu

All reports will be available for download

A. CARDIOVASCULAR THEMATIC GROUP:

Coordinator: *Dolores Helena Rivero*

Joint experimental subprojects

Responsible person: Dolores Helena Rivero

In the cardiovascular thematic group, the first studies utilizing the biodiesel generator engine of USP Atmospheric Pollution Laboratory were carried. The financial resources for the acquisition of the generator, including parts, accessories and the renovation of the physical space provided by LPAE – FMUSP for the assemblage of the system coupled to the inhalation chamber, were obtained through the support of FAPESP (proceeding no. 2007/57747-0). The studies resulted in the Master´s degree dissertation conclusion of the student Jôse Mara de Brito, with the monograph named: “Acute cardiovascular and inflammatory toxicity induced by the inhalation of diesel and biodiesel fuels particles”, defended on 19/03/2010. The student had a CAPES scholarship, validity time: 03/2007 to 03/2009.

Two Master´s degree students and two Scientific Initiation students are already engaged in the next studies of the cardiopulmonary group.

The partial results were presented in the 7th congress of Toxicology for developing countries, from September 6 to 10, 2009, South Africa. The above work had a great repercussion during the presentation in the congress and the oral presentation, when the importance of this research line at economic and worldwide level was highlighted, not only focused on environmental issues, but mainly on human health issues. This study was awarded with the 1st place Award as the best oral presentation.

The work was submitted to publication in the Toxicological Sciences magazine with the title: “Acute cardiovascular and inflammatory toxicity induced by inhalation of diesel and biodiesel particles” and is now printed.

Main results:

The effects caused by atmospheric pollution have been largely studied in the last decades, due to the prejudices caused to human health, to ecosystems and to materials; and one of the

main responsible agents for the emission of pollutants in the atmosphere are the automotive vehicles moved by diesel. Literature has shown that various chemical substances present in the air cause great disorders, not only in the respiratory system, for passing from lungs to the circulatory system, thus affecting other physiological functions, such as the circulatory system, the nervous system, among others. Within this context, the analysis of the chemical specificity of different fuels emissions (diesel, biodiesel in the proportion of 50% - B50 and 100% - B100) is extremely relevant to understand the effects of such emissions in the atmospheric air and in the cardiopulmonary system. Thus, the proposal of the present project was not to evaluate the engine performance, but rather develop a diesel particles generation system coupled to a inhalation chamber for small animals, in a controlled emission system, in order to evaluate the impact of such emissions generated by the exhaustion of diesel fuels, B50 and B100, in the cardiopulmonary system of Balb/c mice. In the experimental design, Balb/c mice were exposed for 60 minutes to the exhaustion material of diesel fuels, B50, B100, and during the exposure period some parameters were real-time monitored ($PM_{2.5}$, CO (carbon monoxide), temperature and humidity) and filters were sampled for afterwards elementary characterization and characterization of polycyclic aromatic hydrocarbons (PAHs), a punctual collection was carried out with a canister for analysis of volatile organic compounds (VOCs); records of cardiac frequency variability (CFV), cardiac frequency (CF) and blood pressure (BP) were obtained immediately before, 30 and 60 minutes after the exposure; 24 hours after, the bronchoalveolar lavage (BAL), blood, bone marrow and pulmonary tissue were collected for analysis of inflammatory and systemic parameters. This study showed that the B100 fuel exhaustion reduced the emissions of mass, soot, metals, CO, PAHs, compared to diesel and B50; the square root of the sum of successive differences between RR intervals normal adjacent to the square (RMSSD) increased in the control, the low frequency (LF) increased in the group exposed to diesel exhaustion ($p < 0.01$) and B100 ($p < 0.05$) compared to the control group, and CF increased in the group exposed to B100 exhaustion ($p < 0.05$) compared to the control group; blood: the average corpuscular volume (ACV) increased in the group exposed to B100 and diesel exhaustion ($p < 0.01$) compared to the group exposed to B50 exhaustion and the control group ($p < 0.001$), the mean corpuscular hemoglobin concentration, or MCHC, reduced in the group exposed to B100 exhaustion compared to the group exposed to B50 exhaustion ($p < 0.001$) and the control group ($p < 0.05$), the leukocytes increased in the group exposed to B50 exhaustion when compared to the group exposed to diesel ($p < 0.05$), blood platelets increased in

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the group exposed to B100 exhaustion when compared to the diesel group and to the control group ($p < 0.05$), reticulocytes increased in the group exposed to B50 exhaustion when compared to the group exposed to diesel, the control group ($p < 0.01$) and B100 ($p < 0.05$); bone marrow: metamyelocytes increased in the group exposed to B50 and B100 exhaustion compared to the group exposed to diesel exhaustion ($p < 0.05$); bronchoalveolar lavage (BAL): neutrophytes increased in the group exposed to diesel and B50 exhaustion compared to the control group ($p < 0.05$), the macrophages increased in the groups exposed to diesel exhaustion material ($p < 0.01$), B50 and B100 ($p < 0.05$) compared to the control group. We conclude that B100 fuel reduces the emissions of pollutants in the atmosphere, opposed to this, B50 increases the emission of pollutants in relation to diesel; however, B50 and B100 fuels showed to be as toxic as diesel, because they promoted pulmonary, systemic inflammation and an unbalance in the autonomous nervous system.

Clinical subproject: Air pollution and physical activity in individuals with cardiovascular and chronic respiratory diseases.
Responsible person: Ubiratan de Paula Santos
With no quantitative results so far.

As specified in the timetable presented in 2009, the following activities were carried out during these 12 months:

I. Three (03) research team meetings were held with those involved in the project.

II. Equipments acquisition initiated.

- 01 machine for ergospirometry test
- 01 plethysmograph
- 01 display for monitoring blood pressure, ECG, temperature, and breathing rate

III. Scholarships requested

- 01 Mid-level CNPq scholarship: Paula Chaves Sampaio, started in August 2009

IV. Selection of volunteers for the study

According to criteria defined within the project, the selection process for volunteers was started. Thirty (30) patients with moderate asthma were chosen. We opted for not completing the selection process for lack of a clear study start-up date, which depends on

the purchase and installation of the special container that needs to be adapted at the premises of the Heart Institute.

V. Study protocol

- Evaluations will be carried out Mondays through Fridays, always during the late afternoon;
- Evaluation will be scheduled in pairs and carried out once in the container with its windows open, breathing external air (near Rebouças Ave.). For the second evaluation, the container's windows will be closed with external air captured and filtered for particulate matter and other pollutants;
- Evaluation order for the pairs will be determined through a random drawing;
- All evaluations will be either conducted or monitored by a physician;
- Volunteers will be received at the container at 2:00 pm and will be placed in waiting for two (2) hours for internal air acclimatization;
- After acclimatization, the following tests will be carried out, from 4:00 to 5:00 pm, in this order:

  a) Quantity of nitric oxide in exhaled air

  b) Simple spirometry

  c) Venous occlusion plethysmography employing the isometric exercise protocol used in the Rehabilitation and Physiology of Exercise Unit at the Heart Institute

  d) Logging of blood pressure and ECG data

  e) Progressive cardio-respiratory stress test; ramp type, to be carried out to the limit of the volunteer's tolerance on an ergonometric machine equipped with an electromagnetic breaking system

  f) Post-exercise quantity of nitric oxide in exhaled air

  g) Post-exercise simple spirometry

  h) Post-exercise venous occlusion plethysmography

  i) Post-exercise logging of blood pressure and ECG data

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Subproject: Behavioral and Oxidative Stress Evaluation of Rats Exposed to Air Pollution in the City of Porto Alegre.
Responsible person: Claudia Rhoden

The general objective of the project (for the whole proposed period) is to promote the Institutional technological progress, as well as the qualification of human resources to enhance the relations with excellence research groups, strengthening the network of researches on the chemical characterization of atmospheric pollutants, action mechanisms, effects and toxicological evaluations. With this, we believe that the execution of this proposal will allow the consolidation and expansion of the post-graduation program, correcting the existing regional asymmetries.

Specific objectives for the whole study period:

1) Stimulate the technological qualification addressing the employed methodologies/models, strengthening the research infrastructure;

2) Investigate the participation of oxidative stress as modulator of damages to health resulting from exposure to air pollution;

3) Investigate possible behavioral alterations (anxiety, depression and memory impairment) promoted by the exposure to air pollutants in different ages;

A doctor´s degree thesis (USP Experimental Physiopathology PPG) carried out in our laboratories in partnership with USP-Experimental Atmospheric pollution Laboratory: student: Ana Cláudia Zanchi: “Study of alterations in the development, behavior and brain biochemistry of male adult rats exposed to ambient level of air pollution during the intrauterine phase and the lactation period” (2010).

(2) A paper sent for publication at Inhalation Toxicology: “Pre and post-natal exposure to ambient level of air pollution impairs memory of rats: the role of oxidative stress” (2009).

a) the exposure to ambient level of air pollution during the intrauterine life and lactation period (up to the 21st day of life) did not cause alteration in the development of male baby rats;

b) the exposure to ambient level of air pollution during the intrauterine life and lactation period (up to the 21st day of life) caused the reduction of the short-duration discriminative memory; increase of the habituation capacity and did not modify the spatial memory;

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c) the change of environment did not modify the short-duration discriminative memory; the change of environment – from filtered to non-filtered – promoted an increase of habituation; the change of environment did no cause alteration of the spatial memory;

d) the exposure to ambient level of air pollution during the intrauterine life and lactation period (up to the 21st day of life) caused the increase of lipoperoxidation in the cerebral and striate cortex; it did not promote the modification of the lipoperoxidation rate in the hippocampus;

e) the exchange of environment – FNF (filtered to non-filtered) – promoted the increase of lipoperoxidation in the hippocampus; it did not promote the modification of the lipoperoxidation rate in the cerebral and striate cortex;

f) the exposure to ambient level of air pollution during the intrauterine life and the lactation period (up to the 21st day of life) caused a reduction only of the SOD activity, with no alteration of the catalase activity or of GSHt concentration in the cerebral cortex; in the hippocampus and in the striate cortex there was no alteration of SOD activity and of catalase, nor modification of GSHt concentration.

g) The exchange of environment – FNF (filtered to non-filtered) - caused the reduction of GSHt, it did not modify SOD activity and catalase in the cortex; in hippocampus, it promoted a reduction of GSHt, without modification of SOD activity and catalase; in the striate cortex, it promoted the reduction of SOD activity, without alteration of catalase and increase of GSHt concentration;

h) the exposure to ambient level of air pollution during the intrauterine life and the lactation period (up to the 21st day of life) caused an increase of the higher Cd concentration in the total blood of animals, and the non-exposed group presented a higher concentration of antimonium in the total blood; there was no modification in the concentration of lead;

i) the exchange of environment – FNF (filtered to non-filtered) - caused an increase of the higher Cd concentration in the total blood of animals; the exchange of environment did not cause the alteration of antimonium and lead concentration;

j) the exposure to ambient level of air pollution during the intrauterine life and the lactation period (up to the 21st day of life) did not cause the alteration of copper, zinc, selenium and manganese concentration in the total blood of animals;

k) the exchange of environment – FNF (filtered to non-filtered) - caused an increase of copper, selenium and zinc concentrations, but it did not change the concentration of manganese in the total blood of animals;

In summary, the exposure of adult male rats during the intrauterine phase and lactation period to ambient level of atmospheric pollution in the city of Porto Alegre did not cause alteration in the development of those animals, though it reduced the short-term discriminative memory, increase the adaptation capacity and did not modify the spatial memory. The oxidants at cerebral cortex level have an important role in the mechanism responsible for the impairment of the discriminative memory, and are not involved with the reduction of spatial memory.

For the knowledge and technology items, the following goals and respective activities were planned:

1) Reduction of knowledge asymmetries in the field. This goal involves the following activity: a) Training of personnel of other locations in the developed techniques. Physical indicator achieved.

2) Exchange with other institutions and laboratories involved with the theme. This goal involves the following activities: a) Meetings with reports of the applied technology; b) Discussion of results; c) Compilation of results

We have attained the physical indicators for the 3 proposed activities: meetings were held. Such meetings involved the group of Prof. Paulo Saldiva (FMUSP) and Prof. Fernando Barbosa Júnior (USP-RP). The results presented in this report were partially discussed with these professors.

For the education and science divulgation items, a goal was planned for 2009, namely: Course of sensitization for the issue: air pollution and health, which involved the Conduction of a short-duration course. Physical indicator achieved.

I Extension Course on Atmospheric Pollution and Health

Coordinator: Prof. Dr. Cláudia Ramos Rhoden

Laboratory of Oxidative Stress and Atmospheric Pollution

- UFCSPA -

This event with national coverage is intended for the whole academic-scientific community of the health and environment fields, in addition to government authorities, private sector and non-governmental organizations representatives that somehow are involved with the highlighted issue (see Programming).

Our objective, with the organization of this event, was to raise the awareness and the scientific debate and understanding about the diseases caused to men´s health due to the exposure to atmospheric pollution, as well as the possible social consequences of this process.

The lecturers of the course are mostly members of INAIRA.

For the human resources formation item, the following goals and respective activities were planned:

1) Three scientific initiation scholarship students. This goal for the year 2009 involves: a) Selection of the student; b) Training/guidance of the student. The accomplishment of this goal is related to the selection of graduation students from the Biomedicine course (who have received a scientific initiation scholarship obtained by the Laboratory of Oxidative Stress and Atmospheric Pollution of UFCSPA), namely: Maurício Moresco, Alan Fleck, Giordano Toldo

2) Selection of master´s degree student. Goal accomplished. Selected student: Alessandra Ciccone, PPG in Health Medical Sciences.

3) Selection of CIEE intern. Goal accomplished. Selected student: Fernando Teixeira, PUC/RS Biology course. In addition to the selection, the student has already been trained and is working with bioterism activities (which goal was of 2010) and animals care activities in the Exposure Chambers.

B. EDUCATION THEMATIC GROUP

Coordinator: Nilva Campina

Between January 2009 and January 2010, the research project of online learning Network in environmental education and public communication of science had its goals progressed as shown below. The work was initiated through the elaboration of an investigative questionnaire on the environmental perception. Weekly meetings were carried out with the Education team in order to discuss the different perspectives of environmental perception, and to align the questionnaire in

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a concise way according to the project objectives. The questionnaire has the objective of verifying the environmental perception of the course participant teachers, as well as the profile of such educators. Such data are very important for the conduction of discussions during the course with teachers, and allow a more precise evaluation at the end of the course, when indicators are surveyed.

The team meetings allowed the discussion of the questionnaire intentions and the elaboration of the questions according to our objectives.

After the elaboration of the first version, the questionnaire was forwarded for analysis by the INAIRA Epidemiology group researchers, who have suggested some modifications. Concomitantly with the questionnaire construction process, we stayed in contact with a statistics professional that helped us guiding the instrument validation process, and with the study of the city where it would be applied for the validation of the questionnaire.

Thus, some criteria were listed for the site selection: a) municipality without the course; b) facilitated political access for the application in the public sphere; c) middle to large scale education network for the application of 300 questionnaires at Fundamental or Middle level Education, according to the guidance from the statistics professional.

The municipality of Santos encompassed the defined criteria and was selected for the instrument validation. The validation process occurred according to the following stages:

a) application of the questionnaire for 10 Fundamental Education teachers of a municipal school in Santos;
b) analysis of questions comprehension and necessary adjustments;
c) reapplication of the questionnaire for the same group of teachers a week later;
d) comprehension analysis and final adjustments;
e) application of 300 (three hundred) questionnaires in 9 (nine) schools of Santos municipal network. At this stage, schools were selected with the aim of encompassing the whole zoning of the municipality insular area, and those schools allowing easy access to the manager were selected;

The questionnaire has 33 closed questions, which makes it an objective instrument, with information easy to analyze. The contents of the questionnaire can be accessed in the complete report of the Education group.

For the application of the questionnaire, Mirassol D´Oest/MT was visited by the Education

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team. In this meeting, in which the Education Secretary of the municipality and a researcher from INAIRA Biomonitoring group also took part, the questionnaire, its objectives and the application manners were explained. Upon the consent from the Secretary, the date for application was defined as being the first presence class to be given in the beginning of the school year of 2010. Despite the goal being the application of questionnaire in a city still in 2009, the Education team decided to postpone it, after various discussions and pondering, for considering important that the application would occur at the same time as the beginning of the process of the course with teachers. We thus intend the questionnaire to be a trigger of discussions on environmental and educational issues.

The elaboration of the online course to be given occurred through biweekly meetings with the Education group researchers, throughout 2009. In parallel, periodical meetings with the FMUSP Telemedicine team started. The interaction of both teams was very important in the moment of elaborating the course because the Telemedicine group analyzed in detail the feasibility of the pedagogical proposal presented by the Education team, according to the format of Distance Education. During such meetings, it was possible to discuss the whole course structuring process, including different contents to be adjusted according the reality of each city and the characteristics of the mediatic resources to be utilized – videos, Tele or Web Conferences and Cybertutor. The programmatic contents of the course can be found in the complete report.

In the period, two Scientific Initiation and one post-doctor´s degree scholarships were provided to this thematic group.

The abstracts of two papers were presented in the 5th World Environmental Education Congress in Canada (May 10-14, 2009, see website, Reports and Testimonies) and two papers in the V EPEA – Meeting of Researches on Environmental Education held at the Federal University of São Carlos in 2009.

C. ENDOCRINOLOGY THEMATIC GROUP

Coordinator: *Elaine Maria Frade Costa*

This group has not generated quantitative results yet.

Subproject 1: Effects of atmospheric pollution on the female reproductive system: genic, molecular, immunological, endocrinal and clinical aspects in the female rat/mouse model.

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Responsible person: Elaine Maria Frade Costa

To evaluate the impact of atmospheric pollution in the production of sexual steroids, in a first stage we will study the adrenal of female rats submitted to various pollutants. After submitting them for a certain period of time to such pollutants, the animals will be sacrificed, morphological and molecular analyses of the adrenal glance will be done, and hormonal dosing will be carried out to determine the alterations in the production of androgens and its byproducts. The differential genic expression in various situations will also be quantified. Data will be compared to data of animals raised in pollution free environments. For this project, we have one doctor´s degree student and two scientific initiation students. In a later stage, the ovarian steroidogenesis will be the focus of attentions. The selection of the endocrinal system to be studied matches the clinical branch of the project, considering that patients with hyperandrogenism diagnosis coming from various regions and submitted to different environmental pollutants levels will be evaluated.

