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Studio della componente organica ed inorganica del particolato atmosferico

SETTORE SCIENTIFICO DISCIPLINARE DI AFFERENZA: CHIM/12, Chimica dell'ambiente

Tesi di Dottorato di Angelika Hofer, matricola 955836

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FOREWORD

In the three years of my PhD-thesis, I gained experience in sampling airborne particulate matter and chemically analysing its pollutant content. This work in its part dealing with the quantification of the organic and inorganic fraction of the atmospheric particulate matter has a general introduction where the topics are treated in general, the other chapters are divided in two parts:

Part 1: Sampling with the filter system

The first part reports on the sampling with the traditional method: the particulate is deposited on filters kept in an automatic sequential sampler. On these samples, only the organic fraction (PAH, TC – EC – OC, levoglucosan and its isomers) constituting the atmospheric particulate matter have been analyzed.

Part 2: Biomonitoring

The second part discusses the use of *Tillandsia aeranthos* (Loiseleur) L.B. Smith plants as biomonitors for organic and inorganic constituents of the ambient particulate matter. It includes a one – year sampling campaign, where plants were exposed to different outside emission scenarios (urban -, industrial-, semi-harbor area) but also to the indoor ambient air (orchid greenhouse of the Botanical Garden of Padua, and two working places: a public office and a garage). The analyses of PAH and metals in plants were carried out and the results interpreted.

The obtained data were presented at different conferences:

- The part of PAH on filter samples was presented in a poster titled "*Impacts of $PM_{2.5}$-bound Polycyclic Aromatic Hydrocarbons on Common Air Toxicity during Wintertime in Venice – Mestre (Italy)*" on the 13th EuCheMS International Conference on Chemistry and the Environment ICCE 2011. Further, they contributed to the data two articles are based on:

    - Masiol M., Hofer A., Squizzato S., Piazza R., Rampazzo G., Pavoni B. (2012): *Carcinogenic and mutagenic risk associated to airborne particle-phase polycyclic aromatic hydrocarbons: A source apportionment.* Atmospheric Environment, 60: 375-382, ISSN 1352-2310, 10.1016/j.atmosenv.2012.06.073

    - Masiol M., Centanni E., Squizzato S., Hofer A., Pecorari E., Rampazzo G., Pavoni B. (2012): *GC-MS analyses and chemometric processing to discriminate the local and long-distance sources of PAHs associated to atmospheric $PM_{2.5}$.* Environmental Science and Pollution Research, 19 (8): 3142-3151; Issue "*International Conference on Chemistry and the Environment in Zurich – ICCE 2011".*

- The data of the carbonaceous fraction were presented during the poster session of the conference *XIII Congresso Nazionale di Chimica dell'Ambiente e dei Beni Culturali* (Taranto, Italy) with the poster titled "*La determinazione del carbonio in atmosfera – confronto di due metodi".*

- Data of *T. aeranthos* were presented partially on the 14th EuCheMS International Conference on Chemistry and the Environment ICCE 2013 with the poster "Monitoring the air quality in Venice – Mestre with *Tillandsia aeranthos".*

Acknowledgements

I would like to thank Prof. B. Pavoni for giving me the chance to complete my studies at University with the PhD – degree and that he let me carrying out the research on air pollution with *T. aeranthos*.

In addition, I thank the great team of ARPAV – collaborators Gian Maria, Paulo, Franco, Alberto, Enzo e Luca for their help and sustainment with the loan of their instruments for data analyses, the providing of meteorological data and the coffees drunk together.

Further, I am grateful to the collaborators of the Botanical Garden of Padua, Roberto and Nicola, for taking care of *T. aeranthos* plants exposed in their orchid greenhouse and to Prof. Buffa for making this possible.

For sending me the meteorological data requested monthly for the industrial area (Malcontenta), I thank Egisto Rampado of EZI.

Furthermore, I thank Mauro for his help with discovering the application of the different tools of STATISTICA, Khan for helping me with the meshing of the plants, and Francesca and Alice for filling the lab with laughs. For his help with the analyses of the lipid content in *T. aeranthos* I would like to thank Andrea. I would like to thank also Nic for his patience and help with the bureaucracy during my studies.

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Introduction

Air pollution became one of the most discussed topics over the last sixty years, following the "Great Smog" event occurred in London in December 1952 (Brunekreef, 2008). Air pollution is defined by the World Health Organisation (WHO, 2013) as the "contamination of the indoor and outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere".

Contaminating agents are generated by the emission of particles of different dimensions and shapes from anthropogenic as well as from natural sources on earth and in the space. Singularly emitted particles alone would not cause so many problems, but as it lays in the nature of elements and particles to accumulate in the atmosphere, they influence climatic conditions, visibility and radiative transfer (Seinfeld and Pandis, 2006), and human's health all over the world (Brunekreef, 2008).

The removing of these particles depends on different weather scenarios. As not every time perfect weather conditions are prevailing, many strategies have been developed to decrease the emissions of them in order to reach the established limits imposed by authorities on an international (WHO, 2005), European (EC, 2008) and national (DLgs 155/10) scale. To detect if those measures have been successful, the air quality needs to be monitored continuously.

The used monitoring methods generally vary from physical and chemical measurements with active and passive sampling stations, on line or laboratory based methods, different supports to catch different kinds of particles, up to the biological ones.

In general, sampling methods include campaigns where filters (quartz- or glass fibre or polyurethane filters (PUFs)) are exposed to the ambient air under controlled conditions. Those filters are further analysed in order to detect the concentrations of inorganic (metals, ions) and organic compounds observed in the air: Polycyclic aromatic hydrocarbons (PAH), their

oxygenated (oxy-PAH) or nitrated (n-PAH) forms (Durant et al., 1998), total, elemental and organic carbon (TC, EC, OC, respectively) and levoglucosan (Seinfeld and Pandis, 2006). Another type of sampling air particles is the biomonitoring, which uses different biota as biomonitors or bioindicators for different pollutants such as heavy metals (Wannaz et al., 2012) and PAH (Skert et al., 2010).

1.1 Particulate matter (Aerosols)

All the substances in a microscopic or submicroscopic size larger than a molecule existing, under standard conditions (Standard Ambient Temperature and Pressure, *SATAP*, with T = 298.15 K, P = 100 kPa), as a solid in the atmosphere under normal conditions are defined as “particulate matter” (PM). The term “particulate matter” is often interchanged with the term “aerosol”, where an aerosol is defined as a “stable suspension of liquid or solid particles in a gas” (Colbeck and Lazaridis, 2010). Aerosols are divided in primary aerosols and secondary aerosol, depending on their origin. Primary aerosols are composed by directly emitted particles from the Earth’s surface, whereas the particles forming the secondary aerosol are generated in the atmosphere by gas-to-particle conversion processes (fig. 1). The particles’ size varies from the size of molecules (few nanometers (nm)) up to macroscopic dimensions (tens of micrometers (μm)) (Seinfeld and Pandis, 2006).

The primary aerosol with particles’ diameters greater than one micrometer (μm) derives mainly by mechanic actions from

a) natural sources:
   i. biomass burning and particles of herbal origin;
   ii. sea spray;
   iii. volcanic eruptions;
   iv. windblown dust;
   v. cosmos and atmosphere.

b) anthropogenic sources:
   i. burning of fossil fuels;
   ii. transportation;
   iii. industrial emissions;
   iv. incinerating plants;
   v. production and use of chemical substances;
   vi. mining industry (Colbeck and Lazaridis, 2010).

Secondary aerosols include particles with a diameter in the range between 0.1 and 1 µm, are produced by chemical transformations from gas precursors e.g. volatile organic compounds (VOCs) in the atmosphere.

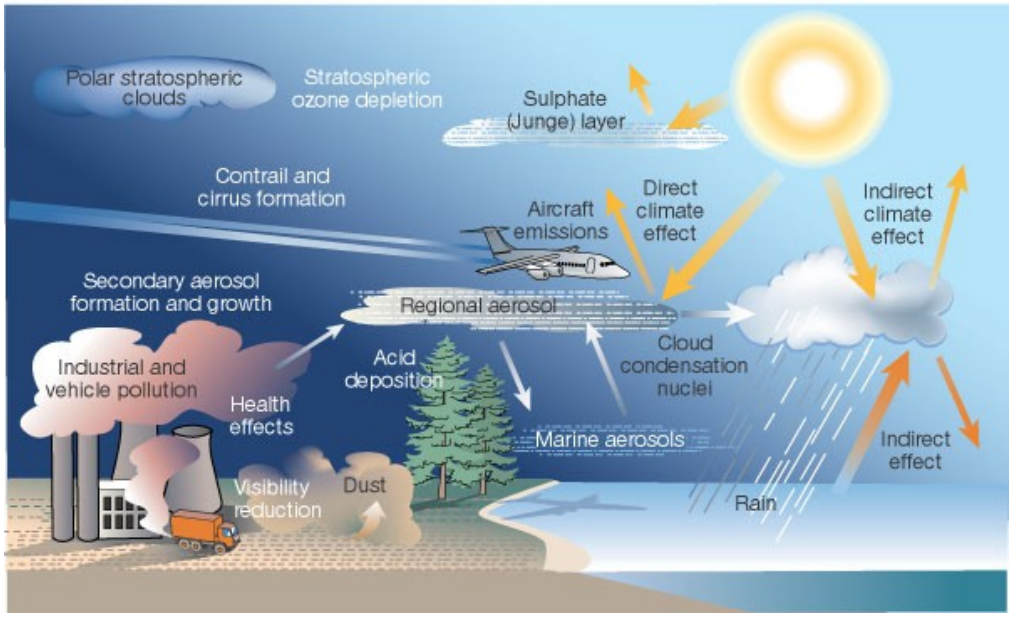


Fig. 1: Formation, removal and effects of aerosols (Kolb, 2002)

Because of the highly varying particle sources all over the world and the different climatic conditions prevailing in different places, the composition of aerosols changes within their geographic position. The atmospheric PM is composed by various organic and inorganic compounds in different amounts, depending not only from the emission sources, but also from the climatic conditions. Those compounds can be divided in major (> 1% of the total mass of PM) and minor (< 1% of the total mass of PM) components of the PM. As the major elements of urban areas are the same all over the world with variations only in their proportion, the term “bulk chemical composition” describes the relative abundance of them (Harrison and Yin, 2000).

As described by Seinfeld and Pandis (2006) and Harrison and Yin (2000), the major components are:

- Sulfate concentration in the air depends on regional emissions of SO_2 which is oxidised in the atmosphere;
- Nitrate is formed predominantly by the oxidation of NO_2;
- Ammonium is formed during the neutralisation process of sulfates and nitrates;
- Chloride derives directly from sea spray and from road de-icing salt during winter time, and in a second step from the atmospheric transformation of hydrochloridric acid vapour emitted by industrial sources;
- Carbon (elemental, organic, black) is mainly emitted by combustion processes of fossil fuel as soot;
- Crustal material is composed by soil dusts and windblown rock-derived minerals. It differs in composition and reflects this way the local geology and the surface conditions;
- Biological materials include bacteria, spores, pollens, fragments of cellulosic plant material. The biological material has generally coarse size dimensions. Therefore, it has often been characterized as organic carbon.

The size distribution of aerosols is crucial for determining their dynamics (transport, deposition, residence time) in the atmosphere, and it is expressed as the “number of particles per size fraction” (Colbeck and Lazaridis, 2010).

Because of the variety of particles’ shapes and sizes, an equivalent factor was needed to link a numerical value to a particle property or behavior. Therefore, the equivalent aerodynamic diameter (D_{AE}) was established. It is defined as the “diameter of a particle with a density of 1000 $kg*m^{-3}$ (water) that has the same settling velocity as the particle in the question” (Szymanski, 2011). D_{AE} permits the distinction and classification of the particulate in different categories (Englert, 2004):

- D_{AE} > 10 µm: Total suspended particles (TSP);
- D_{AE} ≤ 10 µm: Coarse Particles or PM_{10}: inhalable fraction, reaches larynges;
- D_{AE} ≤ 2.5 µm: Fine Particles or $PM_{2,5}$: reaches bronchia;
- D_{AE} ≤ 1 µm: PM_1: reaches the terminal bronchia (Molinaroli and Masiol, 2006);
- D_{AE} ≤ 0.1 µm: Ultrafine Particles (UFP) or $PM_{0.1}$: reaches alveoli (Englert, 2004).

The atmospheric aerosol is polydisperse and shows mainly four distinct modes:

1. nucleation mode: particles have a diameter <10 nm and they are formed by nucleation processes;
2. Aitken mode: Particles (10 nm < d <100 nm) are originated by condensation and coagulation of high temperature combustion products (e.g. industrial emissions). The nucleation is homogeneous.
3. accumulation mode: Heterogeneous particles in the accumulation mode have dimensions between 0,1-1 µm and derive from
   a) coagulation and condensation of more nuclei of the Aitken mode in order to form aggregates;
   b) coagulation of drops formed by condensation of vapours at low volatility.
4. coarse mode: Particles produced by natural mechanical processes (Seinfeld and Pandis, 2006; Molinaroli and Masiol, 2006).

Modes from the nucleation mode up to the accumulation mode include similar particles: the major part is constituted by organic compounds, whereas soot, toxic heavy metals, transition metals, PAH, viruses are present in minor concentrations. Particles of crustal, urban or oceanic origin take part of the coarse mode. Throughout coagulation, the particles in the Aitken Nuclei Range (D_{ae} ≤0.1 µm, UFP) become particles with a D_{ae} between 0.1 µm and 2.5 µm (PM 2.5) (Molinaroli and Masiol, 2006).

Particles in each mode have different atmospheric residence times, varying with the concentration and the composition of the troposphere itself. The residence time of particulate matter in the troposphere varies from few days until few weeks, because the sedimentation velocity of particles with D_{AE} > 2.5 μm is higher than those of smaller particles.

There are two mechanisms how particles can be removed from the atmosphere: or by dry deposition (gravity – driven), or by wet deposition (rain, snow or fog). This way, they reach other environmental compartments such as the biosphere, the geosphere and the hydrosphere (Kolb, 2002; Ravindra et al., 2003).

During the dry deposition, the sedimentation velocity of particles with a D_{AE} > 10 μm is given by the Stokes' law

$$v_S = \frac{d_P^2 \rho_P g}{18\eta} \qquad \text{eq (1)}$$

where

v_s = sedimentation velocity;

d_p = particle diameter;

ρ_p = particle density;

g = gravitational acceleration;

η = air viscosity.

During the wet deposition, particles with a D_{AE} ranging between 0.1 and 1 μm are bound in water droplets as they act or as cloud condensation nuclei (CCN) forming this way clouds ("rainout"), or they aggregate with other particles of aerosol in droplets of already existing clouds ("washout"). Another process of wet deposition is the so-called "sweep out", where particles reside under the clouds are brought to the earth surface by collision with the water droplets. Further, particles deriving from natural sources are removed more easily by wet deposition rather than those from an anthropogenic source (Molinaroli and Masiol, 2006, and references therein).

1.1.1 Effects of aerosols

The particles' concentrations in the aerosols depend on the chemical and meteorological conditions in the atmosphere such as wind direction and velocity, temperature, relative humidity, precipitations, altitude and the presence of clouds. A decrease in the vertical mixing of the air caused by inversion phenomena lead to an increase of the amount of particulate matter in the lowest layer of the atmosphere. Therefore, aerosols interact both directly and indirectly with the Earth's radiation budget and climate (IPCC, 2007). Directly it results in the back – and side scattering of the incoming sunlight, influencing so the planetary albedo, as well as in the reduction of visibility (Seinfeld and Pandis, 2006). Indirect effects are given by an increase of the number of CCN available for the cloud formation and the effects on the height, life time and water content of clouds, as well as their chemical composition. The consequence can be acid rain, which causes problems to monuments and fronts of buildings (Huang et al., 2009; Khan et al., 2010), (Kolb, 2002), and adverse health effects to humans and all the other organisms living in the affected environment.

1.1.1.1. Effects of aerosols on humans health

Many studies (e.g. Harrison and Yin, 2000; Pope et al., 2002; Simkhovich et al., 2008; Brunekreef et al., 2009) have demonstrated, that elevated ambient PM levels lead to an increased risk for human's health in terms of acute or chronic morbidity and, finally, to mortality (Franklin et al., 2009; Pope et al., 2009). The key parameter for respiratory deposition is the D_{AE} of the different particles (fig. 2).