Activities carried out from 08/01/2009 to 08/01/2010:

Introduction to the adrenal study in female rats and the training of the doctor´s degree student already concluded. The student dedicated to morphological analysis and molecular techniques in collaboration with the Discipline of Histology and Structural Biology of UNIFESP-EPM, where he obtained his master´s degree. Currently, after defining the immediate objectives of the work, we are in the phase of putting together the groups of animals to start the experiments. The scientific initiation students will start the project follow-up.

Subproject 2: Study of the effects of particles coming form atmospheric pollution in the city of São Paulo in the reproductive system of the zebrafish (Danio rerio): histological, morphological and molecular analysis.

Responsible person: Elaine Maria Frade Costa

The hypothesis of certain environmental compounds being hazardous for living beings and for their respective descendants has created a new investigation field in environmental toxicology, dedicated to the study of substances susceptible of producing endocrinal alterations in living beings. In vivo and in vitro findings show that diesel can contain compounds that modulate the activity of sexual hormones and the genic expression. In this study, zebrafish (Danio rerio) will be

utilized as a biomarker, an interesting model to evaluate reproductive factors considering that it contains genetic characteristics similar to human beings, and also for having a short life cycle. This first stage of the work has the objective of evaluating the cumulative effect of exposure to diesel exhaustion particles in the sexual development of the zebrafish (Danio rerio).

Activities carried out from 08/01/2009 to 08/01/2010:

During this period, animals maintenance and survival standardization was done, as well as the purchase of pre-test materials. The standardization of the exposure of the water-soluble fraction of the diesel exhaustion was also done.

The animals were purchased in an aquarium shop and are being kept in the laboratory of atmospheric pollution of São Paulo Medical School.

To obtain the water-soluble part of diesel exhaustion particles (DEP), one part of it was added to four parts of chlorine-free water in a glass bottle. This solution was agitated by an agitator for 2 hours, and then the insoluble part was discarded. The final solution was filtered with cellulose filters of 2.5µm porosity.

To determine sublethal concentrations to be used in the definitive experiment, a pre-test with varied concentrations of DEP is being conducted. Tested concentrations are 0.5; 2; 4; 6 and 8 µg/L. Fishes were placed in 1L aquariums with 4 adult fishes each, and behavioral and survival parameters are being observed for a period of 96 hours. Each test will be done in duplicate.

The dilution was examined through X ray fluorescence spectrometry analysis before and after the exposure of animals.

Remark: There was an alteration in the project materials and methodology. Due to time constraints, it was not possible to accumulate the amount of particulate matter necessary for the experiment, and because of that we are now using DEP.

Subproject 3: Effects of atmospheric pollution in the female reproductive system: genetic, immunological, endocrinal and clinical aspects – Clinical Study
Responsible person: Elaine Maria Frade Costa

This subproject was incorporated to subproject 4.

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Subproject 4: Effects of atmospheric pollution in the female reproductive system: genetic, immunological, endocrinal and clinical aspects – Molecular Studies
Responsible person: Elaine Maria Frade Costa

The analysis of possible interactions between atmospheric pollutants and polymorphisms in genes involved in the development of the reproductive system, especially in the process of ovarian differentiation, and the clinical manifestations resulting from such interactions were not yet described.

The objectives of this work are: 1 - analyze the association between components of atmospheric pollution and molecular alterations in the genes CBX2, TCF21, FOXL2, STRA8, BMP15 in women bearing gonadal and reproductive development disorders. 2 – research abnormalities and/or polymorphisms in genes involved in the ovarian differentiation in 46,XX patients bearing gonadal and reproductive development disorders, comparing the frequency of identified allelic variations with a control group of normal women. 3 – analyze the effects of atmospheric pollution components in the enzymatic activity of polymorphisms described in the cytochrome P450, CYP3A7, in female patients bearing ovarian development and function disorders.

The possible role of the gene-atmospheric pollution interaction in the etiology of the disease will be evaluated from the clinical and molecular points of view.

Activities carried out from 08/01/2009 to 08/01/2010:

1 – Elaboration of the Terms of Free and Informed Consent for the collection of material (blood and/or scratch material from the oral mucosa) of patients of Hospital das Clínicas bearing female sexual development disorders and women not bearing female sexual development disorders (normal controls) for the study of different genes involved in the ovarian formation and function.

2 – Standardization of techniques utilized for the expansion (PCR) and sequencing of candidate genes in this study. Standardization of polymorphisms research techniques utilizing restriction enzymes.

3 – Start of the analysis of genes CBX2, TCF21, FOXL2, STRA8, BMP15 and inclusion of the study of the gene SF1/NR5A1 in the DNA extracted from leukocytes of the peripheral blood of patients in treatment in the Departments of Medical Clinic – Endocrinology and Gynecology

bearers of female sexual development disorders (60 patients with 46.XX gonadal dysgenesis and premature ovarian failure).

4 – Selection of 112 women bearers of SOP in treatment in the clinics of Gynecology and Endocrinology of HCFMUSP, and of 50 normal women. All these patients have already their DNA samples extracted from peripheral leukocytes. The research of the polymorphic variant CYP3A7*1C, in the groups of patients and controls, was already concluded, which was present in heterozygosis in 1.7% and 3.4% of the alleles, respectively.

5 – Elaboration and validation of the questionnaire directed for the characterization of female sexual development disorders and for the evaluation of the influence of the exposure to atmospheric pollution components in different forms of clinical presentation.

D- AGING THEMATIC GROUP

Coordinator: Wilson Jacob Filho

This group has not yet generated quantitative results.

The project has planned an experimental study with healthy elder individuals who will be submitted to different climatic conditions (different temperatures), in a controlled environment, in which cognitive function, balance and muscular strength tests will be conducted.

This controlled environment would be the container for the study in humans. Since such container has no prevision for conclusion, we are searching for a solution as for the site for the conduction of evaluations.

The projects were submitted to the local Ethics Commission.

E – EPIDEMIOLOGY THEMATIC GROUP

Coordinators: Paulo Afonso de André and Alfésio Luís Ferreira Braga

Subproject 1: Evaluation of the participation of automotive sources (diesel and gasoline) for environmental concentrations of fine particulate matter (PM2.5) and their impacts on the human health in 6 metropolitan regions of Brazil: Recife, Belo Horizonte, Rio de Janeiro, São Paulo, Curitiba and Porto Alegre. An evaluation based on population-based epidemiological studies.

Subproject 2: Evaluation of the adverse effects to health of fine particulate matter and ozone in areas of influence of sugarcane straw burning, through population-based epidemiological studies.

Responsible person: Alfésio Luís Ferreira Braga

These projects did not yet generate quantitative data.

The projects of evaluation of air pollution by automotive sources and its impact on human health in 6 Metropolitan Regions of interest and in areas of sugarcane straw burning had their time schedules affected by the disqualification of the suppliers of filters for the sampling of atmospheric particulate matter, base of the project to allow the calculation of concentration and elementary analysis of the particulate matter in the atmosphere of such locations.

Millipore was our provider of membranes for this field sampling since the beginning of our laboratory activities. However, between 2007 and 2009, the filters presented a great amount of failures, which was responsible for the rejection of a great amount of them. Despite our insistent complaints, also with the involvement of the quality guarantee and technical assistance area of that company, no corrective action was adopted by the supplier.

Therefore, at the end of January 2009, we were obliged to disqualify Millipore and initiate the selection of a new company for the provision of 37 mm polycarbonate membranes.

Due to the reduced number of manufacturers of such material, in February 2009, we purchased a pilot lot of 20 membrane boxes from the representative of the Whatman company, to allow an evaluation of the material. Since such materials are imported, with delivery time of 60 days, we only received the materials at the end of March, when we started the tests. At end of May 2009, in a contact with the representative of Whatman, we were informed that that company has been purchased by GE group, which keeps its own sales group in Brazil, and that the commercialization of the product was still being decided.

Only in the beginning of August 2009, we had the definition of the sales channel for the Medical School, by the own GE Healthcare Life Sciences, when we could request a quotation and negotiate the acquisition of the first lot of filters. In August 18, 2009, we sent a request for the provision of the first 40 boxes of polycarbonate membranes, which delivery occurred between October and November 2009.

Therefore, considering the stage of filters conditioning and shipment preparation, as well as the alignment of field sampling activities, which depends on the availability of local personnel, the field sampling stage was reprogrammed to be initiated at the end of February 2010, after the mobilization of personnel.

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Thus, there will be a 6 months delay in the original time schedule.

The collection of health data will be done concomitantly with the obtainment of air quality data.

A course of statistic analysis techniques leveling for air pollution and health studies is being organized, and shall be started as soon as the data collection is concluded as well as its respective data processing.

Subproject 3: Implementation of the Air Quality Model Integrated with the Environmental Risk Analysis Model.

Responsible person: Maria de Fátima Andrade

This subproject has the objective of developing a platform of air quality models coupling with epidemiological models, resulting in the description of the formation and physical-chemical processes involving the atmospheric pollutants and their occasional impacts in terms of morbidity and mortality to human health. For the conduction of such integration, models of sources description (movable and stationary), meteorological models for the description of pollutants atmospheric transportation, models of atmospheric chemistry, and risk evaluation models are necessary. Thus, the objective of this subproject is the implementation of a combined model of air quality and pollutants impacts on health, with the description of the spatial and temporal behavior of pollutants, especially of the Particulate Matter and of Ozone. This will be done by adding one more module of calculation of impacts to health to the already existing air quality modeling tools. In the current stage, this coupling was already done and tests for the evaluation of results are being conducted. The next stage is to provide the results at INAIRA website.

Summary of obtained results:

In the Department of Atmospheric Sciences, the Weather Research and Forecasting atmospheric Chemistry - WRF-Chem model, which manages in an integrated manner the description of meteorology and chemistry, is operational. This model is being already utilized in other works of LAPAt members.

The first stage of the work consisted of the application of the WRF-Chem model for the example case of RMSP (Metropolitan Region of São Paulo). For the initial and contour conditions of chemical species, data from the automatic network of CETESB located in the RMSP and in the interior of São Paulo state, and also the re-analyses of NCEP in case of meteorological variables,

were utilized. For the inventory of emissions, two procedures are being utilized. One procedure is based on the official inventory of emissions of CETESB for RMSP and for the regions of São Paulo state interior (Baixada Santista, Campinas, Sorocaba and São José dos Campos), and also on data resulting from concentration measurements and emission factors of various gases (CO, NOx, COVx) obtained from measurements conducted inside of tunnels in São Paulo, such as reported by Martins et al. (2006); Andrade et al., 2004; Ynoue, 2004. Another procedure, in development stage, shall cover the entire territory of Brazil, and is based on the identification of urban areas from satellite images. In summary, in this first stage, we intended to allow the application of the model by evaluating its effectiveness in simulating the concentrations of chemical species (gases and particles) for the further adaptation and application of the model to evaluate the pollutants impact on the health in the study area. The evaluation of results and the validation of the model are being done by comparing the simulated data with those observed at CETESB stations, present in the RMSP and in the interior of São Paulo state, and with data obtained in experiments conducted by LAPAt – ozone surveys conducted in the RMSP and measurements of emission factors inside tunnels of the city of São Paulo.

The final goal of the work consists of obtaining a module of evaluation of the pollution impact on health that utilizes the concentrations of pollutants predicted by air quality models (the WRF-Chem model in this work), and that can be utilized for various purposes, among which the estimation of impact in different scenarios of vehicular contributions on human health and the economic appraisal of the pollution impact on health.

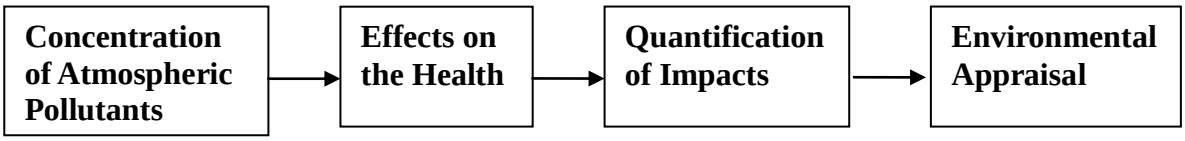
Subproject 4: Economic appraisal of morbid-mortality due to atmospheric pollution in Metropolitan Regions

Responsible person: Prof. Dr. Simone Georges El Khouri Miraglia

The objective of this research is to appraise the impacts on health due to atmospheric pollutants concentrations. The appraisal of effects adverse to health due to the exposure to atmospheric pollution will be done in terms of the loss of life years, through the DALY (Disability Adjusted Life Years) methodology, which was elaborated by the World Health Organization and by the World Bank to create a universal "currency" to express the costs of consequences to health that is independent from regional monetary characteristics. This instrument was specifically

developed for the definition of public policies based on the cost-effectiveness relation. The model calculates the number of life years lost due to some factors (in this case, atmospheric pollution) taking into consideration the life expectancy of the population. The life expectancy reduction is then transformed into a monetary estimation. This type of approach was successfully utilized in São Paulo by our group in previous studies (2005).

The approach of environmental economic appraisal intends to attribute monetary values to the positive and negative impacts of anthropic activities. The obtainment of monetary values becomes important as a factor that subsidizes the decision-making process in the determination of public and environmental policies.



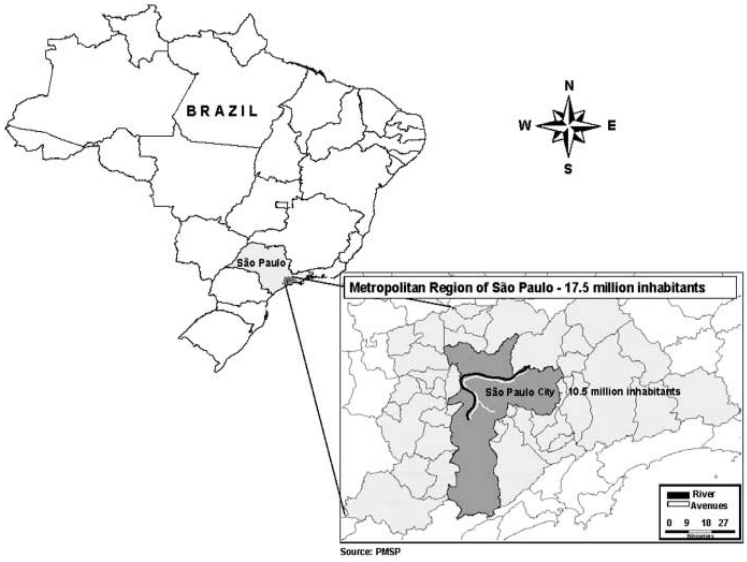
In the case of the present study, the analysis of atmospheric pollution in metropolitan regions, the procedure to conduct the research shall necessarily start from the results of other research groups. The first stage consists of the determination of the environmental exposure standard to which the population under analysis is subject; then, epidemiological studies shall be conducted in order to determine the deleterious effects of atmospheric pollution in the population health. From the obtained dose-response relation, the magnitude of impacts is determined, thus allowing the conduction of the environmental appraisal.

As we can notice, the environmental appraisal study depends on other groups which previous stages need to be concluded so that the appraisal models can be fully applied.

In the period of reference, the team compiled the morbid-mortality data from the epidemiology group, and conducted an initial analysis of the economic appraisal of atmospheric pollutionadverse effects. The survey of data included the life expectancy tables updated and published by IBGE to be used in the DALY methodology. The research in reference covered six national capitals: São Paulo, Rio de Janeiro, Curitiba, Salvador, Porto Alegre and Recife. This preliminary result is in the form of a scientific article, in phase of revision for re-submission in the periodical.

Scientific initiation students were involved in the project since the survey of data until the learning of economic appraisal techniques application, and elaboration of abstracts and the

scientific article.



In order to evaluate another consequence to health in metropolitan regions attributed to atmospheric pollution, the group surveyed birth data per gender in São Paulo, as well as particulate matter concentration data in order to evaluate a time series spatially in the different regions of the city, with different pollutants concentration gradients, obtaining results that confirm previous findings (Lichtenfels et al, 2007). Such result was submitted in a scientific congress, and is being described in a scientific article under elaboration.

Another initiated methodological approach consists of the survey of data in order to update an estimation of atmospheric pollution onus in São Paulo in terms of public health (Miraglia et al, 2005). With the aim of applying the DALY methodology. For such a purpose, the processing of data concerning mortality caused by respiratory and cardiovascular diseases utilizing the international diseases classification (CID-10: IX, X), as well as the revision and selection of dose-response curves regarding the mortality associated to atmospheric pollution published in literature, were conducted.

Therefore, despite this research group being dependent of the other groups and, thus, being always at the end of the general project time schedule, bearing all occasional alterations of the schedule, this group currently has a considerable database and important preliminary analyses indicative of the methodological guidance in progress.

The planned time schedule is of 6 months for the appraisal of effects in health, considering the reception of epidemiological results, and of 2 years for the appraisal of the results of gender analyses associated to air pollution in São Paulo, and for the update of total pollution onus in São Paulo through DALY methodology.

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Subproject 5: Economic appraisal of morbid-mortality due to atmospheric pollution in sugarcane production cities.

Responsible person: Prof. Dr. Simone Georges El Khouri Miraglia

Sugarcane had always a highlighted position in the Brazilian economy. In recent years, the sugarcane industry and consequently the cultivated areas considerably increased to meet the national and international demand for ethanol production. Due to the use of burning as the most utilized pre-harvest method, the emissions resulting from this process cause the deterioration of the air quality, with repercussions in impacts to the human health.

Under such circumstances, this project has the objective of quantifying the onus associated to these events in monetary terms through an environmental economic appraisal tool. The socioenvironmental costs resulting from morbi-mortality cases will be estimated, such as the increase of hospital admissions, use of medicines, loss of productivity and measures adopted for the prevention and treatment of air pollution related diseases.

Similarly to the project **"Economic appraisal of morbi-mortality due to atmospheric pollution in Metropolitan Regions"**, the present study of environmental appraisal is dependent on other groups which previous stages need to be concluded to fully apply the appraisal models, in special the results produced by the epidemiology group.

In addition to the appraisal of epidemiological results, in order to aggregate other information in the analysis of this relevant environmental impact, we decided to incorporate another methodology to evaluate the impacts of ethanol production, when Prof. Dr. Luiz Alexandre Kulay of USP Politechnic School Pollution Prevention Group was invited to participate in the group.

The Life Cycle Assessment (LCA) is an environmental management tool that allows the evaluation of aspects and potential impacts associated to a product, analyzing various stages from the extraction of raw material from nature that gets into the productive system (cradle) to the disposal of the final product in the environment (grave). The organized set of environmental aspects of the studied product is called the life cycle inventory, being constituted of values regarding the energetic consumption, atmospheric emissions, liquid effluents and solid waste, among others (Camargo 2007).

The motivation to introduce ethanol in the Brazilian energetic matrix is manifested in environmental, economic and social levels. In environmental terms, the adoption of the so-called

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renewable raw material for the production of biodiesel allows significant benefits in comparison with petro-diesel, such as the closure of the carbon balance, the preservation of natural resources and the reduction of emission levels of atmospheric pollutants resulting from the combustion process.

Ethanol shows to be feasible in the economic level for reducing expenses and dependence on the importation of petroleum and its byproducts, in addition to foster the agribusiness that takes place around it, which leverages development in the rural zone.

Such bias reveals a potential for the fuel also in another very important field of activity: the social field. Considering that its production chain stimulates the creation of jobs, also using the productive capacity of the familiar agriculture – this model employed in the production of oil producing plants such as castor beans and babassu – biodiesel stimulates the social inclusion in various areas of the country.

An appraisal conducted on the socioenvironmental impacts of the introduction of an additive that allows the mixture of diesel and ethanol revealed amounts of total benefits much higher than the costs associated to the introduction of this technology in São Paulo (Miraglia, 2007).

Considering that the environmental problem that most affects big urban centers is the air quality, measures that reduce the emission of atmospheric pollutants are encouraged since environmental and social gains are evident. Therefore, if the atmospheric pollution in big Brazilian urban centers results, above all, from vehicular emissions, and more specifically from diesel vehicles emissions, the utilization of biodiesel or ethanol added to diesel shall improve the atmospheric scenario of metropolitan regions, as well as contribute for the minimization of the global warming problem, considering that, as mentioned before, the carbon balances of renewable energy sources are positive when compared to fossil fuels.

The present research is in the stage of data survey and of methodological discussion of the Life Cycle Assessment of projects for the application in the case of sugarcane process.

In order to successfully carry out the present initiative, the Life Cycle Inventories (LCI) are being produced for ethanol production.

With such results, the economic appraisal of environmental and economic impacts of this productive process will be conducted, in order to subsidize the decision-making process in the selection of the best option from the economic-environmental point of view, from which the presented conclusions will result. In addition, insofar as the other groups conclude their researches

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on the effects of the pollution resulting from sugarcane burning in producing regions, other economic appraisal models will be applied in order to monetarily evaluate such impact. The planned time schedule is 6 months for the appraisal of effects on health considering the reception of epidemiological results, and 2 years for the appraisal of the results of the life cycle analysis of the ethanol productive process.

This project has not yet generated quantitative results.

Two Scientific Initiation scholarships were granted for Environmental Engineering students: Luis Fernando Amato Lourenço and Fernando Rodrigues da Silva, both students of SENAC University Center, scholarship students, and one UNIFESP Chemical Engineering student of Scientific Initiation as volunteer, Rafaela Andrade.

F – DEVELOPMENT THEMATIC GROUP

Coordinator: Thais Mauad

Subprojects 1 and 2: Evaluation of the effects of intrauterine and postnatal exposures to environmental pollution in maternal and fetal organisms, and in the health of children in the first two years of life: clinical study.

Responsible persons: Rossana Francisco and Sandra Vieira

There were no quantitative results yet.

The following was planned for this phase of the study: purchase of equipments and consumption material, development of protocols, sensitization of UBSs teams and start of pregnant women selection.

As for the purchase of equipments, there were difficulties for the acquisition of the ultrasound equipment, which was installed in December 2009.

In the definition of the protocol, the need of including nutritional evaluation of pregnant women, newborns and children was verified. A new research protocol was outlined and forwarded to FAPESP, and then approved in January 2010. To coordinate the nutritional evaluation, the researcher Sílvia Saldiva was included in the team.

For the measurement of pollutants, there was also the need of changing the previously proposed equipment, with the need of enhancing it.

Development of equipment for gauging atmospheric pollutants in residences and individuals: filters were developed at INAIRA Pollution Laboratory, adapted in plastic hangers and

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installed at residences and in small ID cards to be borne by the individual for 1 week. The equipment was tested in exposures with 30 days duration in 80 residences and 40 individuals.

As for the sensitization of field teams, a pilot project was executed as well as the following stages:

Formation and training of the field team. 2 teams were formed, one for each Basic Health Unit involved: V. Dalva and Jardim Boa Vista. The teams were composed of 1 supervisor and 10 community agents each. Before training, sensitization lectures about the effects of atmospheric pollution in the human health were given to UBSs professionals. The training of community agents belonging to the family health teams of UBSs was oriented to: a) application of the questionnaire and obtainment of demographic, clinical data, morbid background, domiciliary physical environment and exposure to atmospheric pollutants; b) installation and manipulation of domiciliary and individual pollution monitors. The training was conducted in the study site, by the responsible researcher through lectures, group discussions and pollutants gauging equipment demonstration, followed by practical exercises of exchange and removal of filters. The field work was supervised by a professional also previously trained, belonging to the UBS and in daily contact with community agents.

Subproject 3: Evaluation of the effects of gestational and postnatal exposure to air pollution in the maternal organism, fetal development and its consequences to the adult life health: experimental study.

Responsible person: Thais Mauad

In this subproject, a complete toxicological study of the reproductive efficiency of the placenta and umbilical cords is proposed, including morpho-functional modifications, as well as of the fetal and postnatal pulmonary development in mice exposed to diesel, biodiesel and particulate matter (PM2.5) smoke from vehicular origin; taking into consideration different exposure periods (periconceptional, gestational and post-gestational periods).

Each topic of this study will be approached by smaller projects subdivided according to the type of exposure, thus classified as follows: A) ambient air pollution, B) diesel exhaustion, C) biodiesel exhaustion, and D) concentrated particulate matter

So far, studies of categories A and B were conducted. The others will be conducted along

the next period. Partial results are presented as follows:

A) ambient air pollution

1 – Effects of exposure to concentrated particulate matter with vehicular origin in the development of testicles: experimental study in mice.

The reproductive process is directly affected by the environment. Countless toxic substances present in the air, such as heavy metals (Cd, Pb) and organic compounds (PAH), are associated to adverse effects in the reproductive function, both in females and males. In the past ten years, a series of publications indicate that air pollution is also a risk factor that compromises the reproductive function. Epidemiological and experimental evidences show an association between exposure and reproductive problems, such as infertility, subfecundity, ovarian failure and behavioral alterations. Our research group is making lots of progress in the knowledge of the effects of polluted air exposure in the female reproductive health. Utilizing mice as model and exposure chambers, receiving ambient air (polluted) or filtered air, we showed that, even at moderate levels, mice growing exposed to environmental pollution presented a smaller number of born alive baby mice and a higher rate of implantation failures. Also, we showed that there is a reduction in the males/females ratio and alterations in the estral cycle and in the number of ovarian follicles. In addition, exposed couples presented a significant increase of time necessary for copulation, reduction of fertility and gestation rates, and of the average rate of post-implantation losses. We also verified that the exposure to unfiltered air is associated to placental alterations, showing reduction in volume, in diameter and in the surface area of maternal blood spaces, to an increase of the surface area of fetal capillary vessels and in the diffusion conductance of the placenta. Morphological alterations in the umbilical cord were also found. In addition, our findings give support to the idea that fetuses are one of the population subgroups most vulnerable to the effects of air pollution, probably due to the immaturity of the immunological system and for having a higher life expectancy after exposure. In this study, we evaluated the effects of chronic exposure, for 3 generations, in the development of testicular development and morphology of mice semen. The exposure was conduct in the garden of São Paulo University Medical School in exposure chambers (Fig. 3), assembled side by side: the first one receiving ambient air (polluted chamber), and the second receiving filtered air (clean chamber). We evaluated two groups (average of 10 animals per group) of third generation (G3) mice of animals that copulated and grew inside

exposure chambers receiving filtered air (F) or nfiltered air (nF).

The average concentration in the chamber receiving ambient air was 27.08 $\mu g.m^{-3}$ (CV = 43%), and the values were similar to the concentration measured in the external environment. However, the average value (7.79 $\mu g.m^{-3}$; CV = 55%) was significantly smaller in the chamber receiving filtered air (P<0.001), which represents a reduction of 71% in the concentration of $PM_{2.5}$. The average ambient values of NO_2, CO e SO_2 were 101 $\mu g.m^{-3}$ (CV = 43%), 1.81 $\mu g.m^{-3}$ (CV = 50%) and 7.66 ppm (CV = 64%), respectively.

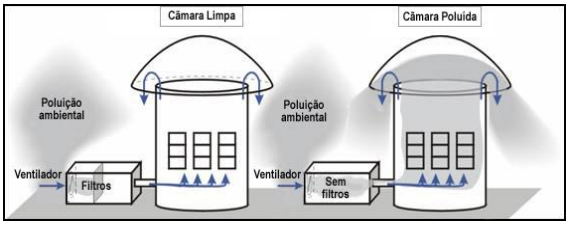

Fig.3: Schematic representation of exposure chambers

Animals exposed to non-filtered air are significantly lighter; however, no differences were observed in the weight of testicles (Fig. 4). Concerning the epididymis, there was a weight reduction. The stereological evaluation did not show alterations in the total volume of testicles and of their compartments, except for the seminiferous epithelium volume, which has a total volume bigger in animals exposed to polluted air. In the evaluation of semen, we observed significant alterations, increase of defective spermatozoids percentages (average F=18%, NF=32%; p<0.01) that includes tail defects, intermediary piece defects and presence of cytoplasmic gout. The presented preliminary results suggest that the exposure to ambient levels of particulate matter in the city of São Paulo might compromise the male reproductive health. Such results are coherent with studies in which mice were exposed to diesel exhaustion.

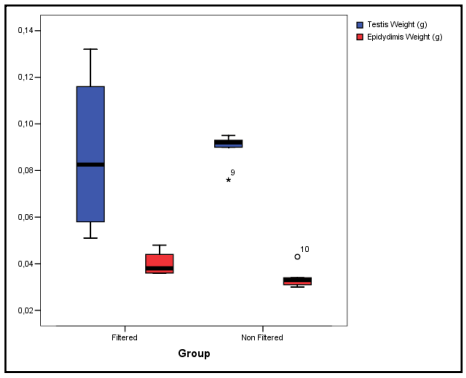

Fig 4: Boxplot of the average weight of testicles and of epididymis of mice exposed to filtered air and to ambient air

A brief literature review gives us clues that this increase of the seminiferous epithelium volume can result from the exposure to estrogenic substances (Robertson et al., 2002); it is a fact that in emissions resulting from diesel burning, estrogenic substances, as well as certain heavy metals capable of interacting with estrogen receptors, are emitted.

2 – Impact of pre-gestational exposure to atmospheric pollution on the embryonary implantation process

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Studies in the field of reproduction are increasingly focusing on the importance of the pre and periconceptional period for the normal development of gestation and of the embryo. It is already known that events, adverse or not, that occur in the initial stages of development might have a continuous influence, along the life, in the health and development of chronic diseases in the adult and in the child. From this perspective, studies that search for unveiling and identifying effects on the child´s health shall include an evaluation of the initial development stages, as well as of the mother´s and father´s health before and at the moment of conception. There are countless examples proving the importance of such association, toxicological, epidemiological and experimental studies reveal that the parental exposure to certain agents such as illicit drugs, medicines, tobacco and environmental contaminants might cause gestational alterations and consequently alterations in the fetus development. We previously observed that the exposure prior to gestation as well as in its initial stages is associated to an increase of post-implantation losses rate, however the causes of this development were not explored. Therefore, in this project, we will conduct a more precise analysis of the effects of exposure to air pollution in the initial stages of embryonary and implantation development that can explain the increase of post-implantation losses.

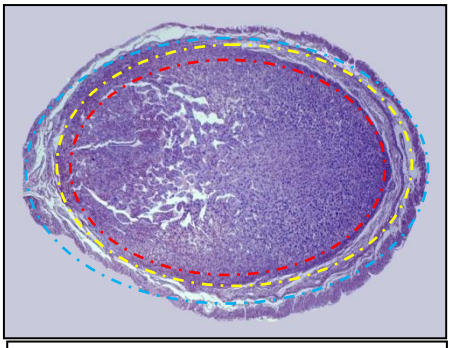

Fig. 5- Histologic section of implantation site (6th day. Trace lines indicate endometrium(---), miometrium (---) and perimetrium(---).

Like in the project previously presented, the exposure to air pollution was carried out at ambient levels utilizing open top chambers. In this case, only second generation females were evaluated to avoid the effects associated to paternal exposure. When reaching the age of 60 days, females copulated, gestation confirmed by the presence of vaginal plug, and 2 lots of females were formed for each exposure, totalizing 4 groups, which when reaching the gestational age of 6 to 8 days were euthanasiated for evaluation of implantation sites. So far, only the evaluation of the reproductive capacity and the morpho-quantitative analysis of implantation sites were concluded. The results are presented as follows:

There was no difference in the time needed for copulation among the groups. This can be explained by two reasons: the small number of animals and for the fact that in this study we only exposed females, different from the previous study in which both males and females were

exposed. Which gives us clues that the reduction of the couple fertility rates observed in the previous study might be associated to the compromising of the male reproductive capacity.

The number of evaluated implantation sites refers to the 8th dpc, because in the 6th dpc visual identification is still not possible. Through visual inspection, we verified that there was no statistic difference in the number of implantation sites per animal. However, we noticed differences in the degree of development of implantation sites, in the number of implantations per animal and in their distribution in the cornua. In animals exposed to filtered air, we noticed a homogeneity in the number, development and distribution of sites. On the other hand, in females exposed to ambient air (polluted), the implantation sites had different development degrees, with some much smaller; although a homogeneous distribution was still kept in the uterine cornua. However, the number of sites in animals exposed to pollution is very heterogeneous. The gestational parameters in the 6th day were not significantly different in relation to the volume of the perimetrium, myometrium and endometrium (Fig. 5).

Fig.6 A: Lung of fetuses exposed to filtered (left) and non-filtered (right) air.

This result can be explained by the initial phase of gestation, although we found significant differences in the number of mastocytes. Mastocytes are involved in the angiogenesis process, thus this alteration in the number of cells might indicate possible alterations in the synthesis of angiogenic factors, as well as a deficient decidual vascularization. In the following stage, we will advance the studies in the evaluation of the expression of nagiogenic factors, quantification of NK cells, and vascularization of such implantation sites.

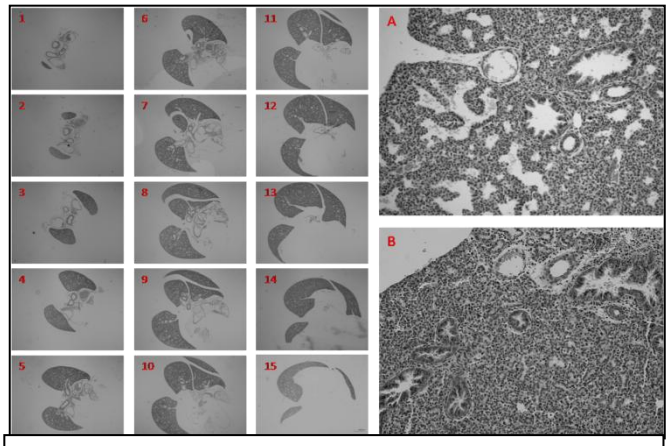

Fig.7- Illustration of the stereological study (1 to 15). Photomicrograph of histological cuts of lung of fetuses exposed to filtered (A) and non-filtered (B) air.

3 – Effects of the gestational exposure to particulate pollution of vehicular origin (in ambient levels) in the organic development fetuses at term: Morpho-quantitative evaluation of lungs

Breathing in big urban centers exposes us to a great variety of toxicants that constitute the air pollution. The developing organism (fetuses and children) shows to be a population specially

sensitive to the noxious effects of air pollution, either for presenting high rates of cellular multiplication and differentiation, immature immunological defense systems and antioxidants, or for presenting a higher exposure resulting from higher breathing rates. We have already shown in an experimental study that the gestational and post-gestational exposure to ambient levels of particulate pollution in the City of São Paulo is capable of compromising the development of lungs. In this study, we will evaluate if the gestational exposure is capable of compromising the intrauterine development of lungs of mice fetuses. We also utilize the chambers to conduct the maternal exposure. The gestation was interrupted at term (18dpc), and a fetus was selected per brood randomly. The fetuses of females exposed to air pollution present significantly smaller body and lungs weight (Fig.6-A). The stereological evaluation of lungs (Fig.7) shows that there actually is a compromising of pulmonary development, increase of mesenchyme volume and density, as well as blood vessels, a reduction in the proportion of saccular structures volume and of their surface area, followed with an increase in the average diameter of airways (Fig. 7). Such alterations suggest a delay in the maturation of saccular structures, which in the future will give origin to the alveolus and alveolar ducts.

B) Diesel exhaustion

1 – Effect of diesel engines exhaustion in the male reproductive system

In São Paulo, as in most of the big world metropolises, the fleet of vehicles is the main source of air pollution; This fleet is moved by a great variety of fuels, ethanol, diesel, biodiesel, gasoline and methane gas. Diesel stands out as one of the main fuels, being the main responsible for the emission of pollutants. The effects of exposure to diesel smoke in the male reproductive system is already well-known, however an approach of a multi-generation exposure focusing on the different periods of life was not well explored yet. In this study, we utilize two generation of Swiss mice exposed in a differentiated way to emissions from a particles generator moved by diesel. The parental generation was divided into two groups: non-exposed (Group P1) and exposed (Group P2). In the first generation, we divided the animals into 4 groups according to the prenatal and postnatal exposure. The first group (Group 1) constituted of animals continuously exposed to filtered air (pre and postnatal period); the second group (Group 2) continuously exposed to diesel exhaustion (pre and postnatal period); in the third group (Group 3), the animals were exposed to filtered air during gestation and to diesel emissions in the postnatal period; and in

the fourth group (Group 4), animals were exposed during gestation to diesel exhaustion and to filtered air in the postnatal period. Thus, we can evaluate the effects of prenatal and/or postnatal exposure on the male reproductive function.