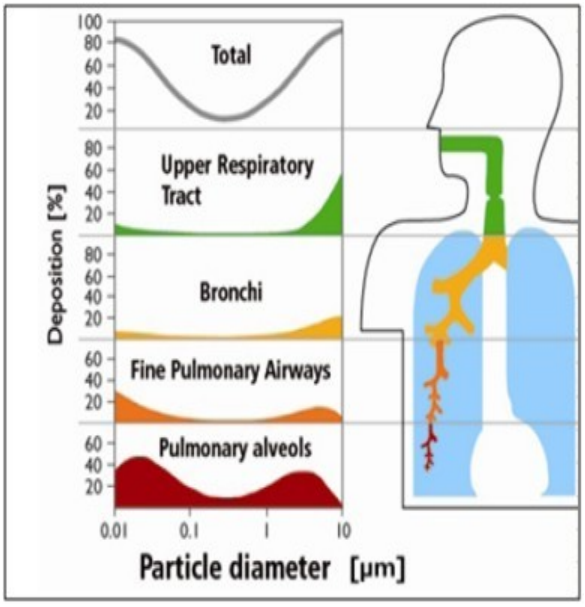


Fig. 2: Respiratory deposition of particles with different diameters. (ICAO, 2005)

When particulate matter enters the respiratory system, it can cause respiratory and cardiovascular diseases, changes in the lung function, lung tissues and structure (ICAO, 2013), coagulation of the blood and arteriosclerosis (Simkhovich et al., 2008). Asthma, pneumonia in older people or even the death of a newborn (Woodruff et al., 2008) as well as incident type 2 diabetes (Krämer et al., 2009) can be the result of densely trafficked areas.

The factors influencing the toxicity of airborne PM are the bulk chemical composition, the trace element content, the strong acid content, the sulphate content and the particle size distribution (Harrison and Yin, 2000). The finer the particles of PM, the deeper regions of the respiratory system they can reach. As those regions do not present any protective mucous membrane, the fine particles can remain in the lungs for longer periods (Amodio et al., 2009;) and some of its compounds such as carcinogenic PAH can influence even the DNA of the host and produce next to respiratory diseases also lung cancer (Pope et al., 2002). However, the human body shows a double defence mechanism where i) the nasal and bronchial ciliate impede the particles to enter; ii) in areas without ciliate macrophages absorb the particles and bring them to the ciliate region (Marconi, 2003).

The risk associated to the PM to produce adverse health effects is connected to the chemical composition, the concentration and the dimension of the constituents of the PM. Those characteristics are influenced by climatic conditions, season and geographical position of the area (Brunekreef, 2008).

1.1.2 Components of PM

The variety of compounds constituting the PM is mainly composed by organic and inorganic particles. Those considered in this work are the following:

1) organic: a) PAH

1. Oxy- and Nitro - PAH

b) EC/OC

c) Levoglucosan

2) inorganic: a) metals

1.1.2.1 Organic compounds

1.1.2.1.a) Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAH) are semi-volatile organic compounds formed by condensed benzene rings which are predominantly composed by carbon and hydrogen atoms. PAH are originated primarily from the incomplete natural or anthropogenic combustion of organic material, or from high-pressure processes. Therefore, they are ubiquitous in the environment (ATSDR, 2009). The different arrangement and the variation in the number of benzene rings forming the single PAH leads towards the formation of thousands of different PAH with differences in their properties and effects on the environment and the organisms living in it. On the basis of their abundance and toxicity, the International Agency for Research on Cancer (IARC) included some of them in their monographs (IARC, 2012). Once emitted to the atmosphere, the weight of a PAH influences the fate of it. The compounds composed by two or three benzoic rings show a low hygroscopicity and a low vapour pressure. Therefore they remain in the gaseous phase (ATSDR, 2009). Because of their high vapour pressure, PAH composed by four or more benzene rings tend to condense or being absorbed on the particulate matter. Some compounds like Pyrene or Benzo(a)Anthracene, can be found in the intermediate gas-particle phase (Seinfeld and Pandis, 2006).

As described by Ravindra et al. (2008), PAH can be formed by

- pyrolysis and pyrosynthesis of organic matter at high temperatures: in conditions of an oxygen deficit and when temperature exceeds 500°C, carbon-carbon and carbon-hydrogen bonds break and free radicals are formed. After a de-hydration, those radicals form rings resistant to thermal degradation, which are called PAH;
- diagenesis of organic matter at low or moderate temperature;
- direct biosynthesis from microbes and plants.

Therefore, the main emission sources of PAH into the atmosphere are:

- **domestic heating**: The low combustion temperatures of petroleum, carbon, gas, wooden materials in the households increases the amount of PAH in the indoor and outdoor air.
- **vehicular traffic**: In urban areas, PAH deriving from exhaust gases vehicles contribute in a significant way to the total amount of PAH in the air.
- **industrial emissions** from the production of aluminium and carbon coke, pesticides and plastics, waste incinerators, oil refineries, power plants.
- **agricolture**: the combustion of organic waste can produce a similar amount of PAH like an urban area.
- **natural events** such as volcanic eruptions and forest fires.

In the atmosphere, the distance of the transport of one single PAH decreases when the molecular weight increases. Therefore, gaseous PAH are suspended for a longer time in the atmosphere. This leads to a wider distribution in space of gaseous PAH compared to those in the particle phase. Each PAH compound reacts in a different way with atmospheric oxidants such as ozone, NO_3, or •OH radicals which favours the decay of PAH and the formation of oxygenated and nitrated PAH (Walgraeve et al., 2010), discussed briefly in chapter 1.1.2.1.a)1. Mainly gaseous PAH with less than four benzene rings are oxidized in the atmosphere at ambient conditions. The low temperatures during winter time favour the condensation and/or absorption of PAH on particles suspended in the air (Ravindra et al., 2006).

Out of the 18 analyzed PAH within this work and displayed in fig. 3,

- PAH with 2-3 benzene rings (Naph, Acen, Ace and Flu) are in the gaseous phase (blue);
- PAH with 4 benzene rings (Phe, Anth, Fluor and Pyr) are in the gas-particle phase (grey);
- PAH with more than 5 benzene rings B(a)A, Chry, B(b)F, B(k)F, B(e)P, B(a)P, Per, IP, D(a,h)A and B(ghi)P are in the particle phase (white) (Kalberer et al., 2004).

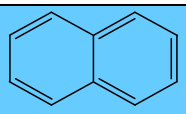
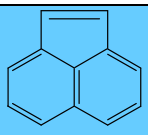
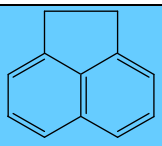
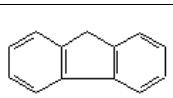
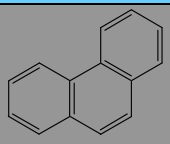
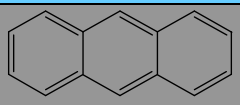
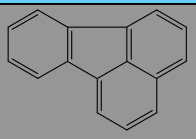
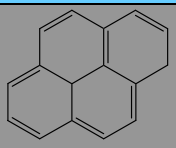
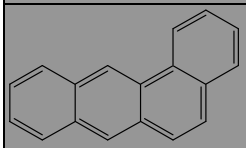
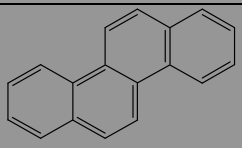
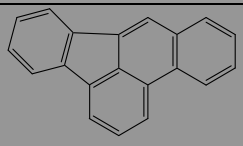
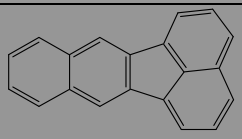
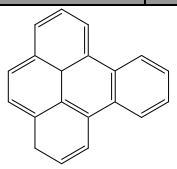
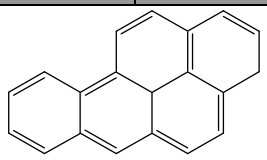
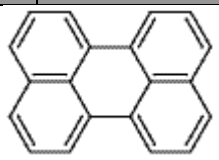
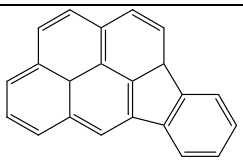
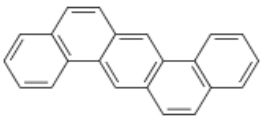
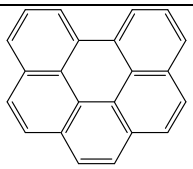
 **Naphtalene –** Naph 2B	 **Acenaphtylene –** Acen ---	 **Acenaphtene –** Ace 3	 **Fluorene –** Flu 3
 **Phenantrene –** Phe 3	 **Anthracene –** Anth 3	 **Fluoranthene –** Fluor 3	 **Pyrene –** Pyr 3
 **Benz(a)antracene –** B(a)A 2B	 **Chrysene –** Chry 2B	 **Benzo(b)fluoranthene-** B(b)F 2B	 **Benzo(k)fluoranthene** - B(k)F 2B
 **Benzo(e)pyrene –** B(e)P 3	 **Benzo(a)pyrene –** B(a)P 1	 **Perylene –** Per 3
 **Indeno(1,2,3-cd)pyrene –** IP 2B	 **Dibenzo(a,h)anthracene -** Db(ah)A 2A	 **Benzo(ghi)perylene –** B(ghi)P 3

Fig. 3: PAH congeners: their structure, name, abbreviation used within the text, and the IARC toxicity class they belong to.

Generally, PAH tend to accumulate in the fine PM with diameters less than 2.5 μm, which can penetrate until the pulmonary alveoli of the respiratory system. Therefore, high amounts of fine particles, in which are included class 1 PAH, found in the air of urban areas can cause many diseases chiefly in children and older people as well as in people with an advanced pathology (Halonen et al., 2008).

The carcinogenicity of PAH is generated by their capacity to interact with the DNA strands by producing errors during the replication and mutations of the DNA, initiating this way the tumour building (Park et al., 2006; Billet et al., 2008; Abbas et al., 2009). The property enhancing the danger of PAH is their high lipophilicity which allows a fast absorption into organism's tissues (Ball and Truskewycz, 2013).

The effect of the analyzed PAH on humans' health vary from class 3 ("Not classifiable as to its carcinogenicity to humans") up to 2B ("probably carcinogenic") and class 2A ("possible carcinogenic") until class 1 ("carcinogenic" for humans) (IARC, 2012), as shown in fig. 3.

Benzo(a)Pyrene is the only class 1 PAH included in the priority pollutant list elaborated by the US-EPA. It derives from the incomplete combustion of fossil fuels, of organic matter, of wood, as well as from asphalt roofing manufacturing and meat char broilers (US-EPA, 2011).

As described by Fagnani, 2010, B(a)P is included in the carcinogenic pollutants, because the formation of the Bay Region and the oxidation of B(a)P (fig. 4) transform the compound in the highly biochemically reactive (+)-anti-BaPDE ((+)-anti-BaP-7,8-diol-9,10-epoxide). The (+)-anti-BaPDE interact with the DNA by producing a DNA adduct which gives the input to the development of carcinogenesis (fig. 4).

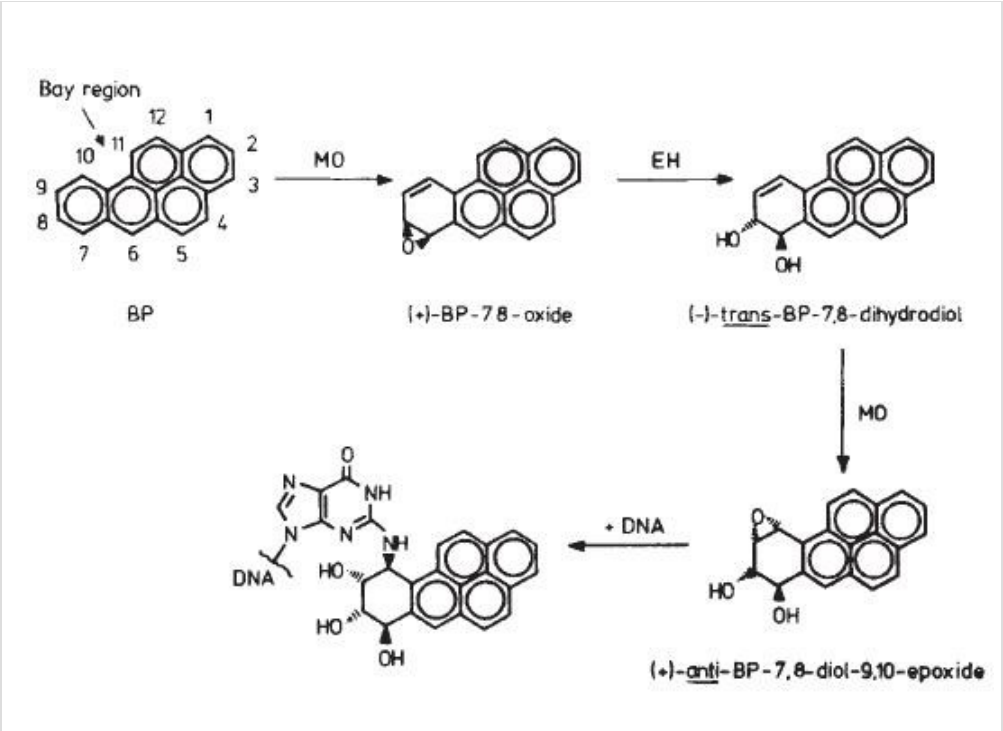


Fig. 4: Metabolic activation of B(a)P, catalysed by monooxygenase (MO) and epoxide hydrolase (EH) to form the most active tumor building isomer (+)-anti-BaPDE which interacts with the DNA strand (Fagnani, 2010 and references therein).

The risk for humans' health is based on the concentration of PAH, and mainly of B(a)P in the air. Therefore, in order to evaluate the exposure risk of a person, the Toxic Equivalency Factor (TEF) (Nisbet and LaGoy, 1992) and the Mutagenic Equivalency Factor (MEF) (Durant et al., 1996) have been calculated for the eight PAH taking part of the IARC danger classes 1 and 2 (B(a)P, Chry, B(a)A, B(b)F, B(k)F, IP, D(a,h)A, B(ghi)P), normalized to the value of B(a)P which is 1 (Table 1).

Table 1: TEF and MEF of the eight class 1 and class 2 PAH.

Congener	B(a)A	Chry	B(b)F	B(k)F	B(a)P	IP	Db(a,h)A	B(ghi)P
TEF	0.1	0.01	0.1	0.1	1	0.1	1	0.01
MEF	0.082	0.017	0.25	0.11	1	0.31	0.29	0.19

The TEF and MEF are used to calculate the Toxic Equivalent Quotient (TEQ, in ng/m³) and the Mutagenic Equivalent Quotient (MEQ, in ng/m³),

respectively (Durant et al., 1996; Petry et al., 1996; Jung et al., 2010). The TEQ and MEQ represent respectively the equivalent carcinogenicity and mutagenicity in regard to B(a)P of a PAH-mixture of the eight above cited PAH.

1.1.2.1.a-1. Oxy- and Nitro - PAH

Oxygenated and nitrated PAH, important components of the secondary aerosol are formed predominantly from anthropogenically originated PAH undergoing photochemical reactions in the atmosphere. As they show one or more carbonylic oxygens or NO_2/NO_3^- (respectively) attached to their general aromatic ring structure, oxy – and nitro - PAH are considered among the key components of the toxicity of the PM present in the air. Even if those compounds have less weight in the total amount of compounds present in the PM than the PAH they are generated from them and they show a greater reactivity when inhaled by humans. Therefore, studies on their carcinogenic and mutagenic effect act on the assumption that those compounds are more harmful to humans than their parent PAH because they don't need any enzymatic activation (Stewart et al., 2010; Walgraeve et al., 2010).

Within this work it was tried with a GC/MS method described by del Rosario Sienra (2006) to detect and to quantify the following oxy – and nitro – PAH: 11H-Benzo(a)fluoren-11-one, Benzanthone, Benz(a)antracene-7,12-dione, 6H-benzo[c,d]pyren-6-one, 9,10-Phenanthroquinone, benzo[b]fluoren-11-one, 5,12-Naphtacenedione, Benzo(a)pyrene-7,8-dione, Benzo(k)fluoranthene-8,11-dione. As only the detection but not a reliable and repeatable quantification of the compounds was possible with the method we used, these compounds will not be further discussed within this work.

1.1.2.1.b) The carbonaceous fraction

The carbonaceous fraction, or total carbon (TC), of the atmospheric particulate matter represents one of the main components of the total mass of suspended fine particles. It is composed of organic carbon (OC) at 70 – 80%, elemental carbon (EC) (also called black carbon (BC)) and inorganic carbon (IC) at 5% (Seinfeld and Pandis, 2006).

The atmospheric carbonaceous fraction can have a primary, natural and anthropogenic, origin (OC, EC and IC) or a secondary one (only OC), when emitted volatile particles condense. Its strong light absorbing properties causes a reduction of visibility and positive radiative forcing which enhances this way the global warming (IPCC, 2007; Ramanathan and Carmichael, 2008). During the last century, an increase in biomass burning and fossil fuel combustion has increased also the input of carbon in the environment (Koelmans et al., 2006).

The total carbonaceous fraction constitutes 18 – 42% of the European $PM_{2.5}$ (Puteaud et al., 2010) and can produce, next to the negative factors on Earth's climate, also adverse human health effects (Maynard and Maynard, 2002; Pope et al., 2002; Barraza-Villarreal et al., 2011).

Black carbon is listed by IARC (2012) as a group 2B carcinogen (possibly carcinogenic to humans), because no genotoxic effect of it could be found, but in the diesel exhaust it normally derives from, it can act as a carrier for PAH and other carcinogenic compounds (Don Porto Carero et al., 2001).