So far, our results show that the exposure does not change the daily individual consumption of ration and water by parental generation animals, both females and males. Such as observed in the parental generation, the individual consumption of ration was the same among groups. However, in relation to water ingestion, the males of groups 3 and 4 presented a smaller consumption. In the parental generation, the body and organs weight (brain, heart, lungs, kidney, suprarenal, epididymis and testicle) did not present significant differences among groups. However, the kidney weight presented a significant reduction ($p = 0.022$) in the exposed group (group P2 – 1.6095 ± 0.0877 g), when compared to the non-exposed group (Group P1- 1.84 ± 0.0402 g), such as the spleen that also presented a significant reduction ($p = 0.012$) in the exposed group (0.0863 ± 0.0040 g) in relation to the non-exposed group (0.09 ± 0.0026 g). The difference in the spleen weight was kept after the adjustment by the animal weight.

In animals of the first generation, the average weight of the kidney shows to be significantly reduced in the prenatal groups (Group 3) and pre+postnatal (Group 2) when compared to the non-exposed group (Group 1). The epididymo-somatic index (sum of epididymis weight/body weigth) also shows to be altered in the prenatal group (Group 3), there is an increase of relative weight when compared to the non-exposed group (Group 1). In the other organs, no alterations regarding weight were verified.

The variation of body and organs weight after the exposure to diesel engines smoke is not defined in literature, because there is no standard for alterations after exposure to pollution generated by vehicular source. Previous studies show that the body weight and of some organs such as lungs and brain of adult mice are bigger, while the weight of testicles and epididymis does not vary. However, in a previous study of our group, in which mice were exposed to particulate pollution in the city of São Paulo, we showed that there is an increase of testicles and epididymis weight in animals chronically exposed. Such results are still too preliminary, the stereological evaluation of such organs will show if there are alterations in the constitution of these organs that justify increases of weight and compromising of their function.

Also in this subproject, we included the preliminary results of the post-doctor´s degree project called "Fetal alterations induced by exposure to atmospheric pollution: a study with focus

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on placentary morphogenesis", Student: Ana Julia de Faria Coimbra Lichtenfels, Fapesp proceeding nr. 07/56479-1.

Various epidemiological and experimental evidences clearly show an association between ambient levels of atmospheric pollution and fetal development disorders. In certain gestational pathologies, we observe that placentary alterations are intrinsically involved and that the correct establishment and differentiation of cells and embryonary annexes preponderantly in the beginning of the gestation are fundamental for the fetus quality/viability. Once the physiopathologic mechanisms are not clearly defined yet, the proposal of this study is to evaluate if the exposure to ambient levels of atmospheric pollution can induce alteration in intrinsic molecular ways at correct placentary morphogenesis, among them, the expression of the hypoxia inducer factor HIF/ARTN involved in the xenobiotics metabolizing ways, the induction of allelic variants of *fgf* and *fgfr* gene, and imbalances in the pro/antioxidant system. Such physiopathologic mechanisms, isolatedly or not, might result in a smaller initial mass of trophoblastic cells and, consequently, in a non-satisfactory placentary development. To better explore the possible effects of atmospheric pollution in the placentary development, we will use a multi-generation test where mice will be exposed to ambient levels of atmospheric pollution in the city of São Paulo. Therefore, the objectives of this work are:

1. Develop a molecular test for the accurate diagnosis of placentary development alterations associated to the exposure to atmospheric pollution;

2. Evaluate the interference of pollution on the accumulation of mRNA of HIF-1α and of protein HIF-2α (ARNT) in the placenta;

3. Analyze the ZnSOD expression in trophoblasts in view of the maternal inhalation of pollutants;

4. Genotype the studied animals as for the alleles of genes of the fibroblastic growth factor (*fgf* and *fgfr*), and correlate the allelic variants with the intensity of placentary and fetal weight reduction through the exposure to atmospheric contaminants.

Until the moment of this report elaboration, the exposure period was concluded, with preliminary results regarding weight, fetal losses and sex ration.

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G - INFLAMMATION THEMATIC GROUP

Coordinator: Marisa Dolhnikoff

Subproject 1: Evaluation of hepatic compromising in the exposure to microparticles emitted by the combustion of diesel/biodiesel.

Responsible person: Dr. Luiz Antonio Rodrigues de Freitas.

The present project had, for 2009, the objective of developing a metabolic syndrome model in mice of the C57BL6 type. The results of the initial experiments showed that the hypercaloric ration, rich in lipids and carbohydrates, continuously administered causes obesity and glycemia alteration in C57BL6 mice, validating the model for use and allowing to plan the second stage of the project of exposing obese animals with hyperglycemia to environmental pollution (concentrator of particles) and to compare the alterations in the kidney to a group of obese animals and with hyperglycemia not exposed, as well as to evaluate the effects of normal mice (controls) exposure in the concentrator of particles. During the year, various projects of validation of the particles concentrator were conducted (see page 65) which will allow, from this second year of the project on, to conduct the experiments with exposure.

In terms of the research results, the establishment of the model for the induction of metabolic syndrome in C57BL6 mice was done: with the follow-up of weight gain and dosage of glycemia in animals, it was possible to define the model for the induction of this syndrome, key for the project development. This allowed the establishment of the metabolic syndrome induction model very similar to the human disease. It is estimated that overweight and diabetes will reach epidemic levels in the world population in coming years. The cellular mechanisms and metabolic ways involved in the hepatic aggression in these diseases are still little known. The establishment of an animal model very similar to the human one allows exploring such mechanisms and better understanding the diseases, including interactions with environmental pollution.

Two graduation students, one master´s student and on collaborator researcher were involved and actively took part in the project, including the practical part and the scientific discussion.

Regarding science divulgation, the following is highlighted: 3 Scientific publications in international magazines, 2 Presentations in congresses, 2 Presentations in research institutions, and 4 Internal scientific sessions at the LPBI- CPqGM/Fiocruz.

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Subproject 2: Effect of pollution in the development of liposaccharide (LPS) induced acute pulmonary lesion

Responsible person: Prof. Dr. Marisa Dolhnikoff

During 2009, equipments and basic consumption materials were purchased for the present project, and the assemblage of experimental groups was planned. The exposure of animals to pollution in the particles concentrator was postponed for the beginning of 2010, considering that in 2009 various projects of the particles concentrator validation were carried out (see pages 3 and from page 65 on of this report), which will allow, from this second year of the project on, to conduct the experiments with exposure.

Subproject 3: Effect of pollution in the rheology of epithelial cells and of the smooth muscle of the lung

Responsible person: Adriano Mesquita Alencar

Searching for the main goals for the first year, of ensuring the necessary infrastructure for the project development, a room was assembled in the Department of Pathology of São Paulo University (USP) Medical School (FM), where materials and necessary resources of a laboratory for evaluation of pulmonary mechanics and of tissue for rodents are being placed; other two rooms in the Department of Telecommunications and Control Engineering (PTC) of USP Polytechnical School (EP), one used for electronic instrumentation and the other, for microcontrollers programming and tests. A laboratorial space of approximately 45m^2 within the General Physics Department (FG) of USP Institute of Physics (IF) was provided for the assemblage of the fourth laboratorial room for the cellular rheology of the project. In parallel, the prototypes of cellular mechanics were developed. Thus, even dedicating most of the time to put together an initial structure for the project, we have already obtained good scientific results with four presentations in conferences:

As for the results, most of the necessary equipments has been already purchased. In partnership with other participant laboratories and institutions, model experiments, already published and as planned, were carried out, and a unified protocol of pollutants manipulation and exposure among subgroups for comparing results was developed. In terms of results, so far

preliminary results of the separation of pollution components were already obtained, as well as data on the cellular rheology and MAPK expression in studied samples, which were presented in international conferences.

In the category of human resources, we include the accomplished goals of selection and start of guidance of 1 master´s student and 1 scientific initiation student, as well as the publication of the first article with results of the group researches.

As for the knowledge and technology transfer goals, these were also fully accomplished. For the cellular rheology, a software utilization manual that we developed was created by the scientific initiation student, and for the utilization of the pulmonary insufflator, the same was done by the involved master´s student. We also started to catalogue the protocols for cell culture and DEP components separation.

A course called "Respiratory Modeling and Mechanics in Multiple Scale" was planned and given in 2009. This course was idealized to provide notions of mathematic modeling and acquisition of experimental signs for researchers involved in the field of pulmonary physiology, with 10 classes given at Fridays, with average duration of 1h30min per class. A mini-course called “Acquisition and Processing of Biological Signs: Basic Concepts” was also developed, with duration of 4 hours in the XIII Brazilian Congress of Biomechanics carried out in São Paulo, on July 28, 2009.

Subproject 4: Pollution Effect in the generation of oxidative stress in encephalic tissue and erythrocytes and in genotoxicity.
Responsible person: Tania Marcourakis

During the first year of the project, the procedures regarding the characterization of the oxidative stress in the encephalic tissue were developed. The main obtained results showed that there was an alteration of antioxidant enzymes in view of the exposure to particulate material. However, the enzymes response varied among different structures. In none of them there was alteration in the production of Melanoaldehyde, suggesting that the action of enzymes was efficient to protect the tissue from lipidic peroxidation. Our results also suggest that the reduction of the glutathione reductase activity in the cerebellum and in the hippocampus might compromise the stock of glutathione, the main non-enzymatic antioxidant agent. Thus, although inconspicuous,

there seems to be an effect of the exposure to particulate material in the Central Nervous system of rats.

Subproject 5: Pollution effect in the innate and acquired inflammation and in the Expression of proteins and generation of metabolites (Omics Study)
Responsible person: Sandra P. Farsky

The LC-MS equipment included in the Project is being imported by FAPESP, and the defined delivery time of the equipment was postponed by the agency, and we haven´t received it so far. Thus, the experiments that are part of the Project “Expression of proteins and generation of metabolites (Omics Study)" are in the initial phase of samples collection for analysis.

A paper in phase of redaction for publication and forwarding of abstracts for national and international congresses in 2010.

The post-doctor´s degree student included in the PNPD Program is now in the Laboratory of Prof. Pierre Thibault, of Montreal University, Canada, training in omics methodologies applied to INAIRA Project.

Professors Sandra Farsky and Ernani Pinto organized the first School of high Toxicology Studies, in November/December 2009, at USP campus of Ribeirão Preto. This was an initiative of the Post-Graduation Programs in Toxicology of USP Pharmaceutical Sciences Faculties where the themes approached in INAIRA were included. Within this context, two foreign researchers with great insertion in Environmental and Occupational Toxicology discussed the current and relevant themes about environmental pollutants. In addition, the study theme of our group within INAIRA, that involves omics studies, was broadly approached by an eminent researcher of this field. It is noteworthy that during the 15 days, approximately 120 post-graduation students from various Post-Graduation programs in the country took part in the event. Inside this School, a post-graduation discipline in advanced Toxicology Topics was created, and given by professors Sandra Farsky and Ernani Pinto.

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Subproject 6: Effects of aerobic physical training in pulmonary alterations induced by different products of atmospheric pollution
Responsible person: Dr. Rodolfo de Paula Vieira

The present project is in development phase with initial evaluation of exposure to physical training. In parallel, structural alterations are being carried out to allow the training of bigger groups of animals (with the acquisition of ergometric treadmills for a bigger quantity of animals) and the evaluation of molecular markers (with the acquisition of equipments and consumption materials for such measurements).

As the main results of this first phase, the DEP exposure model and physical training model was defined, as well as the other necessary techniques for the project execution. The initial results showed that the aerobic physical training carried out at the same time of the intranasal instillation of particles exhaust diesel (DEP) inhibited the neutrophilic infiltration and the accumulation of collagen in the pulmonary parenchyma and the release of nitric oxide exhaled as a result of DEP instillation, showing that the exercise has a protective effect for lungs, even when done in a polluted environment. However, other various evaluations will be conducted in the lungs with the objective of evaluating the possible protective action of exercise in view of DEP instillation in lungs structural cells, such as for instance, in the smooth muscle and in the bronchial epithelium.

The initial results of this study were submitted for presentation in the European Congress of Respiratory Diseases.

Subproject 7: Neurotoxic effects related to pollution: acute, subchronic and chronic evaluation.
Responsible person: Solange Cristina Garcia Pomblum

In this first year of project, equipment was acquired according to the planning forwarded in 2009. The main importation process, a high efficiency liquid chromatograph with UV/VIS/ Fluorescence detectors was finalized and the equipment was already installed. In addition, a -80°C ultrafreezer was also acquired and is installation phase, an indispensable tool to properly store the biological samples for the conduction of analyses of oxidative damages caused by pollutants. For behavioral evaluation, locomotor boxes and Rod route, also with software data analyses, were acquired and are being implemented, with human resources being trained. For the evaluation of DNA damage, a complete electrophoreses system was recently acquired. Therefore, according to

our goals plan sent in March 2009, we are now qualified to conduct the goals for 2010, when experimental and clinical-laboratorial parts will be effectively conducted.

It is noteworthy that the urinary metabolite quantification of benzopyrene (1-hydroxipyrene) was optimized and validated, and is now available for application in the current year. The environmental monitoring part to be conducted in the city of Porto Alegre is in phase of standardization and selection of points which will be used to monitor BTX, benzopyrene and particulate matter by prof. Dr. Adriana Gioda. Therefore, this first stage planned for the execution of the project is following the previously defined time schedule, with some minor alterations.

As for the results, in this phase, the team optimized and validated trough CLA UV/VIS the methodology to quantify the 1-hydroxipyrene urinary metabolite of benzopyrene xenobiotics which is a well-know carcinogenic agent, and current studies are proposing that this is responsible for cardiovascular pathological alterations. Therefore, with the provision of this methodology, new studies might be conducted. In addition, the plasmatic quantifications of carotenoids, which are very important in the cardiovascular risk studies, are in phase of validation. The provision of methodologies within analytical toxicology such as quantification of urinary 1-hydroxipyrene, and blood carotenoids such as lycopene, is very important to reduce regional disparities, considering that in the south of the country it is now possible to conduct such analyses. In addition, the study with traffic controllers in the City of Porto Alegre, evaluating possible cardiovascular risks and oxidative damages is innovative, as well as the in vivo toxicological evaluation, experimental work, to evaluate the damages caused by pollutants emitted through the burning of fuels. Following this line, to follow the INCT execution will allow regional advances, reducing disparities and progress in the sphere of clinical-laboratorial and experimental environmental studies with interdisciplinary nature.

In this first year, a master´s student is working with carotenoids, possibly defending her thesis until March 2011, another master´s student was selected to start work in March 2010, and two doctor´s degree students are planned to start in March 2010. Thus, there will be 2 master´s students and 2 doctor´s degree students, each of them with at least one Scientific Initiation student. This subproject will allow the conclusion of at least two master´s and two doctor´s degree dissertations, in addition to at least 8 Scientific Initiation orientations. This will allow the qualification of professionals in the theme, in areas with lack of qualification in the country, which are environmental and occupational Toxicology.

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H – BIOMONITORING THEMATIC GROUP

Coordinator: Regiani Oliveira

For a higher biomonitoring integration in different regions of the country, this thematic subgroups will be presented as a whole.

The central objective of this theme is the development of alternative approaches for the exposure identification and for the definition of sources participation in areas without a proper air quality monitoring.

We propose the use of vegetal biomonitoring and passive tubes as instruments for the conduction of environmental sorting and, if possible, to estimate the risk for human beings. In the capital of São Paulo, a study will be developed with the objective of validating the biomonitoring with plants and the passive monitoring of pollutant gases as a low cost tool to estimate the risk to human health in the exposure to atmospheric pollution. This project will be developed in the Laboratory of Experimental Atmospheric Pollution (LPAE) of São Paulo University Medical School (FMUSP), coordinated by INCT/INAIRA researcher, Regiani Carvalho de Oliveira, and will be developed by the biology graduation students Amanda de Florio Barizon and Felipe Duarte Coelho de Sousa. The aforementioned project has financing of the CNPq Universal Call to Bid (proceeding no. 481334/2009-2).

In parallel to the environmental sorting, carried out with the passive monitoring and vegetal bioindicators, we also propose the active monitoring of particulate matter with aerodynamic diameter 2.5µm ($PM_{2.5}$), for 24 months, for then characterizing the particles. The active monitoring of particulate matter concentrations will be implemented during 2010, when the monitoring with biomonitor plants will be fully consolidated in all points proposed for evaluation in the Biomonitoring subprojects. The active monitoring of $PM_{2.5}$ concomitant in all study sites and to the biomonitoring with plants will allow a better evaluation of air quality in the study regions, definition of emitter sources participation and identification of the exposure.

The initial proposal of the project aimed to monitor three areas under the evaluation of VIGIAR, Camaçari region, BA, with the subproject "Project of biomonitoring associated to the 4as atmospheric environmental attention areas of Camaçari, BA", the region of Barcarena in the state of Pará with the subproject “Study of atmospheric pollution of industrial and port areas of the municipality of Barcarena, State of Pará”, and in Mato Grosso with the subproject “Pilot project of biomonitoring with *Tradescantia pallida”* in municipalities with high environmental risk in Mato

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Grosso.

Considering that the monitored area in the municipality of Camaçari is too large, the number of researchers and students intended for the conduction of the monitoring with the *Tradescantia pallida* species becomes insufficient for the maintenance of the plants in all sites of the study. It was defined that the biomonitoring will be done with the a species present in the location. The selected species was the *Pachira aquática* Aubl., a species belonging to the Bombacaceae family, and popularly known as Mungumba. The Bombacaceae family is constituted of arboreous plants, distributed in tropical regions, predominantly in the Americas, and is native from the south of Mexico to the north of South America. This species is very much cultivated as an ornamental plant, being specially recommended for urban tree planting in squares and gardens, for having an easy silvicultural maintenance. Since this species is broadly spread in the municipality of Camaçari, it was found in all the 26 points determined for monitoring. The characterization of points is presented in tables 1 and 2. The first collection campaign was carried out in November 2009, and the leaves prepared and analyzed.