The concentration and composition of the carbonaceous fraction vary from region to region, depending on local emissions as well as on climatic conditions (Heald et al., 2008). Urban and biogenic emissions are the main sources of the organic part of the carbonaceous fraction. In rainy regions, the lifetime of EC in the air is about 40 hours, while in dry regions it can stay longer than 1 week in the air (Ogren and Charlson, 1983). As demonstrated by De Gouw et al. (2005), the amount of OC, instead, decreases by about 40% in 48 hours. This implicates that its atmospheric lifetime is in the order of ca. six days which allows that compound to be transported over long distances and finally reach background areas (Squizzato et al., 2012).

Therefore, the European Directive 2008/50/EC (EC, 2008) suggests the analyses of atmospheric EC/OC in rural background locations.

The IC is mainly composed of carbonatic species with crustal origin, such as $CaMg(CO)_2$ or Na_2CO_3 (Seinfeld and Pandis, 2006).

1.1.2.1.c) Levoglucosan

Levoglucosan (1,6-anhydro-*β*-D-glucopyranose) is a monosaccaride anhydride with the chemical formula $C_6H_{10}O_5$ and the molecular structure as shown in fig. 5. This compound is formed by the combustion and pyrolysis of cellulose in combination with its two isomers, mannosan and galactosan. Therefore levoglucosan and its isomers are handled as markers for the combustion of wood (Shafizadeh, 1982; Simoneit et al., 1999; Simpson et al., 2004; Engling et al., 2006; Puxbaum et al., 2007; Křůmal et al., 2010; Mochida et al., 2010; Saarnio et al., 2010). The impact of levoglucosan on the environment has been studied starting from the 1980s (Locker, 1988).

As wood burning is used generally during wintertime for domestic heating while a low insolation is achieved, levoglucosan was demonstrated to be quite stable. The levoglucosan deriving from the outside wood burning during summertime may be depleted by the prevailing photochemistry (Piazzalunga et al., 2011 and references therein).

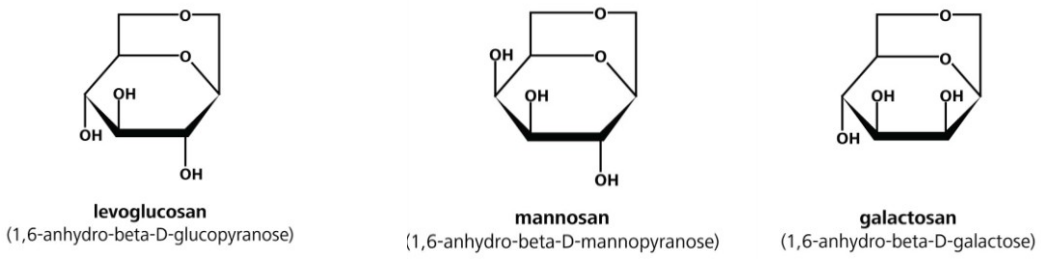


Fig. 5: Chemical structure of levoglucosan and its isomers mannosan and galactosan. (Rick et al., 2012)

Levoglucosan is classified as harmless to the environment but it can be detected in the some adverse health effects to humans (Kocbach Bølling et al., 2009), e.g. irritated mucosas (Riddervold et al., 2011).

1.1.2.2 Inorganic compounds: Metals

Atmospheric aerosols contain in addition to the organic compounds also metals (<1%). More than 40 of them can be found in the atmospheric particulate. Within these metals are found the transition metals (e.g. Mn, Cr, V, Fe, Co, Ni, Cu), which, being present in different oxidation states, participate in various redox reactions.

The origin of these metals is or anthropogenic (e.g. oil and coal combustion, wood burning, dust, waste incineration, brake wear) or natural (e.g. erosion, volcanic eruptions, wind). In table 2 are listed the elements' origins within the sampling area. Depending on their sources, trace elements can be found in either the fine PM or the coarse PM at concentrations varying over almost three orders of magnitude. Anthropogenic sources emitting metals include all the processes working and/or emitting particles at high temperatures such as biomass combustion, fossil fuel combustion, industrial activities and incineration. Those processes release the metals in a gaseous form which can form particles after condensation or gas-particle conversion (Seinfeld and Pandis, 2006).

Elements present in the fine mode have an atmospheric lifetime from some days up to some weeks and can travel over distances up to thousands of kilometers (Seinfeld and Pandis, 2006). Therefore, if those trace elements are found in the fine PM, back trajectories should give an answer if those elements derive from a local source or from a distant one.

The composition of metals in the ambient air can vary in relation to the different weather scenarios, the local sources emitting them, and also the long-range transport. In general, when metals such as Fe, Cu, Ni, Pb, and Sb are detected in the atmosphere, they derive mainly from vehicular sources (Harrison and Yin, 2010; Richard et al., 2011; Lawrence et al., 2013), while industries emit generally Zn, Pb, Mn and Ni (Richard et al., 2011), and S, K, Ca, Mn, Si, Mn, Zn and Cu are associated to wood burning processes (Bukowiecki et al., 2010).

The elements found in the Venetian area within the last ten years and the sources they are attributed to are listed in Table 2.

Table 2: Outline of metals associated to different sources in the Venetian area

Sea spray	Mineral/crustal dust	Road traffic	Engine wear and corrosion	Oil combustion	Biomass burning/Coal fly ash
Cl, Na, Mg, Ca*	Al, Si, K, Ca, Ti, Mn, Fe, Cu♦, Mg◘	Cr, Mn, Cu, Zn, Fe, K♦, Ni, Ca*, Co, Ba, P, Mg◘	Fe, Al, Ti, Mn, Ni, Co, Cu◘	V, Ni♦, Ca, Mg, Ba, Zn, P◘	K*, Ca, Mg, Fe, Al, U◘

* Masiol et al. (2012c); ♦ Masiol et al. (2010); ◘ Rampazzo et al. (2008)

Trace elements were analyzed during different sampling campaigns all over the world (e.g. Querol et al., 2007; Kolker et al., 2008; Rampazzo et al., 2008; Niu et al., 2010; Bermudez et al., 2012), because they take part of different chemical processes in the liquid phase of the aerosol like the oxidation or reduction of different compounds. This way, they influence the amount of free radicals present in the liquid phase (Seinfeld and Pandis, 2006).

Metals present in the atmosphere can influence not only the composition of the aerosol, but also life on Earth. In many biochemical reactions they act as co-factors, but they can become also toxic for cells. As the amount of metals in the environment has increased, eucariotic cells have developed proteins which are sensible to metals' toxicity by activating detoxification mechanisms of the cell. The problem is when the concentration of metals the organism is exposed to is too high. In this case, the cells and organs of the organism cannot detoxify the organism in an efficient way by causing an acute or chronic poisoning with different symptoms (Kováčik et al., 2012). Many metals cause diseases at the immunologic system and they can demonstrate also mutagenic or carcinogenic effects because of their ability to interact with the DNA (Hengstler et al., 2003).

2. *Monitoring air pollution*

The composition of the atmospheric air surrounding us every day has to be monitored in order to guarantee a good air quality to all the organisms living in the environment and, where the exposure limits fixed by the law are exceeded, to invent abatement strategies to reduce the amount of dangerous and harmful compounds in the air.

2.1 Active/passive monitoring

As described by Hayward et al. (2010), air pollution can be monitored in many different ways:

a) active sampling

Active air sampling can be conducted by sequential automatic samplers with a PM – inlet at a pre-established D_{AE} where the air is filtered throughout and the PM deposits on filters of glass fibre, quartz fibre, PUF, or others. In this case, the air flow is constant (at low (2.3 m^3 h^{-1}) or high (30 m^3 h^{-1}) volume, according to EN 14907 for and EN 12341 for $PM_{2.5}$ and PM_{10}, respectively) and filters are changed on a 24 hour basis. This permits the daily observation of more or less dangerous compounds for humans' health in the air. The analyses of those compounds are made in the laboratory.

b) passive sampling

Passive sampling is mainly carried out with biotic samplers such as pine needles, tree barks, lichens, mosses, leaves, grass, animal tissues (de Bruin, 1990) or some artificial substrate (Giordano et al., 2013) like Dacron® (Skert et al., 2010). They are exposed to the prevailing atmospheric conditions and they accumulate certain compounds available in the air. This sampling strategy has the advantage that it is less cost and time consuming and it does not require any further equipment like electricity. Therefore, it is an overall accepted sampling technique since John Evelyn published his book "Fumifugium" in 1661 (De Temmerman et al., 2005). The disadvantage of this sampling method is given by its non-reliability when short-term data are

acquired. Wolterbeek et al. (2010) define in-situ biomonitoring only as an option for retrospective studies of the air quality.

While the active sampling is the officially recognized method to sample airborne compounds (EC, 2008; DLgs 155/2010), monitoring the air quality by the use of biota is still a niche method even if many studies have shown that lichens are good bioindicators of airborne NO_x and SO_2 (ARPAV, 2009), Nicotina tabacum is a good O_3 indicator, whereas mosses mainly accumulate heavy metals (Castello, 2007). Nevertheless, all these plants react in different ways when they are exposed to polluted air. Therefore, the term *biomonitoring* has been established. In a general sense, biomonitoring is defined by IAEA (2000) as the "continuous observation of a defined geographic area with the help of living organisms reflecting spatial and temporal changes of atmosphere, lithosphere and/or hydrosphere". The relevant information is normally deduced from physiological changes of the organism under request or from the concentrations of pollutants in its tissues (Wolterbeek and Bode, 1995 and references therein).

2.2 Biomonitoring

Biomonitoring can be referred to plants as well as to humans. LaKind et al. (2008) define the term biomonitoring in the following way: "The direct measurement of people's exposure to toxic substances in the environment by measuring the substances or their metabolites in human specimens, such as blood or urine."

Biomonitoring is divided into active and passive:

- *active biomonitoring* includes all methods which insert organisms under controlled conditions into the site to be monitored,
- *passive biomonitoring* is the use of organisms or parts or associations of them which appear spontaneously in the ecosystem under survey (SDUDE, 2013).

When an organism responds to a certain level of pollution by changing its neutral behaviour or by accumulating the pollutant in its tissue and giving this way a quantitative result of the environmental quality surrounding it, than it is called a *biomonitor* (Blasco et al., 2008 with references therein).

Organisms or associations of organisms which can be used for the identification and the qualitative determination of environmental factors are named *bioindicators*, while the term *biomarker* is used for organisms where air pollution has caused measurable structural or functional changes at a genetic, enzymatic, physiologic or morphologic level. *Bioaccumulators* are all those organisms which accumulate one or more compounds from the environment (Bermudez and Pignata, 2011).

As described by De Temmerman et al. (2005), plants offers different abilities as air biomonitors in order to give some significant results in air monitoring:

- their integrated response to the polluted climate allows more realistic estimates of the potential risk to flora and also humans than filter analyses;

- the measurable reaction of plants to different air pollutants gives better results of the dose – response factor than dose – response models which can support the elaboration of directives on air quality;

- different organization levels of plants (from the plant cell until the whole ecosystem) can be used for biomonitoring;

- low concentrations of air pollutants can be hardly detected with active sampling methods, but as plants accumulate them, their analyses will be simplified;

- sensitive plants can suffer from the effects caused by certain air pollutants and get visible injuries, while less sensitive plants accumulate the pollutants. This way, direct air pollution effects can be established.

The above listed features can lead towards results such as mapping the air pollutions on a regional or wider scale based on a spatial and temporal distribution, toxic emission sources' identification, the detection of the adverse effects of air pollution on plant communities, ecosystem and the biodiversity in it. Further, in urban and industrial areas, bioindicators can draw public awareness to the air pollution, while bioaccumulators are good indicators of the transfer of toxic compounds to the food chain.

Biomonitoring is mainly carried out by using plants growing in situ, as there is no need to test the suitability of the plants with the surrounding environmental conditions such as seasonal variations in temperature and precipitations. Therefore, the firstly used biomonitors all over the world are lichens and mosses, e.g. Affum et al. (2008) used lichens as biomonitors in Ghana, Montero Alvarez et al. (2006) applied them in Havana City, while Bacci et al. (1986) collected them on the Antarctic Penisula and Giordano et al. (2013) studied the quality of lichens and mosses as bioaccumulators and –monitors in Italy. In South America other biomonitors are very common, namely species of the Bromeliad's subfamily Tillandsioideae. Many biomonitoring studies were carried out with *Tillandsia usneoides* (e.g. Isaac-Olivé et al., 2012) or *Tillandsia capillaris* Ruiz & Pavòn (Bermudez and Pignata, 2011), while the species *Tillandsia aeranthos* was used rarely (Vianna et al., 2011; Benzing et al., 1992), and when it was used it was only in its original environment. In the present study the species *T. aeranthos* has been used for an active biomonitoring survey in Venice-Mestre.

2.2.1 Tillandsia aeranthos

The plant *Tillandsia aëranthos* (Loiseleur) L.B. Smith (written in the further text as *T. aeranthos*) takes part in the family of the Bromeliaceae, subfamily Tillandsioideae, genus Tillandsia, subgenus *Tillandsia*. Until today, more than 500 species of *Tillandsia* have been detected (Luther, 2008). Species of *Tillandsia* are generally epiphytic plants and, therefore, they do not require any soil or other supporting material to get anchored and fed. The plants *T. aeranthos* absorb water and nutrients, and this way also toxic compounds, only from the atmosphere they are exposed to. In its original environment, ranging from South Brazil up to Paraguay, and from Uruguay up to the Northeast of Argentina, this plant is hosted mainly by the mature Tipuana tipu tree (better known as Rosewood) at geographic heights ranging from the sea level (Smith and Downs, 1977) up to hundreds of meters above the sea level (Rainforest Flora, 2013).

As these environmental conditions correspond to those prevailing in Venice-Mestre, and because of its good adaptation property, *T. aeranthos* has been chosen as biomonitor in this area.

Smith and Downs (1977) have discovered *T. aeranthos* during their studies in different regions of South America and described this plant as cited below:

> *Plant flowering 9–32 cm high; stem usually well developed. Leaves many, densely polystichous, usually suberected, rarely slightly secund, 4–14 cm long, covered with fine appressed cinereous scales; sheaths obscure, short; blades rigid, narrowly triangular, attenuate, 5–13 mm wide, often carinate. Scape well developed, erect, slender, glabrous; scape-bracts imbricate, elliptic with linear blades, subinflated, membranaceous, rose, appressed-lepidote. Inflorescence simple with 5–20 flowers in more than 2 ranks, dense or subdense, ovoid or short-cylindric. Floral bracts ovate, usually exceeding the sepals but sometimes only equaling them, acute or the lowest with short filiform lepidote blades, inflated, membranaceous, nerved, glabrous, briht rose in life; flowers sessile, erect or suberect. Sepals lanceolate, acute, 12-19*

mm long, the posterior high-connate, membranaceous, glabrous; petals 17-27 mm long, the blades broadly elliptic, very dark blue; stamens included; filaments plicate. Capsule about equaling the sepals. (Pp. 835 – 837)

This plant became interesting as research object only in the last few years (Papini et al., 2010; Papini et al., 2011), while in the past many actions were adopted in order to eliminate *T. aeranthos* from their host in its original environment (Caldiz and Beltrano, 1989; Bartoli et al., 1993; Kaplan, 1999) by the use of different herbicides such as atrazine, dichlobenil and simazine, applied singularly as well as in combination. The use of those three herbicides is still allowed in the United States, while in Europe their use was banned definitely by the precept 2009/1107/EC (EC, 2009). Therefore, Kaplan (2004) has proposed the use of a non-toxic composition for the control of epiphytic weeds.

But as species of Tillandsia are transported by the action of the wind and, after the reproduction by megasporogenesis (Papini et al., 2011) the resulting plant can be disjointed and go its own way, the control and/or extinction of these plants is proved to be difficult.

Further, plants as *T. aeranthos* using airborne water droplets as important water source, frequently have a rosette growth habit and the so-called “narrow leaf syndrome” which includes a large number and a high flexibility of the narrow situated leaves (fig. 6). This factors increase the water droplet interception efficiency of the plant, as the total water droplets intercepted in rosette plants are proportional to the total leaf area (Martorell and Ezcurra, 2007).

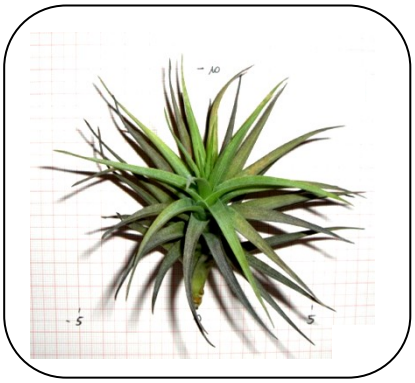


Fig. 6: Narrow leaf syndrome of *T. aeranthos*

2.2.1.1 Water and nutrient uptake in *T. aeranthos*

It is common to almost all the epiphytic plant species of the genus *Tillandsia* to meet their water and nutrient demand by uptake from the atmosphere.

Leaves are equipped with an indumentum of trichomes which guarantees the nutrient and water acquisition by the plant from the surrounding environment. These trichomes obscure the epidermal leaf surface or fully or partially, depending on the taxa. They have a nail – like shape and are formed by a six – step – development already when the plant has only three young leaves (Papini et al., 2010).

Trichomes are composed by an external shield (it extends over the epidermis) and a stem (axis, or stalk (Benz and Martin, 2006)) which connects them to the internal tissues of the leaf. The outmost part of the shield is composed by highly elongated cells which in their totality are named wing, while the center of the shield is composed of four central disc cells (fig. 7). These cells are connected to the mesophyll parenchyma by the stem which consists in one to six cells laying one on the top of another. The apical cell of the stem is called dome cell, whose labyrinth – like structured plasma membrane allows a fast absorption of the solutions and their retention in order to avoid their release back into the atmosphere. When the shield cells lose their cytoplasmatic content by activating their water pumping mechanism, solutions from the surrounding atmosphere caught by the wing enter the shield cells, going through the stem cells and, finally, reach the mesophyll parenchyma (Papini et al., 2010 and references therein).

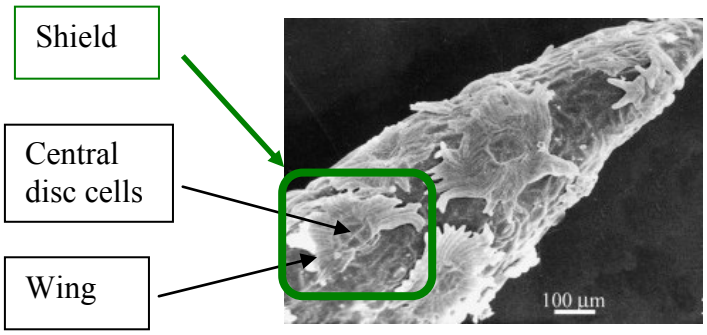


Fig. 7: Trichomes' shield on the epidermis of a leaf of *T. aeranthos* (out of Papini et al., 2010)

Trichomes show next to their ability of acquiring water and nutrients further functions as defender against herbivores and pathogens, or as reflectors of the solar radiation in order to reduce the damage of the photosynthetic physiology as well as the leaf temperature which leads to an increase in the water use efficiency (Haworth and McElwain, 2008). Stomata (or breathing holes) in the epidermis, on the other hand, regulate the plant's gas household and the gas exchange. They are composed of two guard cells on the bottom of the subsidiary cells, while on the top they have the stomatal papillae (Haworth and McElwain, 2008). When the plant is wet, the trichome shield depress over the leaf epidermal and blocks this way the stomata and its respiratory function (Illawara Bromeliad Society, 2008), as it is shown in fig. 8. Stomata play a fundamental role in the metabolism of Tillandsia.

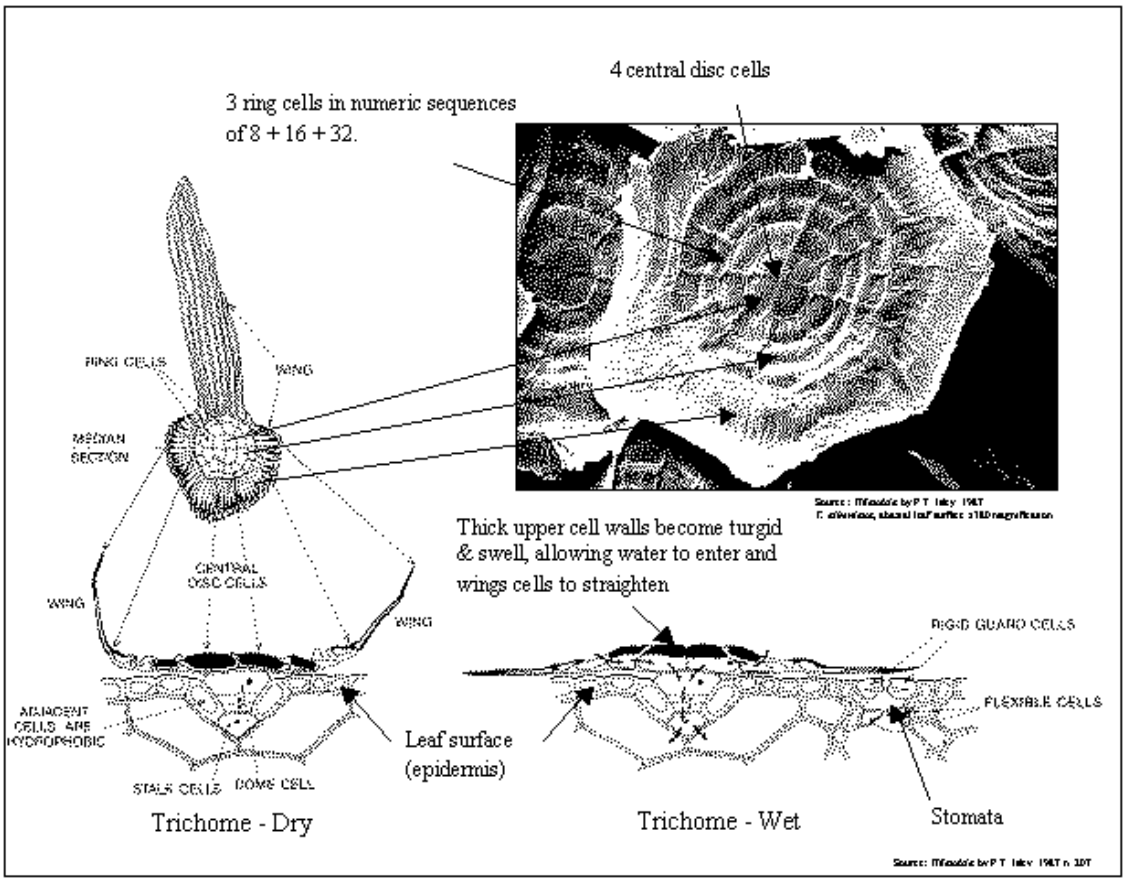


Fig. 8: Stomata of Tillandsia. Out of: Tillandsia: The World's Most Unusual Airplants. (Illawara Bromeliad Society, 2008)

2.2.1.2 The metabolism of *Tillandsia*

As species of Tillandsia mainly live in arid environments, they show the Crassulacean Acid Metabolism (CAM), a nocturnal carbon fixation pathway that avoids the water loss during day time. As they live originally in environments where high temperatures prevail during day time and temperatures not below zero during night time, they have to protect themselves by acquiring water and nutrients only during night time when nor the temperature nor the sunlight can induce the evaporation of them.

The CAM photosynthesis is a cycle divided into two parts, one occurring during night time and one during day time.

During night time, stomata are open to allow the CO_2 to enter. This CO_2 is combined with phosphoenolpyruvate (PEP) and because of the action of the PEP carboxylase, oxaloacetate (OAA) is formed. From OAA is formed malate by the oxidation of NADPH into $NADP^+$, catalyzed by the enzyme NAD^+ malic dehydrogenase. Malate is then stored in form of malic acids in the vacuoles of the mesophyll cells until day time.

During the day, the light induces the release of malate from the vacuoles and its decarboxylation, forming this way next to CO_2 also pyruvate. The enzyme RuBisCo (Ribulose-1,5-bisphosphate carboxylase oxygenase) catalyzes the reaction of the released CO_2 with RuBP (Ribulose-1,5-bisphosphate) contained in the chloroplast, and this way the CO_2 enters into the Calvin cycle to produce on one hand RuBP which guarantees the continuation of the cycle, and glyceraldehyde-3-phosphate (G3P) on the other hand to produce starch, sucrose and cellulose to satisfy the plant's energy demand. The pyruvate is transformed into starch, which will be used during night time to produce the PEP the CO_2 entering then the cell (Forseth, 2010). Fig. 9 shows the summary of the CAM photosynthesis.

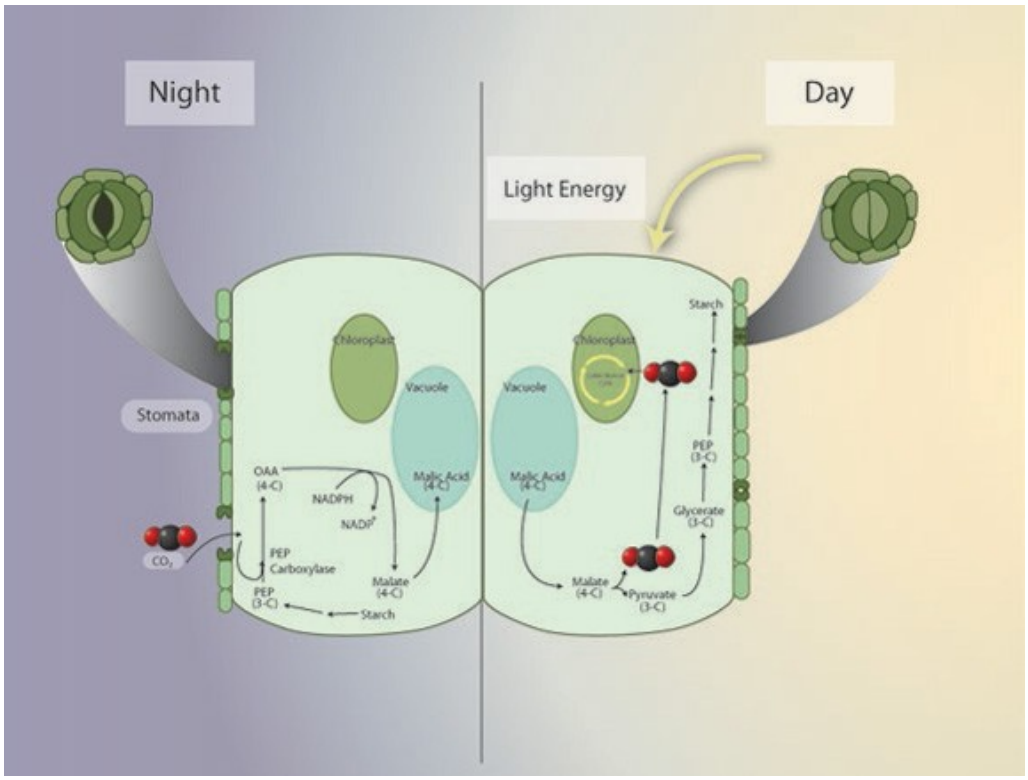


Fig. 9: Summary of the biochemical steps in the CAM photosynthetic pathway (Forseth, 2010).

Between these two phases, there are two transitions when stomata still remain open: a) during the very early light period (sunrise), and b) in the late light period (sunset). This allows a direct assimilation of the taken up CO_2 to carbohydrate when the vacuolar organic acid is exhausted.

However, the different steps of the CAM are driven by different environmental parameters, i.e. temperature, incoming radiation, water, salinity, nutrients, the amount of CO_2 present in the air and their interactions, as well as by plants' physiognomy (e.g. succulents, epiphytic climbers and stranglers, rosettes, trees) (Lüttge, 2004).

3. Legislation

The most recent regulation in terms of air quality is the European Directive 2008/50/EC (EC, 2008), and it was adopted in Italy with the Legislative Decree 155/10 (DLgs 155/10). It abrogates all the previous regulations regarding this matter.

The European Directive is based on the "Clean Air for Europe" (CAFE, 2001) programme. This program's aim is to "establish a long-term, integrated strategy to tackle air pollution and to protect against its effects on human health and the environment" and refers to a series of Directives, including 96/62/EC, 1999/30/EC and 2008/50/EC. These Directives set air quality standards for all 27 EU member countries (Wolff and Perry, 2010). The Directive establishes the monitoring of the ambient air quality in terms of SO_2, NO_x, PM_{10} e $PM_{2.5}$, Pb, benzene, CO, and ozone in urban, suburban, rural and rural background areas all over Europe. Further, the European Member States should not exceed the limit values for SO_2, NO_x, Pb, benzene, CO listed in Annex XI of the Directive. Instead, they should reduce their $PM_{2.5}$ – emissions up to the limit value of 20 μg m^{-3} $year^{-1}$ within 2020.

The Italian DLgs 155/10 (like the European Directive) establishes a unitary policy framework to supervise and evaluate the ambient air quality with the aim of

- identify the objectives to avoid, prevail or reduce adverse health effects as well as negative effects on the whole environment;
- evaluate the quality of ambient air based on standardized national criteria and methods;
- obtain information on the ambient air quality in order to decree abatement strategies;
- maintain the air quality where it is already good or improve it;
- guarantee transparency on ambient air quality information;
- realize a better cooperation within the European Member States to reduce air pollution.

Both the Directive and the Decree designate analyses of EC and OC present in the $PM_{2,5}$ of background areas, but they do not determine a reference method to use for analyzing those compounds. This can cause some problems when comparing data obtained within Europe with different methods (Ficotto, 2011).

In the DLgs 155/10 is also established the monitoring of PAH in the ambient air, even if the Directive 2008/50/EC does not suggest any analyses of those compounds. As this directive abrogated all the previous regulations regarding the ambient air quality, it abrogated also the European Directive 2004/107/EC (EC, 2004), adopted in Italy with the DLgs 152/2007, which established the monitoring of PAH and toxic metals such as arsenic, mercury, nickel and cadmium.

Nevertheless, Annexes of the DLgs 155/10 refer to

- Annex I: data quality of all the compounds cited in the Decree (SO_2, NO_x, PM_{10} and $PM_{2.5}$, CO, benzene, Pb, ozone, B(a)P, As, Cd, Ni, PAH different from B(a)P, Hg).
- Annex II: evaluation thresholds of Pb, As, Cd, Ni, B(a)P next to SO_2, NO_x, PM_{10} and $PM_{2.5}$, benzene, CO.
- Annex III: evaluation of the ambient air quality and location of the measurement stations.
- Annex VI: reference methods.
- Annex XIII: average annual target values for human exposure to As (6.0 ng/m^3), Cd (5.0 ng/m^3), Ni (20.0 ng/m^3) and B(a)P (1.0 ng/m^3).

In art. 5 of the DLgs 155/10 is named also the use of (non-specific) bioindicators to monitor the determined effects of As, Cd, Ni, Hg and PAH in different ecosystems.

4. Aim of the Study

The aim of the study was the determination of the air quality in terms of detecting and quantifying organic and inorganic compounds in the particulate matter in the larger Venetian area with two different methods: 1) active sampling with automatic sequential samplers, and 2) active biomonitoring with *T. aeranthos*.

1) The study this dissertation is the result of, started with the analyses of PAH in 39 filter samples (22/12/2009 - 28/01/2010) which took part of a two year lasting project carried out by the Department of Environmental Sciences, Informatics and Statistics (DAIS) in collaboration with Ente Zona Industriale di Marghera (EZI). The aim of this project was to determine the airborne particulate matter of the Venetian area and its pollutant content in a qualitative and quantitative way. Samplers were set up in three points of the study area, selected to represent the different emission scenarios of this area. The data of PAH and $PM_{2.5}$ in this dissertation correspond to the winter period in the sampling stations of Malcontenta and Mestre (via Lissa). The amount of $PM_{2.5}$ was quantified by gravimetric analyses, whereas the determination of the 18 PAH cited above was obtained by GC-MS analyses.

In a second step, the amount of TC, OC and EC was determined in 119 filters (20/12/2008 - 20/12/2009) by two different methods in order to find a correlation factor to compare data obtained by the instrument generally used for analyses of the carbonaceous fraction in the air with those of an instrument normally used for the analyses of the carbonaceous amount in soil or waste samples.

Further, the marker for wood burning, levoglucosan, and its isomers were analyzed in 106 filters (20/12/2008 - 20/12/2009) to detect if wood is still burned for domestic and industrial heating.

2) The active air biomonitoring was carried out with the plant *T. aeranthos* to assess the suitability of a plant which is native of the South – American continent to quantify the air pollution of the Venice – Mestre area. As the only study about Tillandsia in Italy attempted by Brighigna et al. (2002) was interrupted because of the sensitivity of the species used toward low temperatures, the species used in this study has been chosen with care. The aim of this biomonitoring study was i) to detect the ability of the plant *T. aeranthos* to adsorb and store air pollutants, and ii) being able to analyse airborne organic (PAH) and inorganic (metals) compounds with the same (or similar) methods those compounds are generally analysed on filters.

5. Materials and Methods

5.1 Study area

The study area of Venice – Mestre is located in the north – eastern part of Italy (45°26'19" N, 12° 19' 36" E), in the Po Valley which is known for its highly polluted atmosphere. The study area counts 270 000 inhabitants, most of them living on the mainland. Close to the urban area are located also an industrial district, an airport and an industrial harbour. The atmosphere in the study area receives emissions from different anthropogenic and natural sources:

The industrial district of Porto Marghera includes oil refineries, power plants, incinerators and other activities. In addition, the international airport, high traffic roads and an industrial harbour contribute to the emission of PAH, carbonaceous compounds and metals into the air. As the main land use in the area just behind the urban and industrial area of Mestre and Porto Marghera, respectively, is agricultural, also this factor influences the air quality in that area. Close to Venice, the artistic glass factories on the island of Murano, cause a significant air pollution contribution. During winter time in both areas also the domestic heating contributes to higher concentrations of the analysed compounds (Masiol et al., 2010; Masiol et al., 2012a,b, and references therein).