The subproject “Study of the atmospheric pollution in industrial and port areas of the Municipality of Barcarena, State of Pará", proposes as main objectives the following: (1) evaluate the air quality in the municipality of Barcarena through the quantification of trace levels of metals and polycyclic aromatic hydrocarbons (PAHs) in particulate matter collected in the air in the municipality of Barcarena-Pará, and (2) evaluate the possible routes and effects of pollutants emission in the environment along time utilizing computer and/or statistic models. In parallel with the monitoring of concentration and characterization of particulate matter, PM 2.5, the biomonitoring with *Tradescantia pallida* was also proposed. Since the main objective of the Biomonitoring thematic is the qualification of monitoring institutions with an easily executable and low cost technique, and for these characteristics, capable of being executed in broad areas for a long period of time, we believe that the implementation of the biomonitoring with *Tradescantia pallida* in the region of Barcarena will be very easily diffused in the whole Amazon region. In this first year of the project, a technician from the Instituto Evandro Chagas was qualified to work with mutagenicity tests with *Tradescantia pallida*, and a graduation student, with scientific initiation scholarship, was qualified to work with metal analytical techniques. The active monitoring of particulate matter concentrations will be implemented during 2010, when the monitoring with biomonitor plants will be fully consolidated in all points proposed for the project evaluation.

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In Mato Grosso, monitoring was proposed in two municipalities, Mirassol D´Oeste and Campos Novos de Perecis. Biomonitoring with *Tradescantia pallida* species is in progress in the municipality of Mirassol D´Oeste. The characterization of biomonitoring points is presented in table 3. In this municipality, five points were qualified for monitoring, where four collections of inflorescence were conducted for the micronucleuses test. All conducted analyzes showed negative results, in view of the atypical rainy period as well as the reduction of the sugarcane-alcohol industry activities. The analysis of elementary composition in biomonitor plants leaves was not agreed on in the initial proposal of the project, but with the intention of having a parameter of air quality evaluation common in all subprojects of this thematic, we decided to add this variable to the project. The municipality of Campo Novo do Parecis, initially included in the project, was replaced by the municipality of Vila Rica, considering that the environmental health team was replaced by the municipal administration and there was no support for the continuity of the project. The municipality of Vila Rica, despite the efforts of the Municipal Secretariat of Health and the team, is in its initial phase, and so far the initial protocols were applied, in the same proposed methodology, and is now in phase of “control” vegetative multiplication, for afterwards removal of seedlings for the monitoring points.

In the second semester of 2009, a fourth study area under VIGIAR evaluation was included in the INCT/INAIRA, the Metropolitan Region of Vitória – ES. Two areas of this region were previously studied in the project “Prevalence of asthma and respiratory symptoms in the municipality of Vitória (ES): comparison between two areas with different atmospheric pollution sources identified through biomonitoring", a doctor´s degree project of the Chemical Engineer Dione Conceição Miranda, defended on 28/08/2008, in the post-graduation program of São Paulo University Medical School. The intention of the subproject “Project of biomonitoring associated to the 4As atmospheric environmental attention areas of the Municipality of Vitória – ES”, included in the INCT/INAIRA, is the monitoring of air quality of twelve areas in the Metropolitan region of Vitória, increasing the knowledge about the air quality of the region . The location and characterization of biomonitoring points are presented in figure 1 and table 4. The biomonitoring with the *Tradescantia pallida* plant will permanently fulfill the program of health vigilance related to municipal air quality, in order to outline a more precise profile of the atmospheric pollution exposure, and to allow intervention measures with higher positive impact on health. Two campaigns of *Tradescantia pallida* leaves sampling to determine the bioaccumulation were carried

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out in 2009, the first one in the Winter and the second, in the Spring.

In addition to the studies in areas under evaluation of VIGIAR, two other subprojects are part of this theme: the subproject “National center of low cost technological innovations for the atmosphere monitoring - InovAR" and the subproject "Characterization of the environmental risk in Vale do Paraíba, São Paulo".

The subproject “National center of low cost technological innovations for the atmosphere monitoring - InovAR" has the objective of implementing a laboratory in the Federal University of Bahia (UFBA), designed to develop alternative approaches in the measurement of particles and gases through simplified techniques. The two main objectives of this subproject are as follows: (1st) Develop, adapt or enhance low cost techniques for the air monitoring for purposes of environmental diagnosis and evaluation of risk to human health of exposure/absorption of atmospheric pollutants; and (2nd) Evaluate the exposure risk of the population neighbor to the petroleum and petrochemical industrial area of the north quadrant of the Recôncavo Baiano.

Within the first objective, the development of passive samplers, which are devices capable of sampling chemical species, was selected. The intercalibration with active methods of passive samplers, previously developed by the team, for O_3, NO_2, SO_2 and H_2S, presented a good performance in different types of atmosphere and during different periods: accuracy measurements, based on standard deviations, were within the limit recommended by the European Union, of 25%, for this type of device, and the accuracy was above 80%, a value mentioned in literature, with variation of 2.4 to 12% in the situations tested.

Within the second objective, for the two first years of the subproject, the evaluation of the exposure risk of the population of some municipalities of the north quadrant of the Recôncavo Baiano to polycyclic aromatic hydrocarbons (PAHs), was selected. The study of environmental paths of PAHs and analysis of total exposure risk, integrating data on breathed air, consumed water and fishes ingested by the population of handicraftsmen fishers exposed to emissions of petroleum producing sources, utilizing specific chemical measurements techniques, sensitive and precise, were developed or enhanced in this work. Investigation and evaluation protocols were elaborated in order to allow the replication of similar studies in other tropical areas. Analytical procedures were perfected for samples of atmospheric particulate matter and biological matter (mollusks), and a new procedure of PAHs extraction was developed for particulate matter, utilizing microwave extraction, resulting in the reduction of organic solvents, extraction time and analysis

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cost. The environmental paths and risks of exposure to PAHs were determined in some communities resident in area of Petrobrás influence.

The development of this subproject was very much impaired by a fire of huge proportions occurred in the UFBA Institute of Chemistry, on March 29, 2009. The results obtained and reported in this report were obtained before the fire, and part was obtained in laboratories borrowed from the Fundacentro Regional Center of Bahia and from the UFBA Institute of Geosciences. However, part of the planned goals and activities, especially the installation of new laboratories as well as the development of new passive samplers, could not be developed for depending on middle and high scale equipments, inexistent in other laboratories.

The subproject “Characterization of environmental risk in the Vale do Paraíba, São Paulo”, in addition to aim the analysis of Vale do Paraíba air quality and its relation with health indicators, aiming to quantify the environmental risk for the population, also provides for the educational involvement. In this subproject, the monitoring of air quality with the micronucleuses test in *Tradescantia pallida* and the quantification of ozone in the *Nicotiana tabacum* species were proposed. The two tools were utilized in a previous experiment in the region and proved to be effective for environmental education, contributing for the reflection and discussion of the atmospheric pollution problem in the participant schools. This experience resulted in the publication of an article, Mariani et al., 2009, in the “Environmental Pollution” magazine, a reputed international magazine in the field of environment research.

Concomitant to the monitoring of air quality in the region of Vale do Paraíba, the set up of the project continuity through satellite data validation (MODIS sensor - Terra Satellite), through the comparison of measurements of 2.5 µm particulate material obtained in the surface, is intended. This stage of the project will be initiated when the active monitoring of particulate matter is put into effect.

In the first year of the project, the challenge in the Vale do Paraíba region was to establish partnerships between INCT/INAIRA and municipal authorities responsible for the areas involved in the project, education, health and environment, with the intent of allowing the project development. A long schedule of events was accomplished for the divulgation of the project, such as workshops in public and private schools of basic and/or higher education, workshops and meetings in municipal secretariats, with schools officers and teachers. These events allowed the sensitization

of professionals responsible for the project interaction areas, education, health and environment, of other municipalities of Vale do Paraíba Region, in addition to those five municipalities previously proposed in the study. As a result, a total of eleven monitoring points with *Tradescantia pallida* and *Nicotiana tabacum* biomonitor plants was implemented in the region.

In the original project, the monitoring in five points was planned, thus the increase to eleven points demands a higher number of researchers involved with the project development. This is the current challenge for the continuity of the Vale do Paraíba project. To meet the current personnel demand, projects will be proposed for the promotion of scientific initiation and post-graduation scholarships. In the municipality of Taubaté, we decided to establish a collaboration with the University of Taubaté (municipal institution of higher education), with researchers who already know the project, submitting to the São Paulo Estate Research Support Foundation (FAPESP) a complete biomonitoring project in Taubaté, where we have the objective of implementing the project in 30 points of the Municipality.

In fact, the partnership with Universities, environmental, health and education authorities, and with the society, is the biggest challenge of projects with this theme. The accomplishment of the project proposals and the expansion of an efficient and low cost monitoring to other areas of the country, which gives support to better public policies on air quality, will be the result of partnerships and collaborations established.

Energy dispersive X-ray fluorescence spectrometry

The technique of Energy Dispersive X-ray Fluorescence Spectrometry (FRX-DE) was proposed in the project to evaluate the elementary composition in $PM_{2.5}$ and in samples of biomonitor plants leaves. The possibility of determining both macro elements, such as Calcium-Ca and Potassium-K, as well as trace elements, such as Copper-Cu and Lead-Pb, or also non-metal elements such as Sulfur-S, makes this technique very important in the multi-elementary analysis in biological and environmental systems samples. The multi-elementary determination both in $PM_{2.5}$ samples as well as in samples of biomonitor plants leaves will allow a better characterization of air quality and to identify emission sources of the regions studied in the project.

Along this year, it was defined that the bioaccumulation of marker elements of air contamination will be determined in all the project areas, in addition to the initial proposal for Camaçari, BA. Bioaccumulation is now proposed for the regions of Mato Grosso, the municipalities

of Mirassol D´Oeste and Vila Rica; in Barcarena, Pará, and in São José dos Campos, in the Vale do Paraíba-SP; besides Vitória-ES, the project included in the INCT/INAIRA in the second semester of 2009. The utilization of this technique in all study areas will allow the comparison of air quality and of possible contaminant sources in a big area of the country. Such regions have different contamination characteristics and we believe that we will be capable of characterizing each of them through the knowledge of contaminants of air accumulated in biomonitor plants.

In Camaçari, BA, it was necessary to replace the biomonitoring with *Tradescantia pallida* plant by a local species, *Pachira aquática* Aubl. The first collection of leaves was carried out in November 2009, in 26 points. In one of the municipalities of Mato Grosso, Mirassol D´Oeste, the biomonitoring was already consolidated and *Tradescantia pallida* leaves were collected in six monitoring points in November 2009. The samples were prepared, analyzed, and preliminary results are presented in the preliminary results item. In Vitória-ES, the monitoring with *Tradescantia pallida* is consolidated since the first semester of 2009, and twelve points are being monitored, where samples were collected in Winter and in Spring. The results of such analyses are presented in the “ii. Preliminary results" item.

The first collection of *Tradescantia pallida* leaves in São José dos Campos, in the Vale do Paraíba, SP, will be conducted in March 2010, when plants complete three months of exposure in the study sites, a period necessary for the bioaccumulation of atmosphere contaminants.

The Instituto Evandro Chagas, in Pará, has infrastructure that allows the development of analytical techniques. This institution has an X-Ray Fluorescence Spectrometer, Shimadzu brand, a model similar to that being used for samples analysis in other locations. We propose the qualification of the laboratory for analysis of samples collected in biomonitoring points in Barcarena, forming a center capable of evaluating the bioaccumulation in the entire Region. *Tradescantia pallida* plants are being cultivated and acclimatized in the points proposed for evaluation in Barcarena, and a technician will be trained by LPAE/FMUSP to conduct all analyzes.

The elementary composition of particulate matter samples, $PM_{2.5}$, and of vegetal bioindicators is determined utilizing the X-ray fluorescence spectrometer EDX-700HS, brand Shimadzu (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). Shimadzu´s EDX-700HS utilizes an X-ray generator tube of rhodium (Rh-target tube), 5 to 50kV voltage, 1 to 1000 A current, and a Si (Li) X-ray detector. This equipment was purchased with funds from the Ministry of Health as counterpart of the collaboration from the Laboratory of Experimental

Atmospheric Pollution for the implementation of the VIGIAR project of the Ministry of Health.

$PM_{2.5}$ monitoring will start on March 2010, and daily samplings will be performed for 24 months. The monitoring of one point in each of the areas is planned, as follows: one point in Camaçari-Ba, one point in Barcarena, Pará, one point in Vale do Paraíba, SP, and one point in Mato Grosso.

To determine the elementary composition of bioaccumulator plants leaves, the samples were washed with deionized and distilled water, and dehydrated in a greenhouse at temperature of 50°C, until being totally dehydrated. Dehydrated samples were manually ground in an agate pestle, and carefully homogenized to avoid contamination, and the resulting powder was filtered in a plastic sieve, to obtain homogenic particles sizes. From 0.5 to 0.6 grams of the samples were placed in a cylinder and then received approximately 1 gram of boric acid (H3BO3 p.a.) and then pressed for 60 seconds with 1 ton or 20mPa strength (PCA 4 model press, from Teclago Tecnologia em Máquinas Metalúrgicas, São Paulo, Brazil). The result of this process is a double layer tablet (sample and boric acid) of diameter 20mm that will be taken for FRX-DE analysis.

Edx 700-HS analyses were done at vacuum atmosphere, to increase the sensibility of disperse X-ray energy detection, in samples surface area of 10mm for 220 seconds for high energy elements (Ti-U), and for 400 seconds for low energy elements (Na-Sc). Carbon, in the form of cellulose, was utilized as mass balance. The quantitative results were obtained with the calibration of fundamental parameters, adjustments of the equipment detection sensibility, using a certified standard sample NIST SRM 1547-Peach Leaves (National Institute of Standards, 158, Gaithersburg, MD, USA). All samples were analyzed in this manner for five times. The results are presented in ug g-1.

To data coming from Mirassol D´Oeste, MT, the ANOVA test to evaluate the differences between groups was applied. The significance level of 5% was determined.

To data coming from study areas in Camaçari, BA, and Vitória, ES, or having a higher number of monitored points, it was possible to apply the exploratory principal components analysis (PCA), utilizing the rotation method (Varimax with Kaiser Normalization), in order to identify how component elements of leaves vary as a whole, based on the elementary analyses executed. Variables with 0.7 factor were used to help interpreting run factors, followed by the analysis of main components. The discriminating exploratory analysis was utilized to evaluate if the biomonitoring points presented different bioaccumulation characteristics. Fischer test was applied to determine

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the discriminatory functions

Characterization of biomonitoring points in municipalities where samples were collected for FRX-DE.

In the following figures, tables and map, we present the characterization of points surveyed during the selection of points for biomonitoring with *Pachira aquática* Aubl. plants in Camaçari, BA (table 1) and *Tradescantia pallida* plants, in Mirassol D´Oeste, MT (table 2), and Vitória, ES (table 3 and figure 8).

Ponto de Biomonitoramento	Local	Fonte emissora fixa	Fonte emissora móvel
Gleba A	Rua do Canal	Sem fontes poluidoras fixas	Rua asfaltada com **fluxo veicular**
Mangueiral	Escola Joana Angélica	Bairro a **2 km do Pólo Petroquímico**	Rua asfaltada com **fluxo veicular intenso** – rodovia de acesso a cidade
Gleba B	Pronto Atendimento de saúdel	Bairro a **3km do Pólo Petroquímico**	Rua pavimentada com **fluxo veicular**
Centro	Camara de Vereadores e Praça Abrantes Estação de monitoramento do ar	Sem fontes poluidoras fixas nas proximidades	Ruas asfaltadas com **fluxo veicular intenso**, acesso da frota municipal ao pátio da prefeitura
Bairro Novo	PSF Bairro Novo Horizonte	Empresas que sediam **o Poloplástico e Polo de Apoio**	Ruas asfaltadas com **fluxo veicular intenso** – fronteira a rodovia de acesso ao município e polo – Via Parafuso
PHOC II	Colégio Cosme de Farias	**Próximo a cerâmica**	Rua asfaltada com **fluxo veicular**
Camaçari de Dentro	Praça próxima a UBS	Sem fonte poluidora	Rua asfaltada com **baixo fluxo veicular**
Parafuso	Escola municipal	**Cerâmica Rubi**	Rua asfaltada **com fluxo veicular intenso**

Table 1 *Characterization of biomonitoring points in the municipality of Camaçari- BA*

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Ponto de Biomonitoramento	Local	Fonte emissora fixa	Fonte emissora móvel
Areias	Residência	**Millenium Chemicals**	Rua asfaltada com **fluxo veicular intenso –** via de acesso a orla do município – Estrada do Coco
Arembepe	Praça	**Millenium Chemicals**	Rua asfaltada **com fluxo veicular intenso**
Parque das Mangabas	Casa residencial	Sem fontes poluidoras	Rua asfaltada com baixo fluxo veicular
Machadinho	Praça	Sem fonte poluidora, estação de monitoramento do ar	Rua asfaltada com **fluxo veicular intenso** – via de acesso a orla do município – Via Cascalheira

Table 1 cont. *Characterization of biomonitoring points in the municipality of Camaçari- BA*

Ponto de Biomonitoramento	Local	Fonte emissora fixa	Fonte emissora móvel
Controle	Chácara Santa Maria Localizada a 2 km da urbe do município	Sem fontes poluidoras fixas	Estrada vicinal sem asfaltamento Sem fluxo veicular
Jardim Planalto	Chácara São João	**Usina de processamento de cana de açúcar**; Ponto cercado por canavial	Estrada vicinal pavimentada **Com fluxo veicular**
Sonho Azul	Centro de Saúde Sonho Azul	Distrito é cercado pela **plantação de cana de açúcar** Sem outras fontes fixas poluidoras no entorno	Ruas pavimentadas **Com fluxo veicular**
COHAB	Escola Municipal Irene Ortega	Sem fontes poluidoras fixas	Ruas pavimentadas Ponto de monitoramento distante 200 metros dessa rodovia **Fluxo veicular intenso - rodovia principal de acesso** à cidade
Centro	Prefeitura Municipal	Sem fontes poluidoras fixas nas proximidades	Rua pavimentada **Com fluxo veicular intenso, acesso da frota municipal ao pátio da prefeitura**
Morumbi	PSF Morumbi	**Duas fábricas de cerâmica localizadas a quatro quadras do ponto de biomonitoramento**	Rua sem asfaltamento