Natural sources of particulate matter include the marine sea spray caused by the vicinity to the Adriatic Sea, and crustal material deriving from local and regional sources (Masiol et al., 2010).

Unfavorable weather conditions and orographic features favor the pollutant accumulation in the study area.

The sampling points were chosen on the basis of their demonstrated diversity in emission scenarios. In the cited study two sampling stations (fig. 10) were chosen to monitor the air quality under different emissive conditions: an industrial site (Malcontenta) and an urban one (Mestre). Ana extra station a semi-harbor site (Venezia) was also added. (Masiol et al., 2012a,b,c).

The samples achieved with sequential automatic samplers and analysed in terms of PAH, EC/OC and levoglucosan took part of a broader project with the aim of characterizing the atmospheric aerosol in the Venice – Mestre area by modelling approaches and chemical analyses (Squizzato et al., 2012 and references therein). Therefore, the filter samples analysed within this work give some information on the air pollution just in the industrial site as well as in the urban one.

The biomonitors *T. aeranthos* on the other hand were exposed to the ambient air of all three sampling stations listed above. In addition, some plants were exposed also to the indoor air of i) a public office in Venice, ii) a garage in Mestre and, in order to simulate an environment where *T. aeranthos* plants would grow naturally, iii) the orchid greenhouse of the Botanical Garden in Padua.

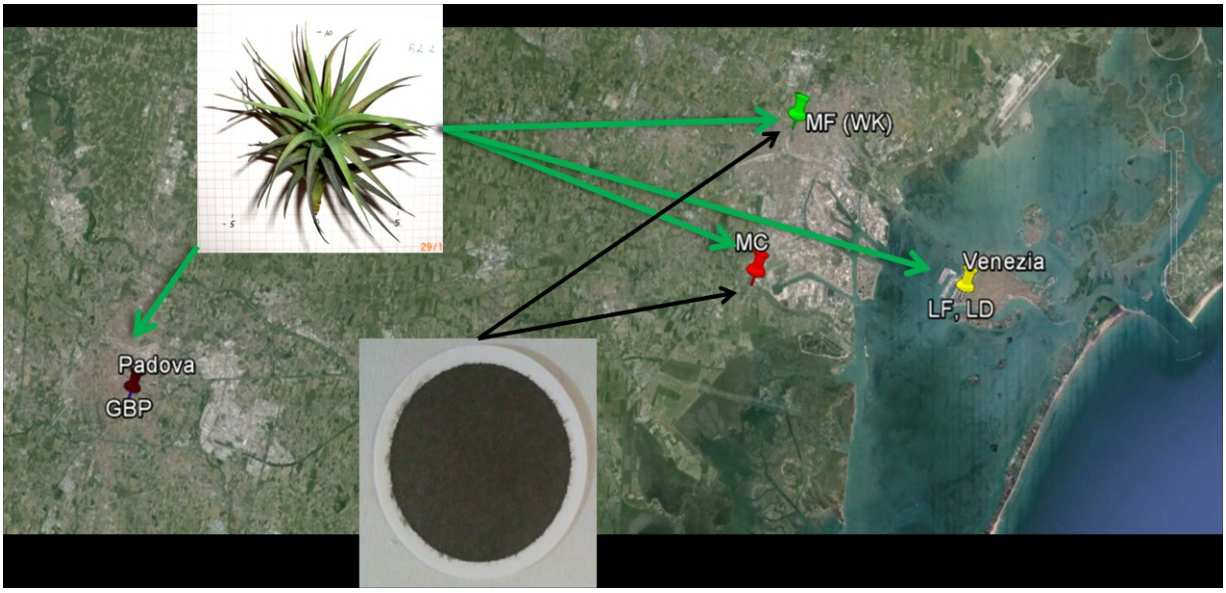


Fig. 10: Sampling area.

5.2 Sampling with filters

$PM_{2.5}$ was sampled on glass and quartz fiber filters (Ø 47 mm; PALL, USA, and Whatman QMA, respectively) with low volume automatic sequential samplers ("Hydra" (FAI), and TCR Tecora). These samplers were set according to the EN14907 (2.3 m^3 h^{-1}) (CEN, 2005) with a continuous sampling over 24-hour cycles from midnight to midnight.

Previously to their exposure, filters were kept for 48 h ca. in a thermostated room with a temperature of 20 ± 5°C and a relative humidity of 50 ± 5% in the Department of Padua of the Regional Agency for the Environmental Prevention and Protection (Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto, ARPAV). After weighing them with a microbalance (nominal precision 1 µg), filters were piled in the samplers. Some filters were kept in the sampler without exposing them to the air flux, for obtaining the so-called "field blanks". These filters were used, together with the blanks during the analyses, to detect a possible contamination of the filters themselves, to enable a correction of the mass of $PM_{2.5}$ and in the calculation of the analytical limit of detection (LOD) together with "extraction blanks" (= only solvent without filter).

After the sampling, filters were kept in the thermostated room for at least 48 h and weighted to determine the amount of $PM_{2.5}$ (in mg) deposited on the filter by gravimetric analysis. Until further analyses, filters were stored at -20°C (Masiol et al., 2012a,b; Squizzato et al., 2012).

5.2.1 Analyses of PAH

The analyses of PAH were carried out on a total of 38 winter data (22/12/2009 – 28/01/2010). The analytical procedure of the extraction and analysis of PAHs is based on the method described by Pavoni et al. (1994) and Centanni (2004):

- **Extraction** of PAHs: glass fiber filters were weighted with an analytical balance (Gibertini) and cut in two pieces with ceramic scissors. One half used for the analyses of PAH was weighted, while the weight of the other was obtained by subtraction. The extraction of PAH from the filter halves was carried out by positioning them in glass flasks of 100 mL, adding 20 mL of dichloromethane (99% Romil Pure Chemistry, UK), and sonicating them in an ultrasonic bath (Branson 5210) for 15 min. The extract deriving from this operation was set apart in another glass flask and the extraction procedure was repeated for two more times, obtaining in total 60 mL of extract that was concentrated in a thermostated bath (35 ± 5°C) under a gentle flow of nitrogen (SIAD ricerche, ≥ 99,9% v) until few milliliters.

- **Clean up**: the reduced extract was cleaned up by passing it through a chromatographic column conditioned with a mixture of n-hexane (>95% SpS Romil, UK) and dichloromethane 3:2 (v/v) and packed with absorbents in the following order:
    - 4 g of silica gel (Macherey–Nagel, Germany)
    - 0.5 g of anhydrous sodium sulfate (ACS ≥99.0%, Sigma–Aldrich, USA).

PAHs in the filter extract were eluted with 25 mL of the mixture n-hexane – dichloromethane 3:2 (v/v).

Silica gel, anhydrous sodium sulfate and cotton were previously washed three times in dichloromethane, air dried and then activated overnight in the oven at 250°C.

The volume of the final extract was reduced to ≈ 1 mL under a nitrogen flow. The remaining extract was transferred with Pasteur pipettes into amber vials. Samples were let dry out at room temperature, recovered with 0.5 mL of isooctane (2,2,4-Trimethylpentane: 99%, Lab Scan, PL) and stored at -20°C until GC-MS analyses.

- **GC-MS analysis:** before analyzing the samples' extracts by GC-MS, they were spiked with 100 ng of the internal standard PAH-MIX 31 (Dr. Ehrenstorfer, Germany) containing the five perdeuterated PAH congeners naphtalene-d8, acenaphtene-d10, phenantrene-d10, chrysene-d12, perylene-d12, as described in EPA – TO13A. 1 µL of the sample was injected in a gas chromatograph HRGC interfaced to a mass selective detector LRMS. The capillary column used was a 5%-phenyl-substituted methylpolysiloxane (DB-5, J&W Scientific, USA), while helium (≥99.9995% v, SIAD, Italy) was the carrier gas at a constant flow of 1 mL min^{-1}. The chromatographic conditions are listed in table 3:

Table 3: Chromatographic conditions.

GC-MS	
Gas chromatograph	HP 5890 series II
Mass spectrometer	HP 5970 B
Injection mode	Splitless
Injected volume	1 µL
Injector temperature	300°C
Carrier gas	helium
Column	DB-5 (50 m x 0.2 mm x 0.33 µm)
Oven temperature program	60°C x 1 min, 18°C min^{-1} to 140°C, 10°C min^{-1} to 252°C, 14°C min^{-1} to 300°C, 300°C for 22 min
Temperature at the interface GC-MS	300°C
Ionization mode	Electron impact (70 eV)
Analyzer	Quadrupole
Analyzing mode	Selected Ion Monitoring (SIM)

The identification and quantification of each PAH congener are based on the detection of specific fragmentation ions of each compound, and their retention time, in the chromatogram. The area of each peak is proportional to the concentration of the single compound.

Data were validated on field blanks and extraction blanks, which were prepared and analyzed following the same procedure as the samples. The obtained values were subtracted from the sample values and the limits of detection (LOD) were calculated for each congener as three times the standard deviation of them (Table 4). When the concentration of the congener was lower than the LOD, the value was interchanged with the value of LOD/2.

Table 4: LOD of each congener (ng/m³).

	Naph	Acen	Ace	Flu	Phe	Anth	Fluor	Pyr	B(a)A
LOD	2.4	6.17	1.17	1.42	0.8	0.55	0.69	0.91	1.84

	Chry	B(b)F	B(k)F	B(e)P	B(a)P	Per	IP	Db(ah)A	B(ghi)P
LOD	0.92	1.76	2.51	0.90	1.23	2.10	0.34	0.31	0.64

The standard reference material SRM1649a (NIST, USA) was analyzed repeatedly to guarantee the quality and accuracy of the analyses, as the recoveries for the analyzed compounds show a percentage of > 75%, where the error was calculated in the following way (Scalabrin, 2008):

$$E = \frac{\sigma}{X} * 100 \qquad \text{eq (2)}$$

where σ = the standard deviation on the repetitions of SRM1649a – analyses;

X = mean of the number of repetitions.

The analyses were calibrated by means of a calibration curve, based on different concentrations (0.1, 0.3, 0.5, 1.0, 2.5 ng mL^{-1}) of PAH-MIX 45 (Dr. Ehrenstorfer, Germany).

Analytical results were “BaP-corrected” to evaluate the carcinogenic and mutagenic potential (TEQ and MEQ) of the PAH-mixture detected. The used equations were the following (Jung et al., 2010):

(BaP-TEQ) $_{\Sigma 8IPA}$ = [B(a)A] * 0,1 + [Chry] * 0,01 + [B(b)F] * 0,1 + [B(k)F] * 0,1 + [B(a)P] * 1 + [IP] * 0,1 + [Db(ah)A] * 1 + [B(ghi)P] * 0,01 eq (3)

(BaP-MEQ) $_{\Sigma 8IPA}$ = [B(a)A] * 0,082 + [Chry] * 0,017 + [B(b)F] * 0,25 + [B(k)F] * 0,11 + [B(a)P] * 1 + [IP] * 0,31 + [Db(ah)A] * 0,29 + [B(ghi)P] * 0,19 eq (4)

5.2.2 Analyses of the carbonaceous fraction

Within this work, two different analytical methods for the carbonaceous fraction have been compared by analyzing portions of 106 quartz fiber filters exposed to the ambient air of the urban and the industrial sampling site. The amount of the carbonaceous fraction in those samples was quantified with a) the Sunset Lab OC-EC Aerosol Analyzer (Sunset Laboratory Inc., USA), an instrument analyzing the carbonaceous fraction by thermal optical transmission, and b) with the TOC - analyzer interfaced with a SSM – 5000A (Shimadzu), an instrument based on combustion / NDIR analyses and normally used only for the analyses of TC in solid samples such as soil or waste.

5.2.2.a) The Thermal Optical Transmission method

The method normally used in laboratory (Quincey et al., 2009, Bernardoni et al., 2011 and references therein) for establishing the concentrations of EC and OC as well as TC contained in a $PM_{2.5}$ sample is a thermal optical transmission (TOT) method, practically applied with the Lab OC-EC Aerosol Analyzer (Sunset Laboratory Inc., USA; fig. 11) in combination with the NIOSH 5040 protocol (US EPA, 2003).

Fig. 11: Sunset Lab OC-EC Aerosol Analyzer

Sub-samples consist in a portion of 1 or 1.5 cm² taken from the remaining subsample of the 106 quartz fiber filters. Analyses were carried out in the laboratories of the Department of Physical Chemistry and Electrochemistry (Universitá degli Studi di Milano) by following the procedure of carbon speciation established by Birch and Cary (1996): sub – samples were exposed to thermal desorption, first in an inert helium atmosphere (He 99,9995% v, SIAD, Italy) then in an oxidizing atmosphere (2% O_2 in He, SIAD, Italy) to become CO_2 which is reduced to methane and is finally quantified by a flame ionization detector (FID).

To create the calibration curve, different amounts (10, 15, 20 µl) of the standard solution *Total Organic Carbon Std 2000ppm* (Reagecon, IRL) were placed on a portion of a preconditioned filter and analyzed. The repeatability of this method is tested on different portions (1.5 cm²) of one out of the samples at the beginning and at the end of one analysis cycle. According to the manufacturer's instructions, uncertainties in EC and OC measurements are of the order of 5% ± 0.2 µg C/cm^2 (Perrone et al., 2011).

As the results obtained by the Sunset Lab OC-EC Aerosol Analyzer had the unit µg C/cm², they were multiplied by the area of the whole filter (A = 10.1787 and 11.6415 cm² for the sampler "Hydra" (FA) (urban site) and the TCR Tecora (industrial site), respectively) and divided by the volume (V = 55±1 m³) the sampler was working on, in order to get all the values in the unit µg/m³ to permit a comparison with the combustion/NDIR method.

5.2.2.b) The Combustion/NDIR method

The combustion/ non-dispersive infrared (NDIR) method (C-NDIR) consists in analyzing TC and EC by a Shimadzu TOC-5000 combined to a SSM-5000A (fig. 12) following EN13137 (CEN, 2001). This instrument is normally used for analyses of the carbonaceous fraction in soils, waste (Kumpiene et al., 2010) and sediment samples (Visco et al., 2005), but in this case, round portions (Ø = 16 mm, total filter area = 3.85 cm² and 3.6 cm² for filters sampled at the industrial and at the urban site, respectively) of each filter were put in the sample holder in order to get analyzed as described below.

The TC was determined by an oxidative combustion of a round sub-area of the filter (A = 1.89 cm²) at 900°C, catalyzed by Cobalt- and Platinumoxide on an Aluminum support. All the carbon present in the sub-sample is transformed into CO_2 and by a flux of oxygen (uncertainty of 3% (CEN, 2005)). The combustion product is transported to the NDIR – sensor of the TOC-5000 for analyses at 4.3 µm (Formenton and Libralesso, 2007). The sensibility of the instrument can be changed manually by interfering on the length of the NDIR- cell, and therefore on the optical path, from high (RANGE 1X) to low (RANGE 30X) depending on the filters' load. TC of the darkest filters was analyzed by choosing the low sensibility option.

To determine the amount of EC in each sample, the high sensibility option was chosen. As the sub-sample destined for EC analyses was pre-treated at 350°C for one hour in a muffle, the sub-samples of the filters lost averagely 36% of their total carbon amount. As organic compounds do not resist at so high temperature and decompose, it was assumed that the remaining fraction was constituted only by EC.

The amount of OC in the samples was calculated for difference (Kuhlbusch et al., 2009):

$$OC = TC - (EC+IC) \qquad \text{eq (5)}$$

In this work, the amount of inorganic carbon (IC) was neglected after testing that $PM_{2.5}$ samples did not contain significant quantities of IC (Ficotto, 2011).

The calibration curve was obtained by analyzing different weights of monohydrated glucose containing 36.36 % carbon. The method of least squares was used to identify the quality and the linearity of the obtained regression curve.

The repeatability of analyses was tested on the standard reference material SRM1649a (NIST, USA) (11 repetitions) as well as on ten filter samples with repeatability limits of 10.8 % and 7.8 %, respectively. The estimated relative uncertainty of this method is 6.4 %.

A method similar to the one described here had been developed by Cachier et al. (1989). The differences with the method used for this study lay in the i) oxidative combustion temperature, ii) detection method of the evolved CO_2, iii) temperature of the precombustion process, and iv) sensibility of the instrument: the area of sub-sample needed with Cachier's method is 3 cm², the one we worked with was almost the half.

Fig. 12: Shimadzu TOC-5000 coupled to SSM-5000A

The results of TC and EC obtained with this method were achieved in mg C/cm² or in µg C/cm² with the instrument set at low or high sensibility, respectively. In order to be able to compare these results with those obtained by the Sunset Analyzer, values were multiplied by the total filter area (A = 10.1787 and 11.6415 cm² for the sampler "Hydra" (FAI) (urban site) and the TCR Tecora (industrial site), respectively) and divided by the volume (V = 55±1 m³) the sampler was set up. So, also these results had the unit µg C/m³.

5.2.3 Analyses of levoglucosan

Levoglucosan and its isomers mannosan and galactosan were analyzed in a total of 119 samples representative for summer and winter wood burning scenarios in the two sampling sites URB and IND during the years 2008 – 2009 in the Venice – Mestre area. “Winter wood burning scenarios” were considered starting with the beginning of the domestic heating season on November, 1 of each year. The method used to detect these markers has been described by Piazzalunga et al. (2010) and was carried out in the laboratories of the Department of Physical Chemistry and Electrochemistry (Universitá degli Studi di Milano).

The analysis of levoglucosan provides the ultrasonic extraction of a filter portion of 1 cm² in ~6 mL of Millipore water and the following analysis with an ion chromatograph (Dionex ICS1000) equipped with an amperometric detector, an isocratic pump, a sample injection valve having a sample loop of 100 mL, and a *Carbopac PA-10 column* (“guard column”,50 mm x 4 mm) and *Carbopac PA-10 anion-exchange analytical column* (250 mm x 4 mm). Sodium hydroxide NaOH (18 mM) was used as eluent. Each analysis took 25 min, followed by a 40 min interval before the next sample analysis in order to regenerate (with NaOH 200mM) and to re-equilibrate the column. Further, waveform B with a slope of 15.93, 18.77, 18.01, a slope standard error of 0.01, 0.00, 0.01 and a correlation coefficient R^2 of 1.00, 1.00, 1.00 for levoglucosan, mannosan and galactosan, respectively, was applied. This waveform was calculated on the response of the analyses of eight standard solutions (Piazzalunga et al., 2010).

For the data analysis as well as for the system control, the Chromeleon software was used. The obtained area was transformed mathematically in absolute ng, which, divided by the sampling volume, leads to the concentration of levoglucosan (ng/m³) in the $PM_{2.5}$ sampled on the filter.

The Limit of Detection (LOD) value was calculated as described by Piazzalunga et al. (2010). In brief, the LOD was obtained by analyzing an analyte concentration which gives a signal equal to the blank signal. LOD of levoglucosan detected this way was 0.47 ng/m³ for analyses carried out within this work.

5.3 Biomonitoring

The biomonitoring was carried out with plants of the bromeliad species *Tillandsia aeranthos* in an almost one year lasting sampling campaign. The plants were purchased from *Gärtnerei Dötterer* (Freiberg am Neckar, Germany). Before exposure they were washed in distilled water, marked and weighted. The complete sampling period started in June, 2012 and ended in June, 2013. The sampling was conducted in three periods, and in each sampling station were located 6 plants (except in the 3rd period, where only 5 plants were exposed). So, in total 102 samples were exposed and analyzed. Samples were collected in each site after exposure times of 3, 6, 9, 12, 15, and 18 weeks. The sampling periods are listed in the following:

1st period: plants were named "(abbreviation of the sampling site), 1 – 6"

1	2	3	4	5	6
28.06.2012 - 19.07.2012	28.06.2012 - 09.08.2012	28.06.2012 - 30.08.2012	28.06.2012 - 20.09.2012	28.06.2012 - 11.10.2012	28.06.2012 - 31.10.2012

2nd period: plants were named "(abbreviation of the sampling site), 2.1 – 2.6"

2.1	2.2	2.3	2.4	2.5	2.6
31.10.2012 - 22.11.2012	31.10.2012 - 13.12.2012	31.10.2012 - 03.01.2013	31.10.2012 - 24.01.2013	31.10.2012 - 14.02.2013	31.10.2012 - 07.03.2013

3rd period: plants were named "(abbreviation of the sampling site), 3.1 – 3.5"

3.1	3.2	3.3	3.4	3.5
07.03.2013 – 28.03.2013	07.03.2013 – 18.04.2013	07.03.2013 – 09.05.2013	07.03.2013 – 31.05.2013	07.03.2013 – 21.06.2013

The sampling sites those plants were exposed in are the following:

- **Exposure to outside ambient air (see fig. 10):**
  - LF: semi – harbour site in Venice;
  - MF: urban site in Mestre;
  - MC: industrial site in Malcontenta;
- **Exposure to inside ambient air:**
  - GBP: Botanical Garden of Padua (orchid greenhouse);
  - LD: public office in Venice (laboratory);
  - Wk: working place – truck garage.

As *T. aeranthos* is an epiphytic plant, it is unanchored to the soil and in its home regions in South America it lives mainly on trees and is transported by the action of the wind from one place to another. To avoid the removal of the plants from the outdoor sampling stations during exposure, plants were anchored in a perforated piece of wood. To prevent the death of the plants which could be caused by an excessive exposure to direct sunlight or rainfall, plants were kept in a tube with holes on both sides to ensure the air circulation (fig. 13).

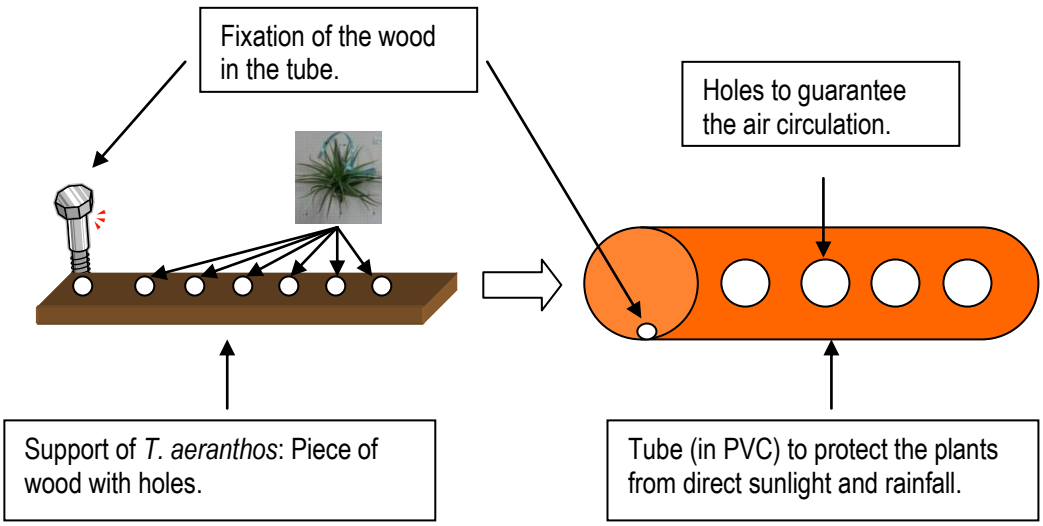


Fig. 13: Example of an outdoor exposure device.

5.3.1 Analyses of plants' properties

a) Weight difference before and after exposure, DW/FW ratio and RWC

Before exposure, every single plant was weighted with an analytical balance (ORMA model bc) and marked. After collecting them, plants were reweighted and the difference in fresh weight ($FW_{exposed} - FW_{initial}$) was detected.

Pignata et al. (2002) calculated the DW/FW ratio in the following way: 1 g of plant leaves was oven dried at 60±2 °C until reaching a constant weight. This method had been experimented initially in this work, and the dry weight / fresh weight ($DW/FW_{exposed}$) was calculated for some samples in this way. But as the water content was still too high for the further analyses mainly of metals, another method was used in order to obtain completely dry samples: After weighting, plants were meshed and kept in the freeze dryer for 24 hours. Then the dry weight / fresh weight ($DW/FW_{exposed}$) ratio was calculated.

The Relative Water Content (RWC) was calculated as described by Bermudez and Pignata (2011) with the following formula:

$$RWC = \frac{100*(FW_{exposed} - DW)}{(TW - DW)} \qquad \text{eq (6)}$$

where FW exposed = the fresh weight after exposure

DW = dry weight

TW = turgid weight*

* The turgid weight was calculated as described by Pereira et al. (2013): 1 g of leaves was kept in distilled water for 24 h at room temperature (20±2 °C) in the darkness. Then, leaves were weighted to determine the TW.

b)Parameters for the interpretation of airborne organic pollutants

1. Lipid content

The method used to analyze the lipid content in the plants was adapted by the methods established by Hara and Radin (1978) and Metherel et al. (2009):

In glass vials (V = 12 mL) were filled in ~0.5 g of freeze dried plant, weighted with an analytical balance (Mettler AE260 Delta Range®). Then, 10 mL of a mixture of n-hexane and isopropyl alcohol (Propan-2-ol RPE, Carlo Erba Reagents, Italy) 3:2 (v/v) were added. The samples were extracted in an ultrasonic bath (Branson 5210) for 20 min and centrifuged (centrifuge ALC 4232) for 30 min. The extract was placed in Al-plates and a second round of centrifugation was carried out with 5 mL of the same mixture (n-hexane and isopropyl alcohol 3:2 (v/v)) was carried out. Two replies of each sample were extracted and analyzed gravimetrically.

To detect the reliability and the repeatability of this method, tests with many samples of different amounts (0.01, 0.05, 0.1, 0.5, 0.7 g) of extra native olive oil (Bertolli, 100% lipid, composed by 70 – 80% oleic acid, 4-12% linoleic acid, 7-15% palmitic acid, 2-6% stearic acid) and oat flakes (Kölln, 6.40% lipid amount) as references were made. The extractions of the lipid part of olive oil and oat flakes demonstrated a recovery of 98 – 99% of the lipid fraction by the application of this method.

2. K_V

As the amount of PAHs detected in the plants can't be compared directly with those achieved by the filter sampling system, the constant K_v was calculated by Simonich and Hites (1994) as described in eq (7) for the five PAH congeners B(a)A, Chry, B(a)P, IP, B(ghi)P and results are listed in Table 5. This values were used to transform the values of PAH - congeners obtained by the analyses carried out within this work from ng/g DW into ng/m³ in order to make them comparable with data obtained by active sampling methods:

$$K_v = \frac{PAH_{veg}}{(lip * PAH_{atm})} \qquad \text{eq (7)}$$

where

- ***PAH $_{veg}$*** = concentration of the PAH congener in the plant (ng/g)
- ***lip*** = amount of lipids in the plant (mg/g)
- ***PAH_{atm}*** = concentration of the PAH congener in the air (ng/m³) achieved by active air monitoring.

ARPAV kindly provided the data of PAH_{atm} for the exposure period of the plants.

Table 5: K_v calculated by Simonich and Hites (1994).

PAH congener	B(a)A	Chry	B(a)P	IP	B(ghi)P
Simonich and Hites (1994):	1.42	1.29	2.47	2.79	3.18

The estimations for PAH_{atm} were executed with the Kv values calculated by Simonich and Hites (1994) not specifically for Tillandsia species, but for other plants and/or parts of plants, and compared to the data obtained by active air monitoring with filters. As the most carcinogenic compound is B(a)P, the main attention was focused on its results.

5.3.2 Analyses of PAH

PAH in the plants were analysed with the same method applied on the analysis of PAH on filter samples (chapter 2.1.1). In brief, 1 g of freeze dried sample was extracted ultrasonically for 20 min in 20 mL of dichloromethane for three times. The extract was reduced up to few millilitres in a thermostatic bath under a gentle flow of nitrogen, purified by passing it through a chromatographic column packed with 4 g of silica gel (FLUKA, Switzerland) and 0.5 g of anhydrous sodium sulphate and conditioned with a mixture of n-hexane and dichloromethane 3:2 (v/v). After the sample extract had passed through the column, the latter was rinsed with 25 mL of the mixture n-hexane – dichloromethane. The sample extract was reduced to 1 mL under a gentle stream of nitrogen, transferred with a Pasteur pipette in an amber vial, let dry at room temperature and redissolved with isooctane. The samples were stored at -20°C until analysis.

Before analysing the samples, they were spiked with 100 ng of the internal standard PAH-Mix 31 (EPA – TO13A). Then, 1 µL of each sample was injected in the GC – MS kindly provided by ARPAV. The calibration curve was established on different concentrations of the standard PAH-Mix 45. The characteristics of the GC – MS are described in Table 6.

Table 6: Chromatographic conditions.

GC-MS	
Gas chromatograph	Agilent 6890
Mass spectrometer	Agilent Technologies 5973 inert MSD
Injection mode	Splitless
Injected volume	1 µL
Injector temperature	300°C
Carrier gas	Helium
Column	HP-5 MS (30 m x 250 µm x 0.25 µm)
Oven temperature program	55°C x 1 min, 16°C min^{-1} to 140°C, 8°C min^{-1} to 252°C, 12°C min^{-1} to 300°C, 300°C for 13 min
Temperature at the interface GC-MS	300°C
Ionization mode	Electron impact (70 eV)
Analyzer	Quadrupole
Analyzing mode	Selected Ion Monitoring (SIM)

The limit of quantification (LOQ, = 3*std.dev. of non exposed “blanks” and “extraction blanks”) was calculated for each PAH congener for each sampling period. But as >95% of the detected values of the congeners Phen, Anth, Fluor, Pyr, B(a)A and Chry were <LOQ, these LOQs are not listed in Table 7. Nevertheless, for further calculations a complete dataset was established by substituting values <LOQ with those of LOQ/2.

Table 7: LOQ (μg/g DW) for each compound for each sampling period.

Congener	LOQ 1	LOQ 2	LOQ 3
Naph	0.011	0.031	0.013
Acen	0.005	0.026	0.005
Ace	0.002	0.003	0.002
Flu	0.005	0.006	0.005
B(b)F	0.027	0.083	0.055
B(k)F	0.013	0.016	0.010
B(e)P	0.006	0.017	0.030
B(a)P	0.032	0.106	0.063
Per *	0.110	0.335	0.193
IP	0.003	0.003	0.008
Db(ah)A	0.006	0.023	0.019
B(ghi)P	0.004	0.004	0.007

*** even if Per showed high LOQs in all the three sampling periods, the obtained values within the samples exceeded the LOQs.**

Also many other research groups (e.g. Brighigna et al., 2002; de Souza Pereira et al., 2007; Rasmos-Jusino and Laboy-Nieves, 2010; Rodriguez et al., 2010) have used the GC - MS method to detect the amount of PAHs in different species of *Tillandsia*.

5.3.5 Determination of metals in T. aeranthos

Metals were detected with the Particle Induced X-Ray Emission (PIXE) method in the laboratories of the National Institute of Nuclear Physics (Istituto Nazionale di Fisica Nucleare, INFN) in Legnaro (Padua). Figueiredo et al. (2001; 2004; 2007), and Isaac-Olivé et al. (2012) have used the TXRF method, while Martínez-Carrillo et al. (2010; 2011) was the only research group which have recently applied the PIXE method for determining metals in different species of Tillandsia. In the past, Sheline et al. (1976) had applied the PIXE analyses to detect metals in species of Tillandsia.

In this work, 100 mg of each freeze dried plant were used to produce a pellet (Ø = 13 mm, thickness 2 mm). Hereafter, these pellets were placed on the sample – holder of the 2.5 MV Van de Graaff AN 2000 accelerator and fixed with a 6 mm wide conductive double sided carbon adhesive tape (NEM – Tape; Nisshin EM Corporation, Japan).

Previously, holes (Ø = 0.4 cm) were made by a puncher in the adhesive to curtail the area the beam strikes the sample. Analyses were carried out with the beam of hydrogen protons at an energy of 2.00 MeV at a current of ≈20 nA and the beam – line at +45°/ 0°. Mittner et al. (1996) had established the PIXE – set up. Each run took 20 min with a mean charge of 16 μC and a mean Chi² at 0.7.

The GUPIX software package was used to fit the X-ray spectra in order to calculate the concentrations, % fit error and limits of detection (LOD) for 26 elements (Mg, Al, Si, P, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Br, Rb, Sr, Zr, Cs, W, Ba, Hg, Pb). All measurements were executed in duplicate. Values <LOD but with an error <33% were substituted with the values of LOD/2.

Further, the repeatability of the method was tested by analyzing the same sample for 10 times which detected an error ranging between 1 – 39%, but as samples showing an error >33% were excluded from further analyses, the corrected error ranges between 1 – 22%.

5.4 Data statistical processing

The data processing was carried out with the use of Microsoft Office Excel, while for factor analyses STATISTICA 7 and the trial version of STATISTICA 10 were used. The Varimax normalized rotation was applied for the principal component extraction and mean substitution, considering the eigenvalue 1 as the minimum. Further, the obtained data were compared to different meteorological parameters, namely temperature, global radiation, relative humidity, rain, wind speed and direction. Validated hourly meteorological data (Teolo) were provided by ARPAV for the sampling stations of Venice, Mestre and Padua, while the meteorological data on an hourly basis for the industrial site were provided by Ente Zona Industriale (EZI) of Marghera (Venice, Italy).

Wind speed and wind direction were processed with WRPLOT (Lakes Enviromental Software) to work out wind roses for the exposure periods.