Table 2 *Characterization of biomonitoring points in the municipality of Mirassol - MT*

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Pontos de Biomonitoramento	Local	Fonte emissora fixa	Fonte emissora móvel
Horto - Controle	Horto Municipal de Vitória em Cariacica, município vizinho em área semi-rural.	Sem fontes poluidoras fixas	Estrada próxima com asfaltamento
US Jardim Camburi	Bairro Residencial no limite do município	Com **fontes poluidoras fixas próximas (Siderurgia)**	Ruas pavimentadas **com fluxo veicular intenso**
Jardim da Penha	Bairro Residencial entre duas vias de grande movimento no município	Sem fontes poluidoras fixas significativas nas proximidades	Ruas pavimentadas com fluxo veicular intenso
US Resistência	Bairro Residencial em região pobre do município, afastado de fontes fixas	Sem fontes poluidoras fixas significativas nas proximidades	Ruas pavimentadas e não pavimentadas **com fluxo veicular regular**
US Grande Vitória	Bairro Residencial em região pobre do município, afastado de fontes fixas	Sem fontes poluidoras fixas significativas nas proximidades	Ruas **pavimentadas com fluxo veicular regular**
US Maria Ortiz	Bairro Residencial em região de classe média baixa no município, próximo ao aeroporto e divisa do município	Sem fontes poluidoras fixas significativas nas proximidades	Ruas pavimentadas **com fluxo veicular intenso próximo**
US Andorinhas	Bairro Residencial pobre no município em região afastada de fontes fixas significativas	Sem fontes poluidoras fixas significativas nas proximidades	Ruas pavimentadas com fluxo veicular regular
US Jabour	Bairro Residencial em região de classe média baixa no município, próximo ao aeroporto e divisa do município	Sem fontes poluidoras fixas significativas nas proximidades	Ruas pavimentadas **com fluxo veicular intenso próximo; aeroporto próximo**
US Bairro da Penha	Bairro Residencial em região pobre do município, afastada de fontes fixas significativas	Sem fontes poluidoras fixas significativas nas proximidades	Ruas pavimentadas **com fluxo veicular regular**
US Santa Tereza	Bairro Residencial próximo ao centro do município	Sem fontes poluidoras fixas significativas nas proximidades	Ruas pavimentadas **com fluxo veicular intenso próximo**
US Ilha de Santa Maria	Bairro Residencial/Comercial próximo ao centro do município	Sem fontes poluidoras fixas significativas nas proximidades	Ruas pavimentadas **com fluxo veicular intenso próximo**
US Vitória	Bairro Comercial/Residencial no centro do município	Sem fontes poluidoras fixas significativas nas proximidades	Ruas pavimentadas com **fluxo veicular intenso**

Table 3 *Characterization of biomonitoring points in the municipality of Vitória - ES*

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Preliminary results of bioaccumulation analysis in biomonitor plants

Camaçari-BA: The exploratory principal components analysis (PCA) identified four principal factors (table 4). The main factor identified is composed of potassium-K and copper-Cu, and the second of manganese-Mg, the third of phosphorus-P and sulfur-S, and the fourth for aluminum-Al. In figure 9, we present the distribution of four factors in the biomonitoring points in the municipality of Camaçari, BA. The Mangueiral point, which is located at 2 km from the Petrochemical Pole and has an intense vehicular traffic, was characterized by factors 3 and 4, which identifying elements are phosphorus, sulfur and aluminum.

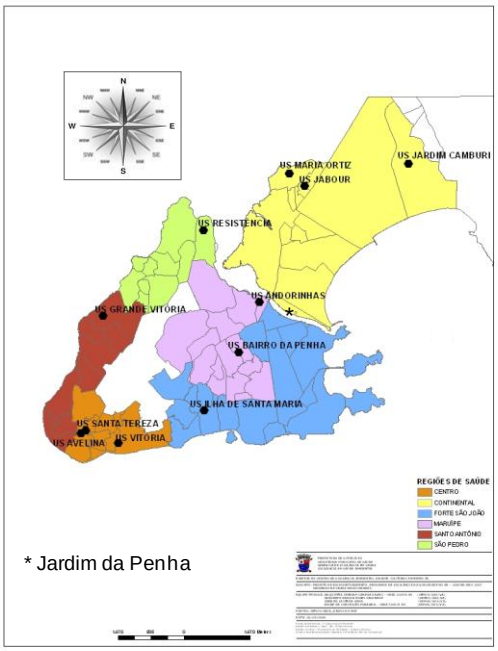

Fig 8 *Biomonitoring map in the municipality of Vitória - ES*

The graphic representation of discriminative functions allows the characterization of samples in 13 different groups (figure 10), with 77% of correctness percentage in the classification of groups. The utilized functions have statistic significance, p>0.0001. Table 6 presents the canonic correlation coefficients between discriminative scores and the groups of the 12 first functions utilized in the discriminative analysis of results.

Rotated Component Matrix[a]

	Component			
	1	2	3	4
Mn	-,377	,628	,330	,168
Fe	-,525	,372	,105	,402
Cu	**,712**	-1,05E-02	,261	,105
Zn	,536	,591	8,311E-02	,152
Rb	,694	-9,92E-02	,166	-6,58E-02
Sr	-,387	-7,36E-02	-,412	,658
Na	6,149E-02	-,680	-1,92E-03	,158
Mg	8,138E-02	**,762**	-,106	-5,78E-02
Al	,123	-8,22E-02	,166	**,887**
P	,131	-,106	**,809**	-4,19E-02
S	,172	,148	**,761**	6,124E-02
Cl	,673	,204	-,228	-8,68E-02
K	**,903**	-,152	,241	4,269E-02
Ca	-,771	-,120	-,300	,431

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 6 iterations.

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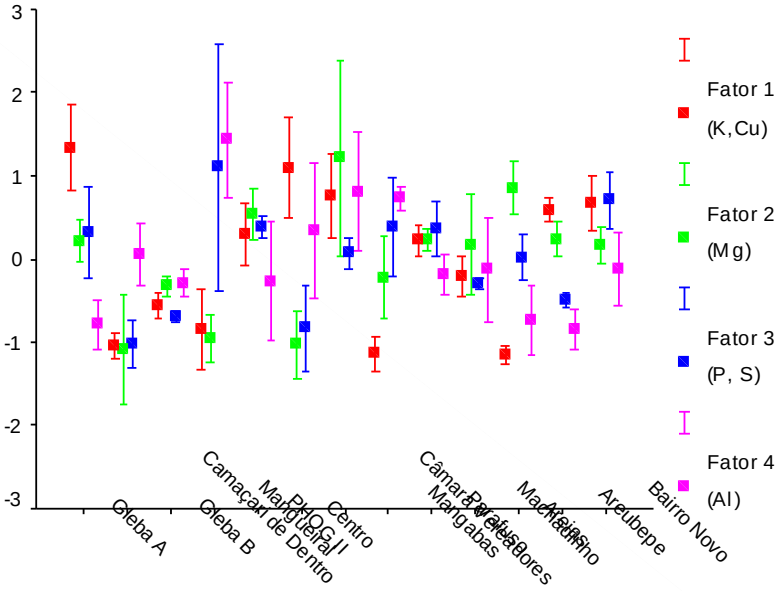

Figure 9 *Graphic representation of distribution in the biomonitoring points in Camaçari, Ba, of the factorial scores, table 5, obtained by the analysis of principal components applied to the results of elementary composition determination (FRX-DE) of samples of Pachira aquática Aubl. leaves, collected in Camaçari, Ba. K, potassium;*

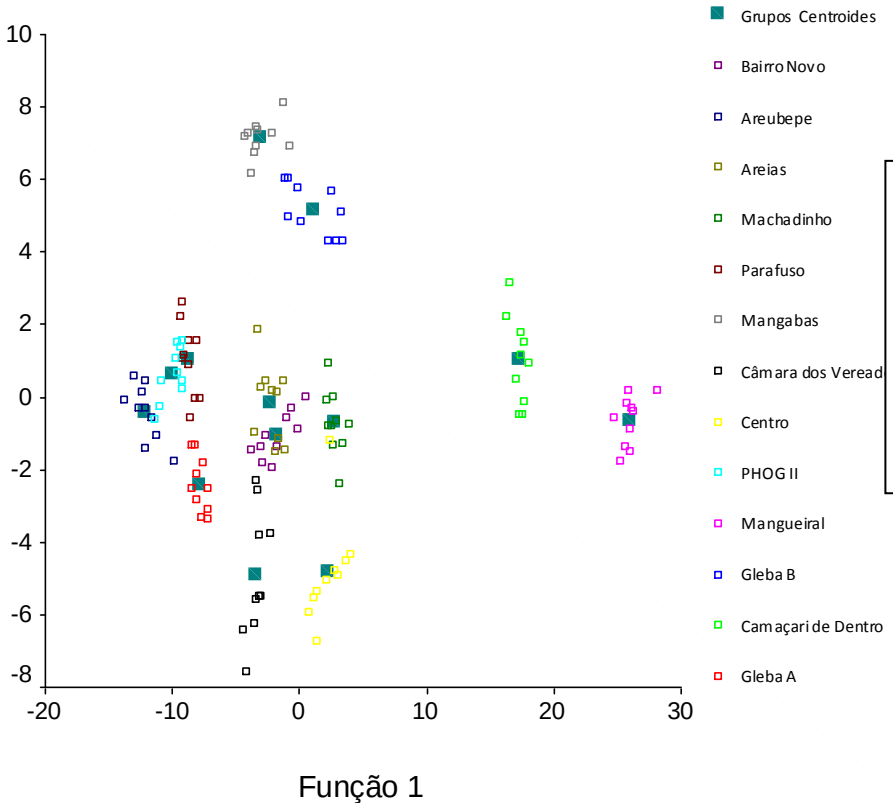

Figure 10 *Graphic representation of functions (1 e 2) obtained by discriminating analysis applied to the results of determination of elementary composition (FRX-DE) in samples of Pachira aquática Aubl. leaves collected in 13 points in the municipality of Camaçari, Ba.*

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Mirassol D'Oeste – MT

The results of FRX-DE analysis for the elements iron, copper, zinc, lead, sulfur, aluminum, calcium, chlorine, manganese, sodium, phosphorus and potassium, averages and standard error, are presented in figures 11, 12 and 13. These are the results of a single collection that occurred after the plants exposure during a rainy period in the municipality, and in which there was no biomass burning. Such results will serve as control for the other two campaigns of the region burning cycle. The first collection will be done in June or July 2010, a period in which the sugarcane burning will be completing an approximate period of three months duration. We intend to conduct the third collection of samples in October. In September, the forest burning starts and the sugarcane burning period will be still active.

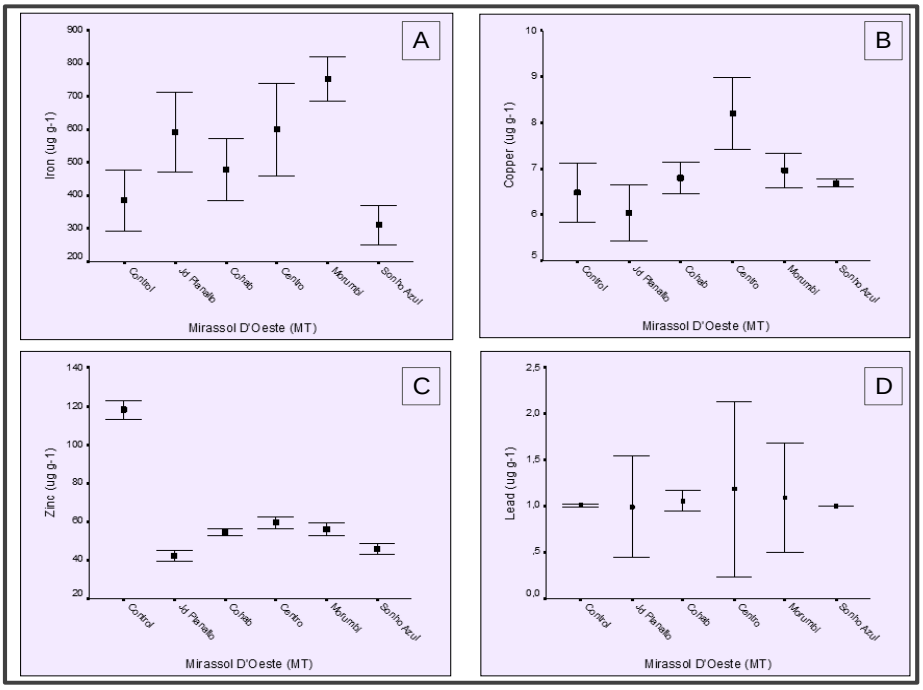

Figure 11 *Graphic representation of averages (standard error) of elements (A) Iron-Fe, (B) Cooper-Cu,(C) Zinc-Zn and (D) Lead-Pb, resulting from the analysis of FRX-DE of Tradescantia pallida leaves samples in the six monitoring points in the municipality of Mirassol D'Oeste, MT.*

The element iron is found in higher concentration in the points Jd. Planalto, COHAB, Centro and Morumbi, in relation to the other points, control and Sonho Azul, $p<0.05$ (figure 11A). In the point Morumbi, a higher concentration of this element was found (figure 11A). The higher concentration of cooper, in relation to the other points, was found in the point located in the center of Mirassol D´Oeste municipality, $p<0.05$ (figure 11B). The concentrations of zinc in the five monitoring points, Jd. Planalto, COHAB, Centro, Morumbi and Sonho Azul, are smaller than those

found in the control point, p<0.0001 (figure 11C). The higher concentrations of sulfur are found in the points Centro and Sonho Azul, and COHAB, p<0.05 (figure 12A). The result found for sulfur in points with higher sulfur concentration, Centro, Sonho Azul and COHAB can be the result of the flow of vehicles in these points. The higher concentration of aluminum was found in point Jd. Planalto in relation to the other points, p<0.0001 (figure 12B). This point is close to the sugarcane processing plant. The control point presented a higher concentration of aluminum than the points COHAB, Centro, Morumbi and Sonho Azul, 0.05.

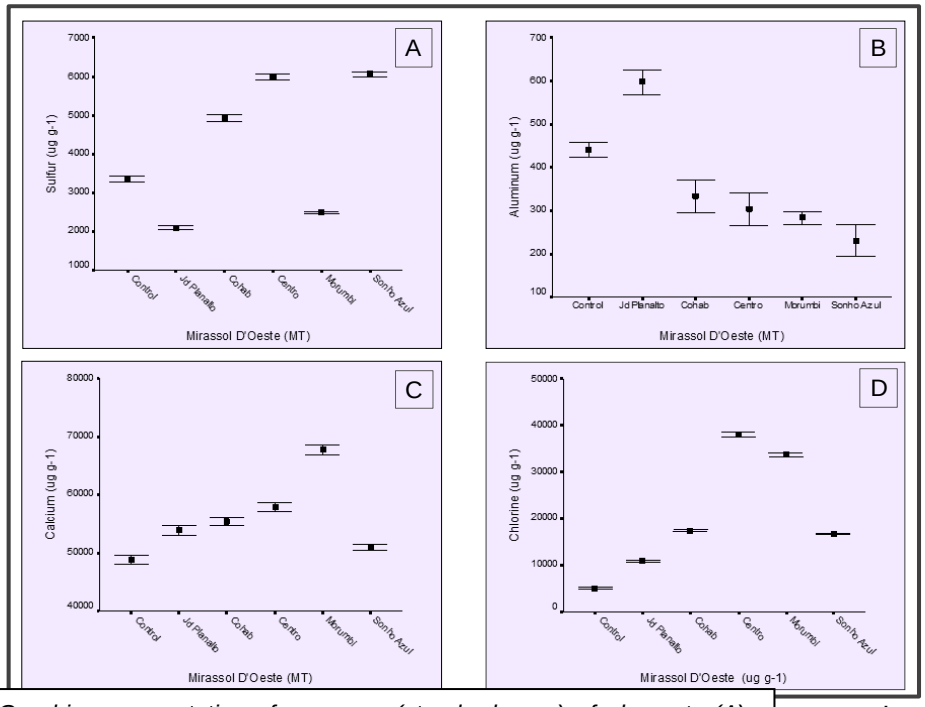

Figure 12 *Graphic representation of averages (standard error) of elements (A) Sulfur-S, (B) Aluminum-Al, (C) Calcium-Ca and (D) Chlorine-Cl, resulting from the analysis of FRX-DE of Tradescantia pallida leaves samples in the six monitoring points in the municipality of Mirassol D'Oeste, MT.*

The control point was selected for being located in a rural zone, far from known fixed and mobile sources, and despite of that the results presented the highest concentration of zinc and a high concentration of aluminum, comparing to the studied points. The regions also present differences in relation to the concentration of macro-elements such as phosphorus, potassium, magnesium, calcium and sodium (figures 13A, 13B and 13C). Some of these elements are found in fertilizers.

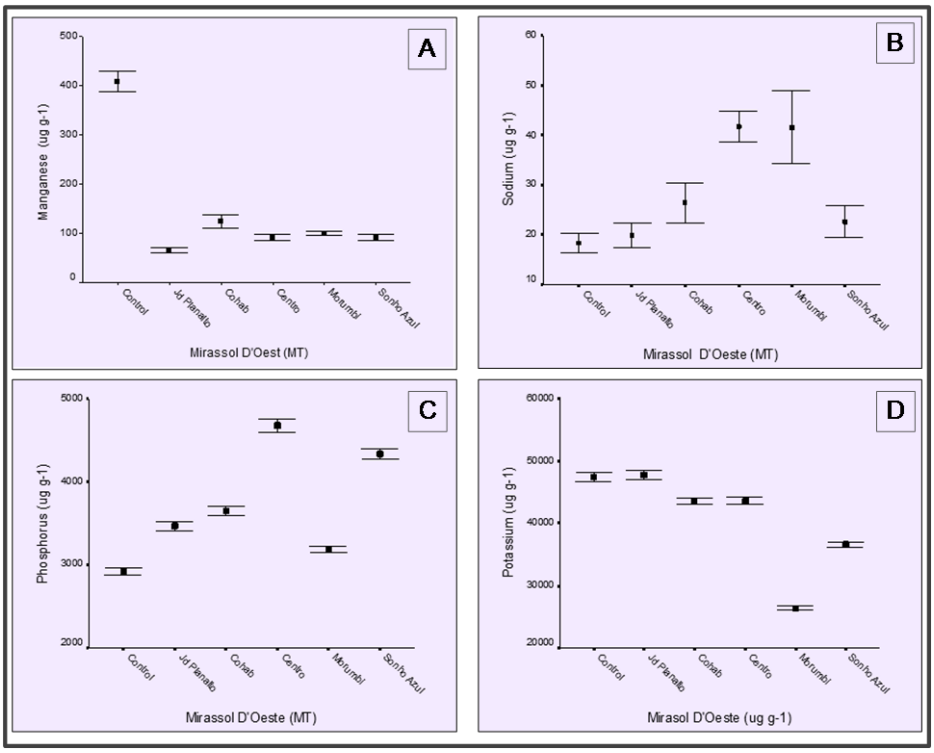

Figure 13 *Graphic representation of averages (standard error) of elements (A) Manganese-Mg, (B) Sodium-Na, (C) Phosphorus-P, (D) Potassium-K, resulting from the analysis of FRX-DE of Tradescantia pallida leaves samples in the six monitoring points in the municipality of Mirassol D'Oeste, MT.*

Vitória-ES

The exploratory principal components analysis (PCA) identified four principal factors (table 5). The main factor identified is composed of iron-Fe, copper-Cu, potassium-K and calcium-Ca. The second is composed of rubidium-Rb, sodium-Na and chlorine-Cl. The third factor is represented by magnesium-Mg and the fourth, by phosphorus-P. In figure 14, we present the distribution of four factors in the biomonitoring points in the municipality of Vitória, ES.

The utilized functions have statistic significance, p>0.0001. Table 6 presents the canonic correlation coefficients between discriminative scores and the groups of the 11 first functions utilized in the discriminative analysis of results.

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Rotated Component Matrix[a]

	Component			
	1	2	3	4
Mn	,332	5,578E-02	,277	-,372
Fe	**,752**	-3,26E-02	-,383	-,237
Cu	**,739**	,514	,159	-,229
Zn	,435	-,353	,510	-9,65E-03
Rb	6,090E-02	**,938**	5,852E-02	5,165E-02
Sr	**,903**	-7,87E-04	,242	,126
Na	,146	**,950**	6,776E-02	-8,19E-02
Mg	-5,03E-02	7,955E-02	**,701**	-1,76E-02
Al	-6,90E-02	-,173	-,849	,102
P	-1,12E-02	1,762E-02	-6,12E-02	**,957**
S	,373	-,177	,571	,592
Cl	,193	**,959**	5,307E-02	-5,20E-02
K	**,786**	,425	-,201	,177
Ca	**,850**	,215	,277	5,396E-03

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 6 iterations.

Table 5 *Factorial scores obtained by the principal components analysis (PCA) utilizing the rotation method (Varimax with Kaiser Normalization) applied to the results of the determination of elementary composition (FRX-DE) in Tradescantia pallida leaves samples collected in the municipality of Vitória, ES.*

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	129,392[a]	52,4	52,4	,996
2	84,102[a]	34,1	86,5	,994
3	16,259[a]	6,6	93,1	,971
4	8,419[a]	3,4	96,5	,945
5	6,186[a]	2,5	99,0	,928
6	,933[a]	,4	99,4	,695
7	,777[a]	,3	99,7	,661
8	,395[a]	,2	99,8	,532
9	,343[a]	,1	100,0	,505
10	,074[a]	,0	100,0	,263
11	,011[a]	,0	100,0	,104

Table 6 *Canonic correlation between discriminating scores and the groups of the first 11 functions utilized in the discriminating analysis of the results of the elementary composition (FRX-DE) in Tradescantia pallida leaves samples collected in 12 points in the municipality of Vitória, ES.*

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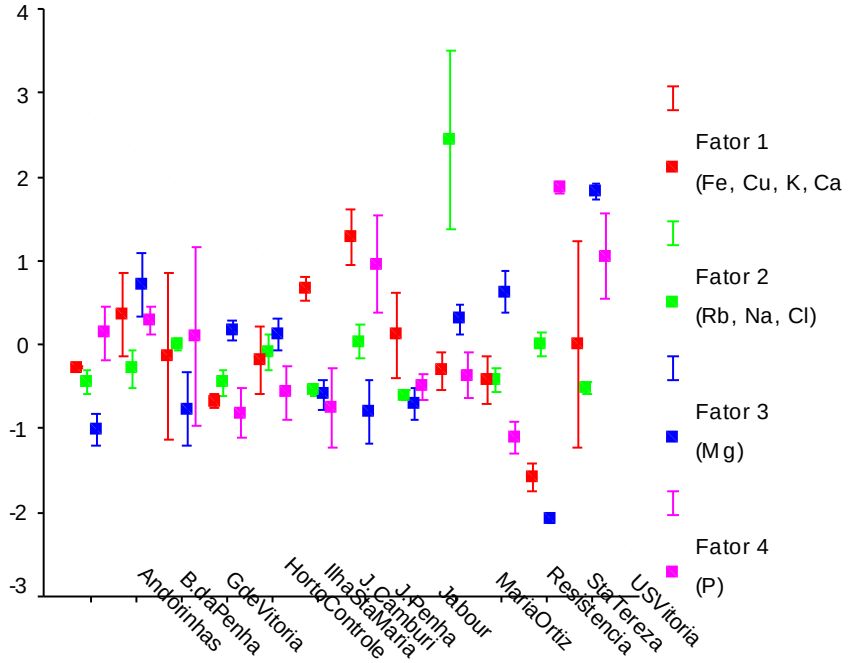

Figure 14 *Graphic representation of the distribution of biomonitoring points in the municipality of Vitória, ES of factorial scores.*

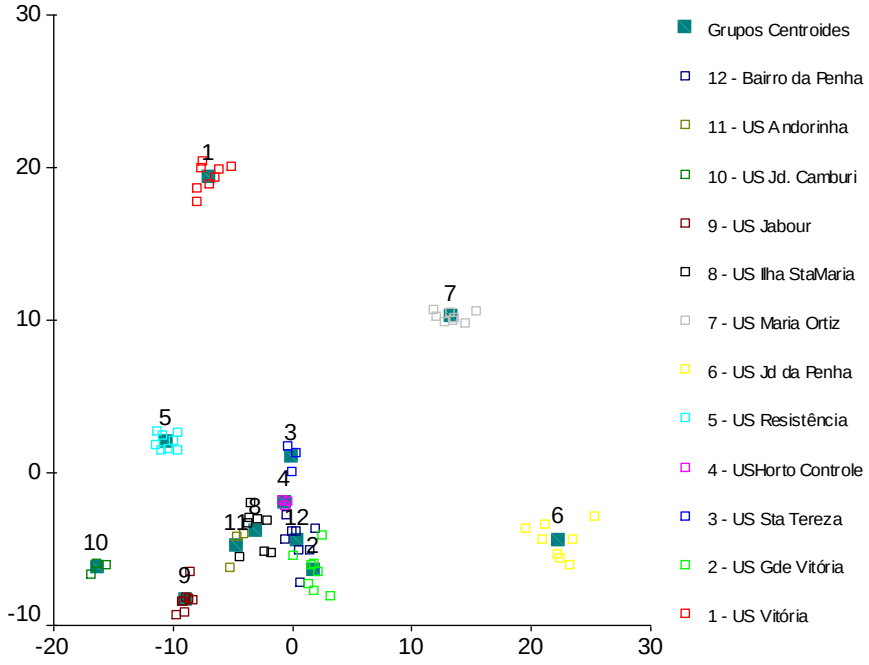

Figure 15 *Graphic representation of functions (1 e 2) obtained by the discriminating analysis applied to the results of the determination of the elementary composition of (FRX-DE) in the samples of Tradescantia pallida leaves collected in Vitória, ES*

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I – CARCINOGENESIS THEMATIC GROUP

Coordinator: Miriam Lemos

Subproject 1: Modulation of pulmonary carcinogenesis induced by urethane in mice by different pollutants: particles generated by traffic, sugarcane burning and by diesel, biodiesel, ethanol and gasoline engines.

Responsible person: Miriam Lemos

In this project, the plan of goals for 2009 included the exposure to diesel and biodiesel in the generator of particles, which in the beginning of 2009 was being constructed at the LPAE. The construction of the equipment would be finished in this year, which actually happened, but there where operation problems that delayed its actual use. We had another exposure system left – the Concentrator of Atmospheric Particles – which construction was concluded in March 2009. Although this type of exposure was planned for 2010, we advanced it for 2009, and before the evaluation of the carcinogenic effects, we evaluated the results and particularities of the new exposure system with a pilot study with the following objective: Explore the possible adverse effects of rats exposure to PM2.5 levels within the limits recommended by the World Health Organization, i.e., 25µg/m^3 (average of 24 hours), for 2 weeks.

The **Concentrator of Atmospheric Particles** uses inertial impactors for the concentration of atmospheric particles with aerodynamic size 2.5um, i.e., PM2.5. We became interested in

Figure 16 *(1) exposure chamber with ambient air (2) exposure chamber with concentrated air with 2.5µm particles.*

Figure 17 *Lateral view of virtual impactors, with exposure chambers in the background.*

studying this size of particles because of the fact that fine particles (PM2.5) are associated to an increase of cancer mortality even after controlling smoke, diet, occupational exposure, and other risk factors, with regional and spatial differences[22]. Such reports push authorities to legislate acceptable pollutants limits. Therefore, with the new scientific discoveries, we were motivated to reprogram our experiments, designing them to answer a very simple question: are the PM2.5 levels of the World Health Organization capable of avoiding adverse effects? This became the relevant question from the scientific point of view in the worldwide context, and thus our efforts were directed in such direction, when we gave rats a dose of approximately 600 µg/m^3 of concentrated PM2.5 from the atmosphere of São Paulo (corresponding to the accumulated dose of 25 µg/m^3 for 24 hours), and then we kept the animals in clean air after such procedure.

After each daily exposure, the animals were transferred to a vivarium located within the CPA, where, through a microenvironmental ventilation system, they received particles free air by passing it through and HEPA filter. Exposures lasted at least 1 hour and at the most 5 hours, depending on the daily external environmental concentrations of pollutants.

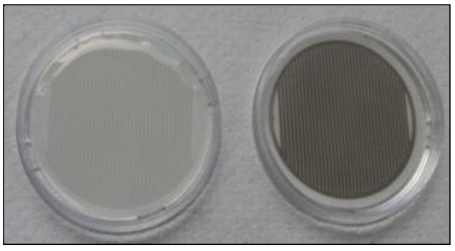

Figure 18 *Aspect of filters of fine particulate matter, with samplings done before (left) and after the passage through the Environmental Particles Concentrator (right). Particles of 2.5µm.*

Figure 18 shows the aspect of fine particulate matter filters placed in the environment outside chambers or after concentration by CPA, while figure 19 shows the environmental concentrations and after concentration of PM2.5 present in the set of experiments.

In addition to gravimetric measurements, specific campaigns of PM2.4 measurement were conducted in the morning and at night, as well as during weekdays and weekends. Such measurements aimed to explore the variability of the traffic and atmospheric conditions, in order to identify pollution sources that influenced CPA. We should remember that the possibility of particles concentration offered by the CPA allowed the collection, in a short period of time (4 hours), of fine particulate matter in enough amounts for the analysis through the employed methods. Black carbon (reflectance) measurements as well as elementary analysis through X-ray fluorescence spectroscopy were carried out. The set of information was submitted to a receptor model, applying the techniques of principal components analysis, with Varimax rotation.

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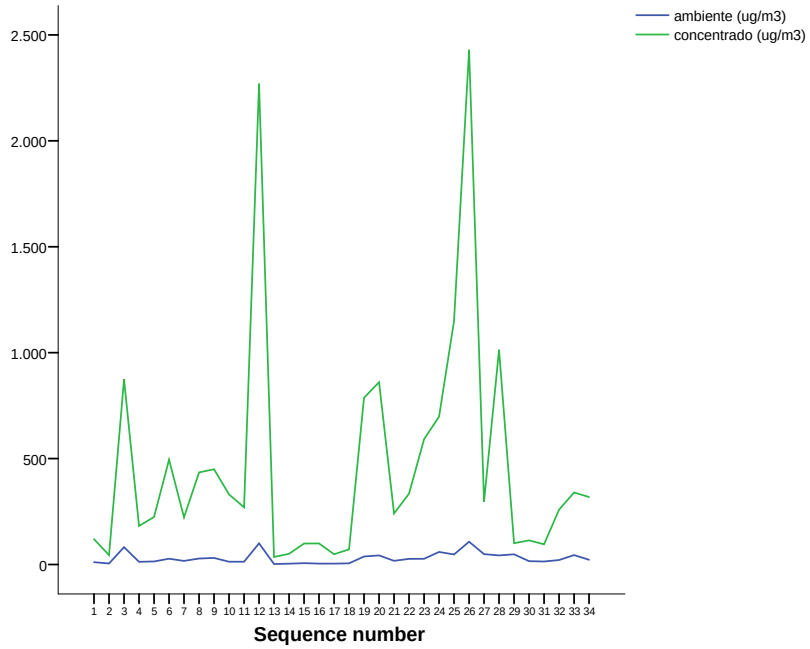

Figure 19 *Graphic representation of environmental values post-concentration of the fine particulate matter.*

The results of chemical composition determinations of the material collected after the concentration are presented in Table 7. Through the analysis of principal components, it was possible to identify three factors that, together, explain 86% of the variability of the concentrated PM2.5 composition. The factors thus determined are presented in Table 8.

The first factor is predominantly constituted of black carbon, Fe, Si and Ca, with significant smaller contributions of Cu, V and S. This factor was associated to the traffic in general, perhaps with a component of soil resuspension (Ca) (MONACI F et al, 2000 and FIGUEIREDO AM et al, 2007). The second component was dominated by V and S, and was interpreted as caused by diesel vehicles, given the association of these elements with the emissions of this fuel (SCHAUER JJ et al, 2006). The third factor, associated to Cu and Ni, was interpreted as being dependent on industrial sources, given the association of these elements with fixed sources (FIGUEIREDO AM et al, 2007 and CARRERAS HA et al, 2009).

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	period											
	morning						night					
	days						days					
	weekdays			weekend			weekdays			weekend		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
$PM_{2.5}$	8	158.10	151.82	3	122.57	63.23	8	197.86	134.77	3	410.07	219.37
Black Carbon	8	18.61	15.59	3	13.27	8.95	8	30.65	29.07	3	53.60	31.56
Mg	8	.02	.03	3	.54	.92	8	.02	.05	3	.00	.00
Al	8	.62	1.07	3	1.56	1.36	8	1.60	2.99	3	4.82	3.51
Si	8	1.54	1.60	3	1.60	2.29	8	2.15	3.50	3	8.01	3.89
P	8	.08	.06	3	1.22	1.97	8	.07	.07	3	.09	.09
S	8	6.63	6.81	3	3.07	1.62	8	8.19	5.97	3	7.97	1.27
Cl	8	2.28	4.56	3	1.01	1.26	8	5.49	12.26	3	7.39	10.05
K	8	1.53	1.58	3	1.09	.75	8	2.24	2.84	3	9.45	6.77
Ca	8	.83	.52	3	.49	.37	8	1.05	1.53	3	1.67	1.08
Ti	8	.11	.10	3	.06	.07	8	.17	.25	3	.47	.28
V	8	.01	.01	3	.01	.00	8	.01	.01	3	.02	.02
Cr	8	.02	.02	3	.02	.03	8	.01	.01	3	.02	.01
Mn	8	.06	.04	3	.68	1.12	8	.06	.06	3	.10	.07
Fe	8	1.62	1.12	3	.73	.67	8	2.63	3.29	3	5.94	3.51
Ni	8	.01	.01	3	.03	.04	8	.01	.02	3	.01	.01
Cu	8	.05	.04	3	.18	.22	8	.33	.72	3	.20	.15
Zn	8	.33	.28	3	.18	.23	8	.32	.33	3	.60	.47
Se	8	.01	.01	3	.02	.02	8	.00	.00	3	.00	.00
Br	8	.02	.02	3	.13	.20	8	.07	.08	3	.09	.06
Pb	8	.02	.03	3	.01	.01	8	.01	.03	3	.00	.00

Table7 *Descriptive analysis of measurements of elementary composition and of black carbon of filters containing concentrated fine particles, collected in different days of the week, and during the day and at night.*

	Factor		
	1	2	3
Black Carbon	0.788	0.278	0.393
Si	0.936	0.137	- 0.009
V	0.328	0.749	0.431
Fe	0.951	0.257	0.053
Ni	- 0.093	0.199	0.888
Cu	0.490	- 0.079	0.673
S	0.193	0.915	- 0.024
Ca	0.920	0.205	0.110

Table 8 *Result of the application of the principal components analysis technique, with Varimax rotation, for the set of data of elementary measurements of filters with concentrated fine particulate matter.*

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After the characterization of the exposure standard, we started to evaluate the effects of such exposure in relation to various parameters such as: alterations in the coagulogram and leucogram, in adhesion molecules and inflammatory mediators in the blood, alterations of the bronchoalveolar lavage, pulmonary histology and mechanics, as well as the effects in other organs such as kidney, spleen and bone marrow. A summary of the main results obtained in relation to these various parameters is presented as follows:

In relation to the leucogram, as well as to the platelet, coagulative and reticulocytes parameters in the peripheral blood, significant differences were not observed between the study groups. The exposure to PM did not modify the number of leukocytes present in the bone marrow nor the number of leukocytes in the blood circulation, in relation to the control animals. The differential counting of circulating cells was also not changed by PM exposure, since the number of polymorphonuclear cells (PMN) and mononuclear cells (MN) were equivalent to the values found in control animals.