With the NOAA/ARL Hysplit (HYbrid Single-Particle Lagrangian Integrated Trajectory) version 4.9 model (Draxler and Rolph, 2011; Rolph, 2011; Masiol et al., 2012a; Squizzato et al., 2012) the air mass back-trajectories were simulated for the PAH concentrations, the carbonaceous fraction and the amount of levoglucosan on the filters. The vertical velocity method and the NCEP/NCAR Reanalysis data were used to simulate the long-range air mass transport at a height of 200 m (right under the boundary layer) going back for five days in the IND sampling site (Masiol et al., 2012a, and references therein) as representative site for the total sampling area. Back-trajectories were carried out at different times during one day (1, 7, 13, 19).

As during winter time reactions of PAH congeners in the atmosphere caused by photochemical reactions should be quite low because of the short day length and the reduced intensity of the sunlight, back-trajectories of five days were simulated and their weight during the total sampling period has been established by clustering the means of all the back-trajectories within 48 hours and with a time label interval of 24 hours.

6. Results and discussion

6.1 Filter analyses

6.1.1 PAH

The determination and quantification of PAH on 38 winter samples, where values <LOD were substituted with the value of LOD/2, showed some significant differences in the composition of the PAH-mixture (Fig. 14).

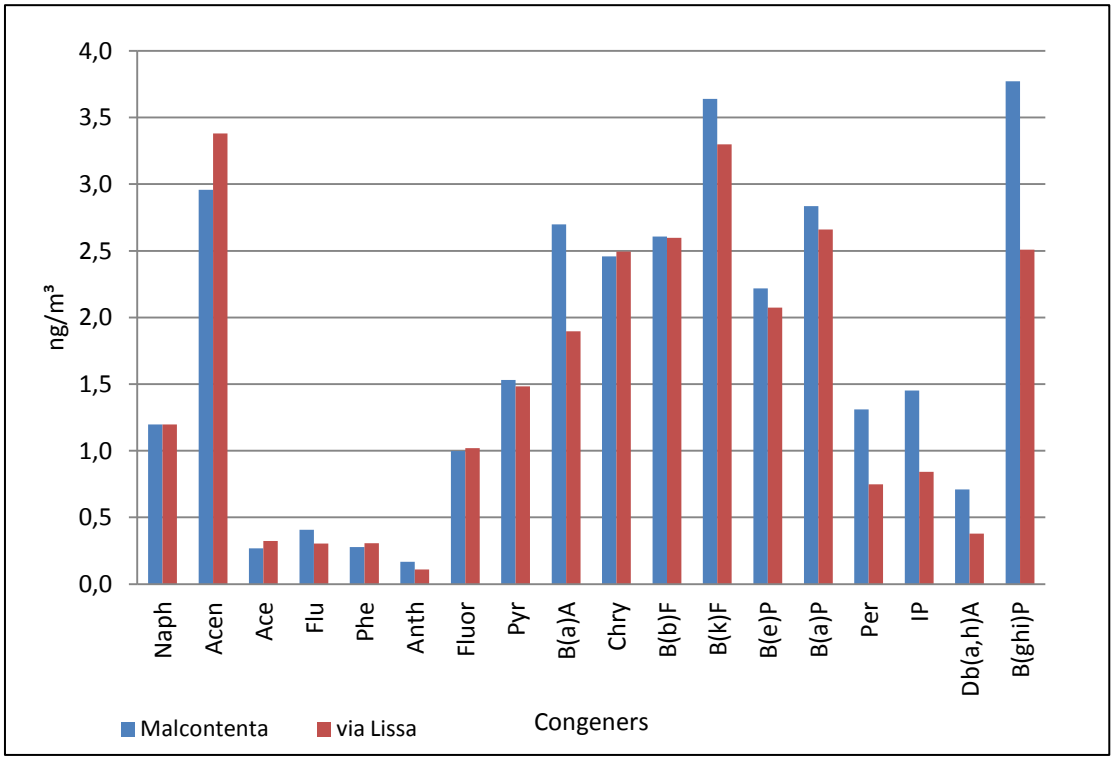


Fig. 14: Composition of the PAH-mixture in the industrial area of Malcontenta (IND) and the urban area of Mestre (URB).

The highest values were obtained for class 1 (B(a)P), class 2B (B(a)A, Chry, B(b)F, B(k)F, IP) and class 3 (Fluor, Pyr, B(e)P, Per, B(ghi)P) listed compounds (IARC, 2013). Therefore, the mixture residents had inhaled during the sampling period (22.12.2009 - 28.01.2010) in the industrial area as well as in the urban area can be considered quite dangerous for their health.

The distribution of PAH concentrations in the two sampling areas was compared by means of boxplots (Fig. 15). In the industrial area the distribution is less uniform than in the urban area. This could be influenced by the difference in the precipitations' quantity, as during the sampling in IND there were no precipitations detected while in URB were revealed some precipitation events (Fig. 16). This is nothing to worrying about, as samples in URB and IND were exposed within different days of the winter season (IND: 22/12/2009 – 13/01/2010; URB: 13/01/2010 – 28/01/2010). As data of the compounds Naph and Acen were <LOD for more than 80%, they were not considered in the further statistical analyses.

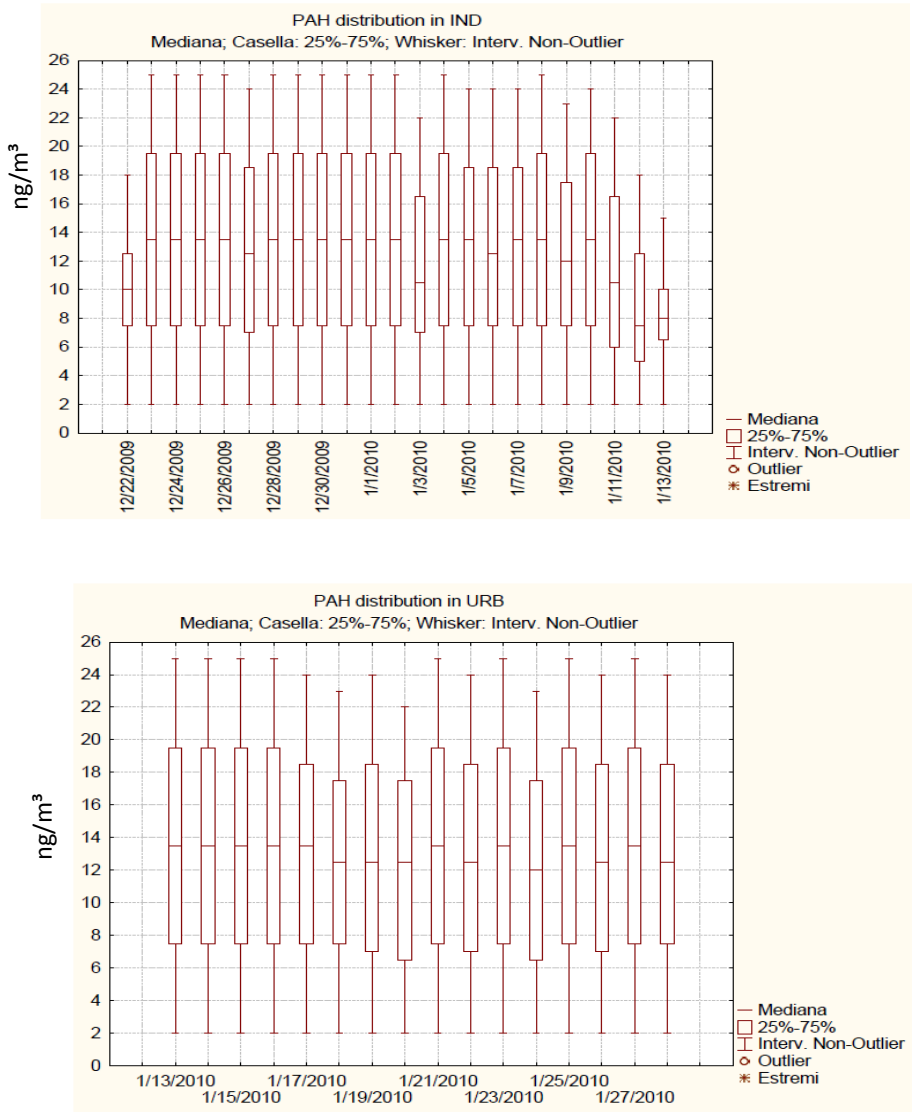


Fig. 15: PAH distribution (ng/m³) in the sampling sites IND and URB.

The single PAH compounds were correlated to each other by the Pearson's correlation (level of significance $p<0.05$), there could be established a good correlation within the total sampling area between $PM_{2.5}$, the sum of the 16 PAH analyzed within this work, the four, five and six ring PAH congeners detectable in the gas-particle and particle phase (Fluor, Pyr, B(a)A, Chry, B(b)F, B(k)F, B(e)P, B(a)P, Per, IP, B(ghi)P), except DB(ah)A. DB(ah)A could not be correlated to any of the other congeners nor in the industrial nor in the urban site.

The correlation between four, five and six ring PAH compounds was detected also in URB and IND, where in URB the compounds Pyr and Per next to DB(ah)A could not be correlated to the others, while in IND also Phe could be correlated to them. The PAH congeners Ace, Flu and Anth could not be correlated to none other compound. In Table 8 are listed the correlations within the total sampling area.

Table 8: Correlations of PAH congeners within the total sampling area.

	PM2.5	Ace	Flu	Phe	Anth	Fluor	Pyr	B(a)A	Chry	B(b)F	B(k)F	B(e)P	B(a)P	Per	IP	Db(a,h)A	B(ghi)P	∑16 PAH
PM2.5	1.00																	
Ace	0.21	1.00																
Flu	-0.33	-0.20	1.00															
Phe	0.17	0.29	-0.14	1.00														
Anth	-0.34	-0.14	0.27	0.07	1.00													
Fluor	0.45	0.06	-0.35	0.55	-0.20	1.00												
Pyr	0.48	0.04	-0.35	0.54	-0.19	0.98	1.00											
B(a)A	0.50	-0.00	-0.31	0.41	-0.16	0.80	0.86	1.00										
Chry	0.59	-0.01	-0.38	0.36	-0.30	0.86	0.91	0.85	1.00									
B(b)F	0.57	0.00	-0.25	0.36	-0.26	0.74	0.82	0.90	0.85	1.00								
B(k)F	0.55	-0.02	-0.30	0.34	-0.32	0.82	0.88	0.91	0.93	0.94	1.00							
B(e)P	0.54	0.01	-0.30	0.28	-0.23	0.80	0.86	0.89	0.91	0.92	0.94	1.00						
B(a)P	0.56	0.01	-0.34	0.26	-0.26	0.79	0.85	0.91	0.91	0.94	0.95	0.98	1.00					
Per	0.23	0.07	-0.21	0.22	0.03	0.49	0.58	0.59	0.61	0.54	0.55	0.58	0.56	1.00				
IP	0.17	-0.01	-0.11	0.26	-0.00	0.52	0.56	0.64	0.53	0.52	0.59	0.63	0.62	0.49	1.00			
Db(ah)A	-0.19	-0.17	-0.16	-0.04	0.11	0.20	0.19	0.16	0.14	0.06	0.06	0.26	0.18	0.22	0.46	1.00		
B(ghi)P	0.33	-0.12	-0.31	0.30	-0.13	0.74	0.78	0.88	0.73	0.80	0.83	0.84	0.82	0.52	0.74	0.32	1.00	
∑16 PAH	0.51	-0.00	-0.32	0.39	-0.21	0.86	0.92	0.96	0.92	0.94	0.96	0.97	0.97	0.63	0.69	0.23	0.90	1.00

If the sum of those data (Annex I) is compared to the weather conditions temperature (T) and precipitation and to the amount of $PM_{2.5}$ detected on the filter (Fig. 16), it is evident a direct correlation between the amount of total PAH and $PM_{2.5}$. On the average, the sum of the 16 PAH congeners in the industrial area showed values of 27.35±1.43 ng/m³, with maximum and minimum data of 78.85±4.08 and 1.76±0.21 ng/m³, respectively. In the urban area instead, the mean value of the ∑16 PAH was 23.04±1.16 ng/m³, with maximum and minimum values of 56.16±3.07 and 10.23±0.45 ng/m³, respectively.

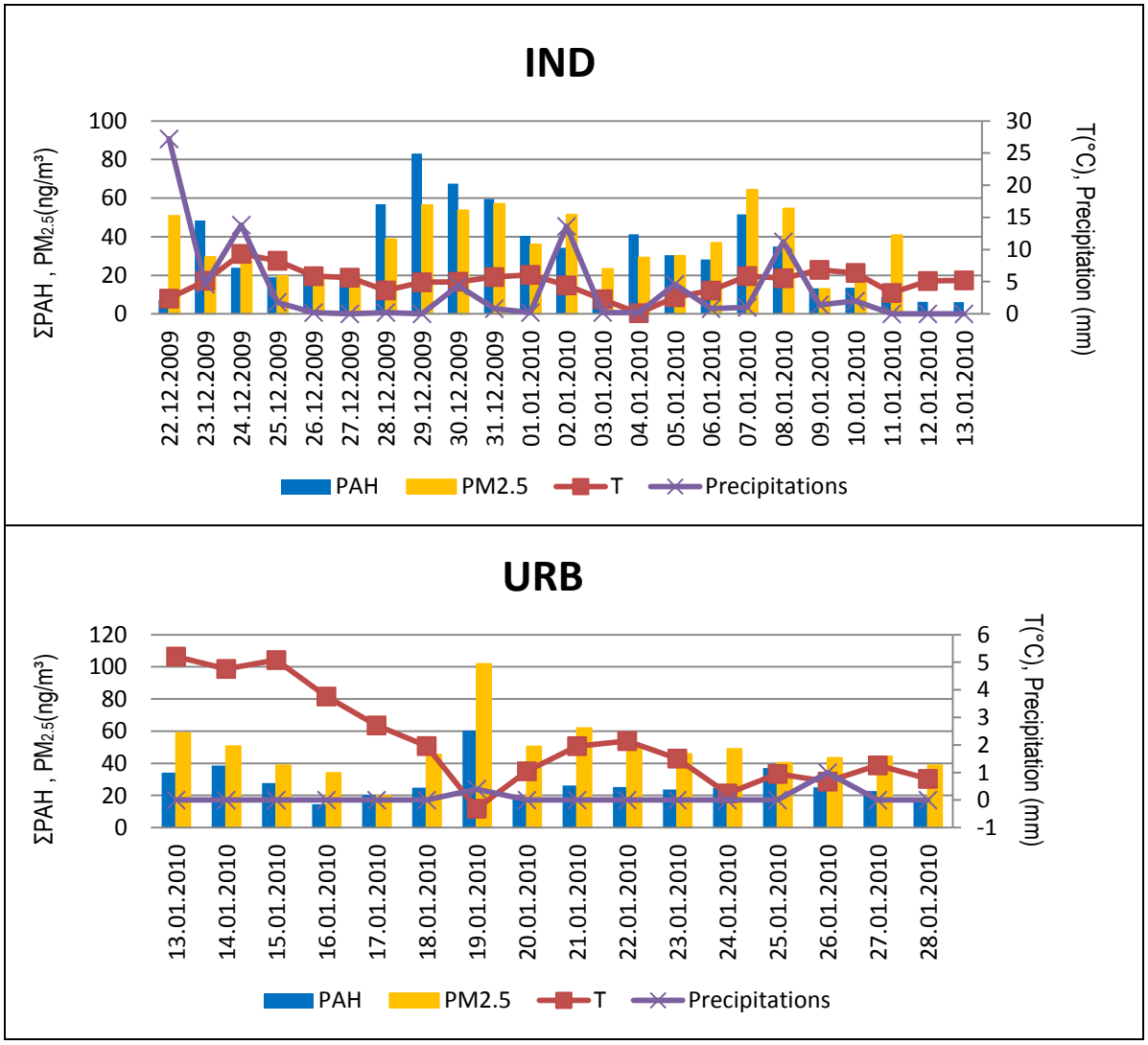


Fig. 16: Correlation between $\sum_{16}$(PAH), $PM_{2.5}$, T, R.H. and the precipitations in the two sampling stations.

In the industrial area and in the urban sampling site as well it can be established no direct correlation between the sum of PAH and the meteorological factors considered. Concentrations of the sum of the PAH in the industrial area ranged between a minimum value of 6.04 µg/m³ and a maximum of 83.13 µg/m³, while those in URB showed a minimum and a maximum value of 14.5 and 60.5 µg/m³, respectively. In Fig. 16 is clearly visible the influence of low temperatures (max = 9.3 and 5.2 °C, min = 0.1 and -0.3 °C for the industrial and the urban site, respectively) and the varying precipitation amounts on the concentration of PAH in the atmosphere of IND and URB.

Further, as the legislation (European Directive 2008/50/EC; DLgs 155/10) has established for B(a)P the mean annual exposure limit of 1 ng/m³, the concentrations of B(a)P in the Venice – Mestre area detected within this study had been observed in detail. As shown in Fig. 17, B(a)P concentrations detected during winter time in the sampling area exceeded the limit in more than the half of the analyzed samples. These high values could be caused by a stagnation of air masses containing high concentrations of PM over the sampling site, as described by Pecorari et al. (2013).