The number of circulating erythrocytes was not modified by PM exposure in relation to control animals.

The effect of PM exposure on the L-selectin and $beta_2$integrin expression in circulating leukocytes was evaluated through flow cytometry tests. The obtained results show that the PM exposure reduced the L-selectin and $beta_2$integrin expression in circulating leukocytes membranes. The reduction of both molecules expression was dependent on smaller expression in PMN leukocytes and in MN.

The effect of PM exposure was evaluated on the concentrations of circulating cytokines through immunoenzymatic tests. Similar levels of IL-1beta, TNF-alpha and IL-6 were detected in control animals and in those exposed to PM.

The presented data show that the PM exposure, despite not changing the number of leukocytes in circulation, modifies the state of activation of such cells, since the expression of both molecules, L-selectin and $beta_2$integrins, were reduced both in PMN and in MN. Based on such results, we can infer that the cellular migration to the inflammation focus might be impaired. It has been well shown in literature that reduced expressions of L-selectin and $beta_2$integrin inhibit the leukocytes inflow in different inflammation models (ALON R *and* ETZIONI A, 2003; PALSVOLSKY R *et al.,* 2007).

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In relation to circulatory aspects of the pulmonary artery, endothelium-dependant vasodilatation was evaluated in the pulmonary artery rings via execution of concentration-response curves to the muscarinic acetylcholine agonist. Pulmonary artery rings of rats exposed for 15 days to concentrated $PM_{2.5}$ (600 µg/m^3) from the air of São Paulo presented a significant reduction of relaxation induced by acetylcholine, in comparison to rats only exposed to filtered air, indicating the development of an endothelial dysfunction in the pulmonary artery of such animals. To verify if the smaller relaxation to acetylcholine found in the pulmonary artery of rats exposed to concentrated $PM_{2.5}$ was associated to alterations in the responsiveness of smooth muscle cells to the nitric oxide vasodilator factor (NO), concentration-response curves to the NO donator, sodium nitroprusiate, were evaluated. No statistically significant differences were observed in the relaxation induced by the sodium nitroprusiate between control and exposed groups.

In addition to the response of vasodilators, in the pulmonary artery rings, the contractile response to KCl (75 mM) and to the phenylefrine alpha-adrenergic agonist was also evaluated. The contractile response to KCl indicates arterial feasibility, considering that those rings contracting less than 0.5 g in response to KCl were excluded, in addition of being an induced contractile response independent from the agonist-receptor interaction. We observed that the contractile effect of KCl administration (75 mM) was of similar magnitude in pulmonary artery rings of rats exposed to filtered air (controls) and to concentrated $PM_{2.5.}$ Similarly, no statistic differences were observed in the contractile response induced by phenylefrine between the studied groups.

In the isolated trachea rings, the contractile response to KCl (60 mM) and to the carbachol muscarinic agonist was evaluated. The contractile response to KCl indicates preparation feasibility, considering that those rings contracting less than 1.5 g in response to KCl were excluded, in addition to induce a contractile response independent from the agonist-receptor interaction. We observed that the contractile effect of KCl administration (60 mM) was of similar magnitude in isolated trachea segments of rats exposed to filtered air (controls) and to concentrated $PM_{2.5.}$ Also, no statistic differences were observed in the contractile response induced by carbachol in the trachea in the two studied groups.

In the isolated tracheal segments, the dilator way induced by the activation of alpha-adrenergic receptors was evaluated, through the execution of curves of concentration-response to the non-selective agonist of alpha-adrenergic receptors, the isoproterenol. It was observed that the exposure of animals for 15 days to concentrated $PM_{2.5}$ was capable of causing a significant

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reduction of the trachea smooth muscle dilatation in response to the administration of isoproterenol in comparison to the group only exposed to filtered air.

The descriptive histopathological analysis of lungs revealed evident alterations in the animals exposed to concentrated particulate matter. The most characteristic finding was observed in the transition region between the terminal bronchiole and the alveolar territory, characterized by the presence of inflammatory infiltrated in the bronchiolar wall and accumulation of macrophages and neutrophiles in the alveolar lights of spaces of acinars center gas exchange. The semi-qualification of inflammation through predefined scores revealed a significant increase of inflammatory lesions in the alveoli of animals exposed to concentrated particles.

As for the increase of mucus production in the respiratory epithelium of airways, the results indicated a significant increase of positive Alciam Blue cells (AB+) ($p = 0.046$, Student´s t-test) and marginal significance for the total of mucous cells (PAS+AB, $p = 0.055$, Student´s t-test).

In relation to the pulmonary vessels, in the results analysis, we observed that the relation light wall increases insofar the vessels are bigger ($p < 0.001$) and that the exposure to fine particulate matter promotes narrowing (constriction?) of pulmonary arterioles ($p = 0.03$), especially in the smaller ones. This type of finding is compatible with other findings of our group, as well as with the relaxation measurements of *in vitro* pulmonary artery, carried out in this same project.

The histological study of the heart did no reveal alterations evident at the microscope examination. Measurements of the arteries and coronary arterioles light/wall relations were also carried out, not revealing significant alterations in view of the exposure to concentrated fine particles of the ambient air.

In the kidney, the proliferation profile and apoptosis were evaluated. The results showed a higher frequency of mitosis and apoptosis figures in animals exposed to fine particulate matter. The finding of a higher rate of hepatocytes renovation in view of the exposure to fine particles was not previously observed, suggesting that the kidney might be a target organ of the action of atmospheric pollutants.

In the analysis of the spleen, we observed an increase of the organ in exposed animals, and that this was due to the increase of the red pulp ($p = 0.033$, Mann-Whitney test). The set of histological alterations in the kidney and in the spleen points to the possibility that the exposure to the fine particulate material promotes subtle though significant inflammatory alterations in organs, other than the lungs.

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Therefore, we can summarize the following results:

LUNGS: inhalation of particulate material for 15 days, in accumulated levels corresponding to the World Health Organization standard (25 µg/m^3, average of 24 hours) caused a discreet inflammatory state of the lungs. There were significant alterations of inflammatory parameters observed at the histological examination, secretor hyperplasia, and increase of *in vitro* reactivity of the trachea. The alterations of the bronchoalveolar lavage measurements and also the *in vivo* bronchial reactivity tests, although not significant, point to the same direction.

CARDIOVASCULAR SYSTEM: There was a significant alteration of the pulmonary artery reactivity, followed by alterations of histological measurements indicative of arterioles thickening in the transition zone between the respiratory bronchiole and the alveolar territory. The morphometric findings of coronary arteries do not indicate effects of exposure to pollutants. The set of results indicate that low levels of exposure to fine particles cause adverse impact in the pulmonary vascular territory.

SYSTEMIC RESPONSE: In the present project, significant alterations in the counting of circulating inflammatory cells number were not detected, as well as of platelets or of the profile of the myelogram. Also, no alterations were found in the plasmatic concentrations of researched cytokines, as well as in the coagulation parameters, fibrinogen and lactate dehydrogenase. The above set of results points to the absence of systemic inflammatory response in the exposed animals. On the other hand, significant alterations were observed in the functional profile of leukocytes, through the reduction of the L-selectin and $beta_2$integrin expression. In addition, the observation of the spleen increase at the expenses of the red pulp and also increments of apoptosis and mitosis estimators in the kidney indicate the existence of systemic effects of the exposure to low levels of fine particles. Therefore, unlike the alterations of the pulmonary territory (bronchi, alveoli and vases), where all measurements point in the direction of damage, the evidences of systemic response do not present internal coherence. Within this scenario, it is wise to conclude that there is no solid base to characterize, in this model, the presence of systemic alterations when of the exposure to fine particles within the standard established by the World Health Organization.

Subproject 2: In vivo and in vitro toxicity evaluation of environmental pollutants; inflammation, oxidative stress, genotoxicity.
Responsible person: Ana Paula Loureiro

Byproducts of carcinogenic cyclic nitrosamines.

Carcinogenic cyclic nitrosamines, such as N-nitrosopyrolidine (NPYR), are found in the diet (for instance, fried bacon, smoked meet, water), cigarette smoke, and are endogenously formed in humans through nitrosation of corresponding cyclic amines. The exogenous human exposure to NPYR is estimated as 0.01 – 0.15 µg per day. However, the contribution of endogenous formation by pyrolidine nitrosation (usually found in the human blood, gastric juice, saliva and urine) can increase the exposure to levels of approximately 10 µg per day. NPYR is a well established carcinogen that mainly induces hepatic tumors in rats and in the respiratory tract of mice and hamsters. Td50 values for the induction of tumors in rats are below those verified for 2-Acetylaminofluorene and vinyl chloride, other potent hepatic carcinogens. There is the need of NPYR metabolic activation to observe the mutagenic and carcinogenic effects, and the critical step is the formation of alpha-hydroxy-NPYR catalyzed by cytochrome P450. In this stage of the work, six adducts were quantified in DNA of rats kidney treated with NPYR at doses 46, 92 and 184 mg/kg of body weight. The rats were sacrificed 16 h after treatment by gavage. Among the DNA adducts, there are three resulting from the DNA reaction with intermediaries of the tetrahydrofuran type, two resulting from DNA reaction with crotonaldehyde, which is a metabolic of NPYR, and one majority adduct involving positions 7 and 8 of guanine, resulting from the reaction of a diazonium intermediary with DNA (Loureiro et al., 2009).

The developed work was presented in the "100th Annual Meeting of the American Association for Cancer Research", carried out in the period from 18 to 29/04/2009 in Denver, CO, USA, and resulted in the article "Mass spectrometric analysis of a cyclic 7,8-butanoguanine adduct of N-nitrosopyrrolidine: comparison to other N-nitrosopyrolidine adducts in rat hepatic DNA", which was published in the periodical "Chemical Research in Toxicology".

So far, data about in vitro cytotoxicity and genotoxicity of solutions contained in light sticks and of a dinitrobromophenol color marker very utilized industrially were obtained. The obtained results were presented in national congresses, and were awarded two prizes. A work in collaboration with Prof. Stephen Hecht of Minnesota University, MN, EUA, allowed the

standardization of the sensitive and selective method for the quantification of DNA adducts endogenously generated through the action of nitrosamine N-nitrosopyrolidine. Some of these adducts are also generated through the reaction of DNA with tetraidrofurane. The work was published in the periodical Chemical Research in Toxicology. Since this nitrosamine can be generated in inflammatory processes, we started a work in which such adducts are quantified in DNA of leukocytes of sugarcane cutting workers, together with the 8-oxodGuo lesion. The project was submitted for analysis by the Ethics Committee of the Clinical Directorate of the Clinics Hospital and of the Medical School of São Paulo University. Afterwards, the opinion report will be forwarded for communication of the Ethics Committee in Research of São Paulo University Pharmaceutical Sciences Faculty. A request for a scholarship for the Scientific Initiation student who will take part in the project was made to FAPESP.

CI Disperse Blue 291

The 2-[(2-bromo-4,6-dinitrophenyl)aze]-5-(dietylamine)-4-metoxiacetanilid colorant is the main component of the commercial compound CI Disperse Blue 291 (DB291), utilized as disperse colorant in fabric dyeing factories together with other colorantes of the dinitrophenil class (Figure 2). Such colorants have in common the 2-[(2-bromo-4,6-dinitrophenyl)aze]-4-metoxiacetanilid portion in their structures, they are mutagenic in tests with S. typhimurium and, during the dyeing process and treatment of effluents, they can be converted into more mutagenicm molecules (containing the phenilbenzotriazole portion, PBTA), which were detected in various rivers that pass through textile industries regions in Japan (Figure 3). Colorants of such class are utilized by textile industries in Brazil, and some studies show that the effluents containing them were responsible for mutagenic activity detected in 2007 in the Ribeirão dos Cristais, which is located in the metropolitan region of São Paulo and is used for water supply.

The generated results, which are still being analyzed, will allow us to understand the mechanisms through which the DB291 colorant induces cellular damages. The understanding of such mechanisms might generate new parameters for the evaluation of toxicity related to the exposure of such class of compounds, which can be useful both for the development of in vitro tests for the monitoring of toxicity of drinking water, as well as for the monitoring of premature cellular alterations in exposed populations.

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Light sticks

Chemiluminescent sticks, called “light sticks”, are utilized by fishing companies and discarded in the beaches. Local residents use their contents as repellents, tanning lotion and medicine for joint pain. We are investigating the reactivity of light sticks solutions, both new ones as well as those collected in Brazilian beaches, and their cellular toxicity. Products from the reaction of light sticks solutions collected in beaches with 2`-deoxyguanosine (dGuo) were analyzed by HPLC-UV-ESI-MS/MS. An adduct with m/z 408 [M+H]+ (addition of 140 Da to dGuo) was purified and characterized through mass spectrometry (MS/MS). The obtained data show an important genotoxic and cytotoxic action of light stick solutions and serve as an alert for public policies in the sense of better regulating their use.

Data obtained so far allow us to conclude that:

1. Light stick solutions are constituted of highly toxic substances for HepG2 cells.
2. Among the solutions compartmented in a light stick tube, the one with the highest toxicity is composed of oxalate, HPA and ftalate, which in the intact tube is separated from the solution containing H202, salicylate and ftalate.
3. Solutions coming from tubes collected at beaches have, in addition to the main components, others that are generated by the reactions occurring as time passes in the presence of sunlight.
4. The components of solutions of light sticks collected at beaches induce the formation of 8-oxodGuo in DNA of HepG2 cells and are highly reactive, causing modifications of biomolecules in vitro.
5. With the structural characterization of a pair of adducts formed from the reaction of dGuo with solution of light stick collected at the beach, we verified that one of the reactive components of such solution is derived from the therein TCPO chlorate oxalate ester.
6. The high cytotoxicity and reactivity of light stick solutions verified in this study shall serve as an alert for information of the target public about these utensils as for the risk to health when such solutions are inadequately used. For having highly toxic substances, such type of waste shall be adequately disposed of.

Data obtained in this stage of the work were presented as a poster in scientific meetings. Part of the results was also divulged as an article in the Ciências Hoje magazine.

9. INSTITUTIONAL COUNTERPARTS

São Paulo University provided a position of administrative assistant, in February 2010, for INCT INAIRA, and Ms. Caroline Fragata was approved in a competitive examination. In the next months, a Call for Bid will be opened for a position of Assistant Professor in the discipline of Environmental Pathology, at USP Department of Pathology.

Mato Grosso state health secretariat offered R$ 3,120.00 to cover the costs of travels and daily allowances for the biomonitoring activities. Mirassol d´Oeste municipal health secretariat provided a location, and human and audio-visual resources that will allow the realization of meetings and presentation of data obtained in the local biomonitoring activities.

In addition, all facilities and equipments of participant institutions declared in the counterpart of the proposal presentation were utilized for the development of the projects at issue.

10. PROJECT OF VIDEO ABOUT INAIRA

According to CNPq request, an educational video comprehending aspects of the researches developed by INAIRA (National Institute of Integrated Environmental Risk Analysis) in the fields of atmospheric pollution, sugarcane and health, will be developed in this project. For the development of the work, the theoretical references of researches of prof. Paulo Saldiva, prof. Alfésio Braga and prof. Ubiratan de Paula Santos will be used.

The main topic to be approached by the video is the cost of choices, both those done by the public administration as well as the more personal and subjective ones, always prioritizing public health and defending the importance of taking this aspect into consideration when making decisions.

Some topics that support the importance of health in the decision-making process will be listed, justifying this point of view with examples of situations showing strong contradictions in the relation between health and public decisions.

The video will start with a brief explanation about the theme to be approached, then it will go straight to the point, because it will not be a long video, through interviews with doctors and

researchers, and also with workers and persons exposed, on a routine basis, to the types of pollution we are talking about.

Contents are not going to be deep. Reasons that support the defended idea will be exposed and illustrated with interviews and situations with people, in an attempt to defend the idea and show to the viewer in a simple and objective way the contents, allowing a good understanding of the theme.

The video will be based on interviews done with researchers and sugarcane cutting workers, and the work will be put together with the information obtained. It is interesting that the interview with the sugarcane cutting worker will be done in the sugarcane plantation, so through the images of the place we can also show the choices done there. The Telemedicine studio will also be utilized for the interviews with researchers, to allow better quality images and audio. The objective of this video production is to make it reach a non-academic public, and thus become a channel of science communication, informing part of what is being researched and also raising the viewer awareness about the theme at issue.

11. PERSPECTIVES AND FUTURE DEVELOPMENTS

INAIRA has as main goal to be a research platform relating environmental alterations with their effects in human health. We expect that this type of evaluation might contribute for the understanding of the human biology, about the pathogeny of diseases (especially the chronic-degenerative ones) and establishes a scientific knowledge about the topic of sustainability, having the quality of life as a point of reference. Within this scenario, we expect that the scientific knowledge produced by INAIRA researchers is translated to be fully divulgated for the Society, as "white papers", journalistic texts and environmental education programs, expanding the environmental debate, in order to converge, in the same conceptual platform, the themes of environment, health and citizenship.

In the specific aspects of science, INAIRA expects to be competitive in the field of how the exposure to pollutants during gestation or in the early post-natal phase modifies the risk of contracting diseases in the adult phase. Epigenetic alterations and their vertical transmission are, as we undertand, the most promising ways of research in our network. We will strengthen transgenerational studies in animals and the cohorts in human beings to deepen our knowledge

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about such issues.

Future climatological scenarios will influence the profile of our researches. The progressive warming of the city, influencing the kinetics of photochemical processes of troposphere, specially represents a point of high interest, and a scenario with high concentration of carbonyls, as occurring in our urban centers. Besides being relevant from the point of view of sciences, this type of knowledge might help formulating urban mobility, and vehicular and fuels technology policies, based on scientific evidences.

In summary, INAIRA aims to constitute the embryo of a national health and environmental center. To achieve this goal, in addition to good research, it is necessary to utilize the results of our works as a way to convince the Society about the importance of the theme. This implies the development of efficient scientific divulgation and environmental education measures, the elaboration of partnerships with various sectors of the government and of the civil society, and learning ways of congregating researchers from different fields of knowledge.

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ANNEX I – REFERENCED LIST OF PUBLISHED ARTICLES

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