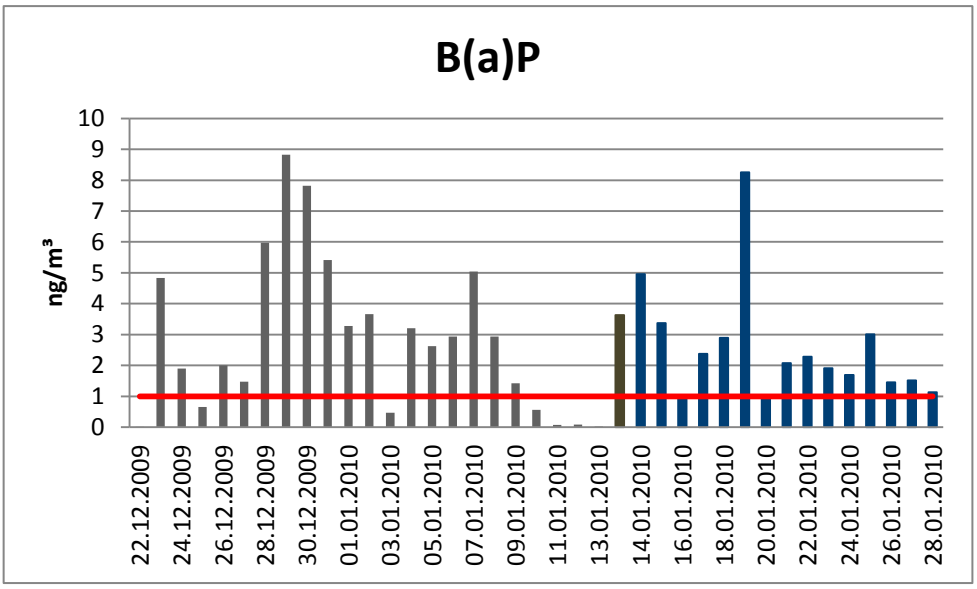


Fig. 17: B(a)P – concentrations within the two sampling stations (grey = IND, blue = URB). Red line = exposure limit established by the legislation (1 ng/m³).

As a result of these high B(a)P concentrations, the **mutagenic and carcinogenic potency** of the PAH – mixture during wintertime was established by calculating the BaP-MEQ and the BaP-TEQ (chapter 3.1.1). Therefore, values of B(a)A, Chry, B(b)F, B(k)F, B(a)P, IP, Db(ah)A, B(ghi)P were elaborated as described in eq (3) and eq (4). The B(a)P - TEQs and - MEQs were calculated for each day (table 9), demonstrating the highest carcinogenic risk in IND on Christmas Eve, from the 28.12.2009 until New Years' Eve as well as on January 9, 2010. In the urban area, the highest exposure risk was attributed to the days January 19 and January 25, 2010.

Table 9: B(a)P - TEQ and B(a)P - MEQ (ng/m³) values calculated for the sampling period.

IND			URB		
Date	**B(a)P-TEQ**	**B(a)P-MEQ**	**Date**	**B(a)P-TEQ**	**B(a)P-MEQ**
22.12.2009	0.68	0.12	13.01.2010	7.18	6.69
23.12.2009	9.16	8.73	14.01.2010	8.47	8.35
24.12.2009	2.99	3.65	15.01.2010	5.00	5.53
25.12.2009	11.85	2.95	16.01.2010	1.48	1.84
26.12.2009	3.66	3.63	17.01.2010	4.59	3.84
27.12.2009	3.07	2.75	18.01.2010	4.56	4.84
28.12.2009	15.37	11.04	19.01.2010	13.99	13.49
29.12.2009	16.51	16.15	20.01.2010	1.99	2.08
30.12.2009	16.56	13.64	21.01.2010	6.23	3.79
31.12.2009	11.57	11.58	22.01.2010	5.69	3.64
01.01.2010	9.57	7.36	23.01.2010	3.72	3.81
02.01.2010	7.76	6.70	24.01.2010	4.89	3.50
03.01.2010	0.87	1.08	25.01.2010	11.67	6.36
04.01.2010	9.25	7.11	26.01.2010	3.02	2.60
05.01.2010	6.59	5.25	27.01.2010	2.72	2.88
06.01.2010	5.53	5.09	28.01.2010	2.23	2.24
07.01.2010	9.84	8.97			
08.01.2010	7.26	5.97			
09.01.2010	17.98	3.00			
10.01.2010	4.60	1.58			
11.01.2010	0.77	0.40			
12.01.2010	0.55	0.19			
13.01.2010	0.25	0.07			

Further, the distribution of the mutagenic and carcinogenic risk was elaborated for the industrial and the urban area (fig. 18). The mutagenic potencies in IND and URB were in average 5.5 (median 5.09) and 4.7 (median 3.8), respectively, while those of the carcinogenic potency were 7.49 (median 7.26) and 5.5 (median 4.7), respectively (fig. 18). Maximum and minimum values of BaP-TEQ detected in IND and URB were 13.99 (1.48) ng/m³ and 17.98 (0.25) ng/m³, respectively. BaP-MEQ on the other hand showed maximum values of 13.49 and 16.15 ng/m³ for IND and URB, respectively, while calculated minima were 1.8 and 1.5 ng/m³ in IND and URB, respectively.

Therefore it can be established that the carcinogenic and mutagenic potency is higher within the industrial area than in the urban one.

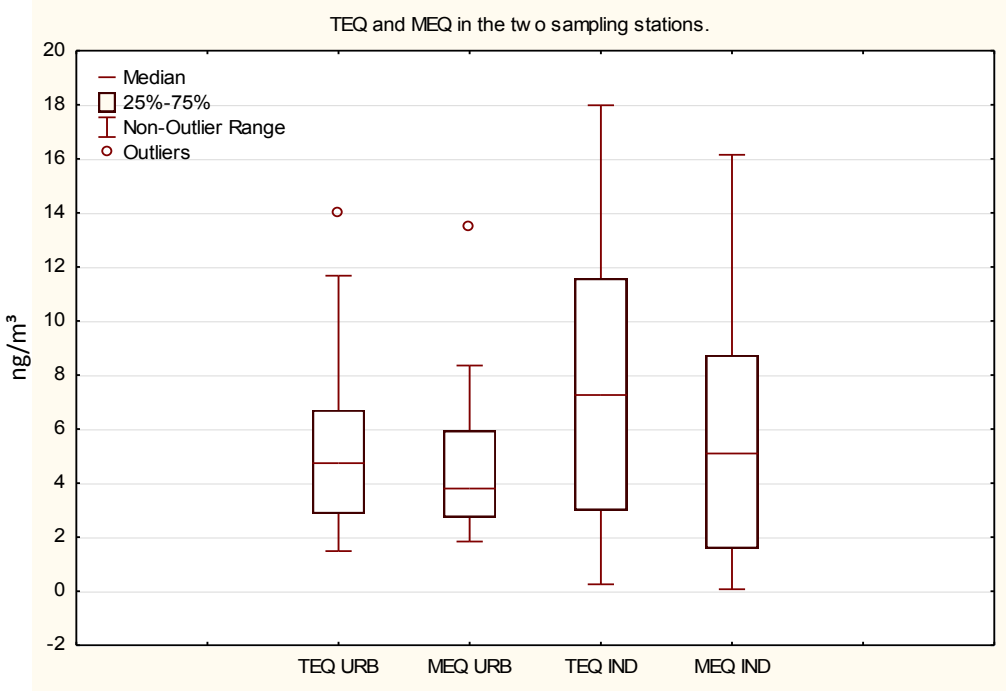


Fig. 18: Distribution of the carcinogenic (TEQ) and mutagenic (MEQ) potency within the two sampling sites.

When comparing the obtained B(a)P-TEQ data to those of other studies (Callén et al., 2011 and references therein; Callén et al., 2012), only the values of URB are comparable (Zaragoza (2012), 0.29 ng/m³; Zaragoza (2011), 0.54 ng/m^3; Athens, 1.6 ng/m^3; Florence, 2.6 ng/m^3; Argel, 3.4 ng/m^3), while those of IND exceeded the mean of the other studied areas.

6.1.2 Analyses of the carbonaceous fraction

The carbonaceous fraction was detected by the two different methods: the combustion/NDIR and the TOT Sunset Analyzer in order to establish a correlation between them. This would allow the use of an instrument normally destined to the analyses of the carbonaceous fraction also for the detection of this fraction in the quartz fiber filters exposed previously to ambient air. Before correlating the data, the means of TC, EC and OC values detected on blanks (table 10) which were analysed in the same way as the samples were subtracted from the values of TC, EC and OC achieved from the samples.

Table 10: Blanks' values of TC, EC and OC (µg/m³).

	TC	**EC**	**OC**
Shimadzu	1.35	0.32	0.77
Sunset	1.09	0.21	0.94

Minimum and maximum values detected with both instruments, the Shimadzu TOC analyzer and the Sunset OC-EC analyzer, are listed in table 11. Blank values are already subtracted.

Table 11: Minimum and maximum values (µg C/m^3) detected with both instruments.

	Shimadzu		Sunset	
	Min	max	min	max
TC	0.16	40.37	1.48	48.41
EC	0.90	36.56	0.58	41.04
OC	0.50	13.88	0.71	25.05

Correlating the data of TC obtained by the two analyzing methods, the correlation factor (Pearson's correlation, $p<0.05$) is $r = 0.976$ (Fig. 19). This correlation of 98% for TC demonstrates that both analyzing methods can be applied to detect the amount of total carbon in air samples.

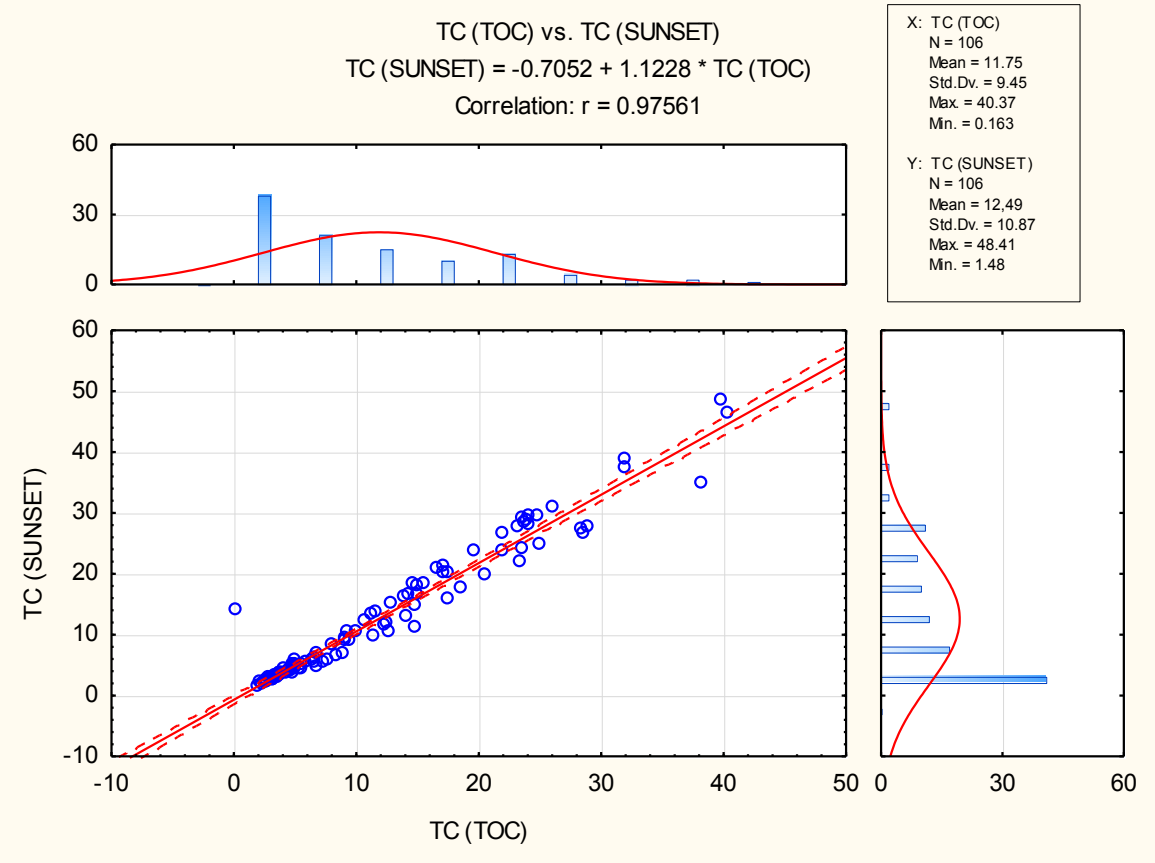


Fig. 19: Correlation of the concentration of the total carbon (µg C/m³).

Data of OC were well correlated, too, with a correlation factor of r = 0.85, showing minimum and maximum values of 0.90 and 0.58, and 36.56 and 41.04 µg C/m^3 for SHIMADZU and Sunset, respectively (Fig. 20). The data of OC referred to the Shimadzu analyzer were obtained by the subtraction of the elemental carbon concentration from the total one, as during the pre-combustion at 350°C in the muffle, the entire organic fraction should be eliminated.

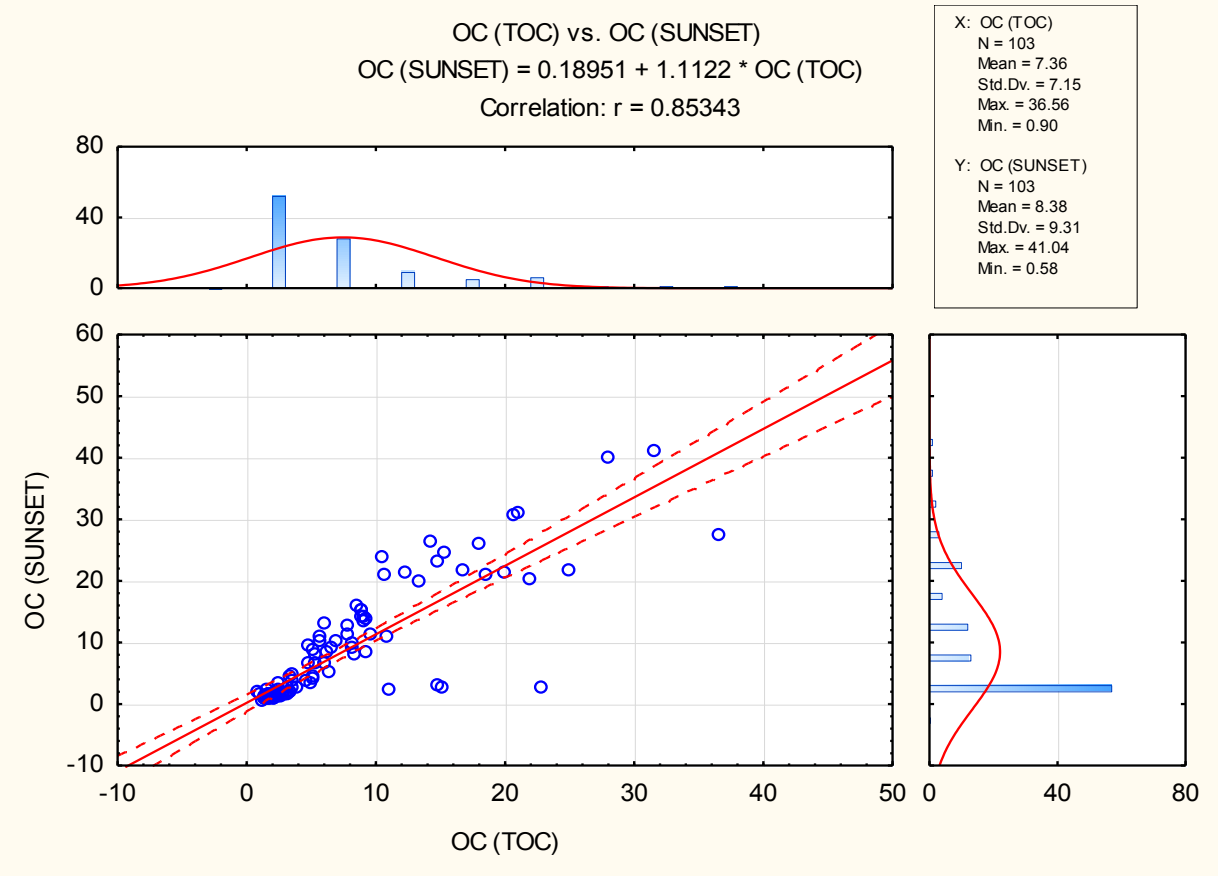


Fig. 20: Correlation of the concentrations of organic carbon (µg C/m³).

Data of EC vary when acquired with the two different methods, as they were correlated only at 46% (Fig. 21). Interferences in EC analyses could have been caused by the pyrolysis which increased the amount of EC, or, on the other hand, phenomena of combustion of native EC occurred during the pre-treatment in the muffle which decreased the amount of EC in some samples (Cachier et al., 1989; Piazzalunga et al., 2013). This conclusion was reached after analyzing 10 test samples, cut in two pieces where one half was pre-treated and one not. Both halves were analyzed with the TOT-Sunset Analyzer.

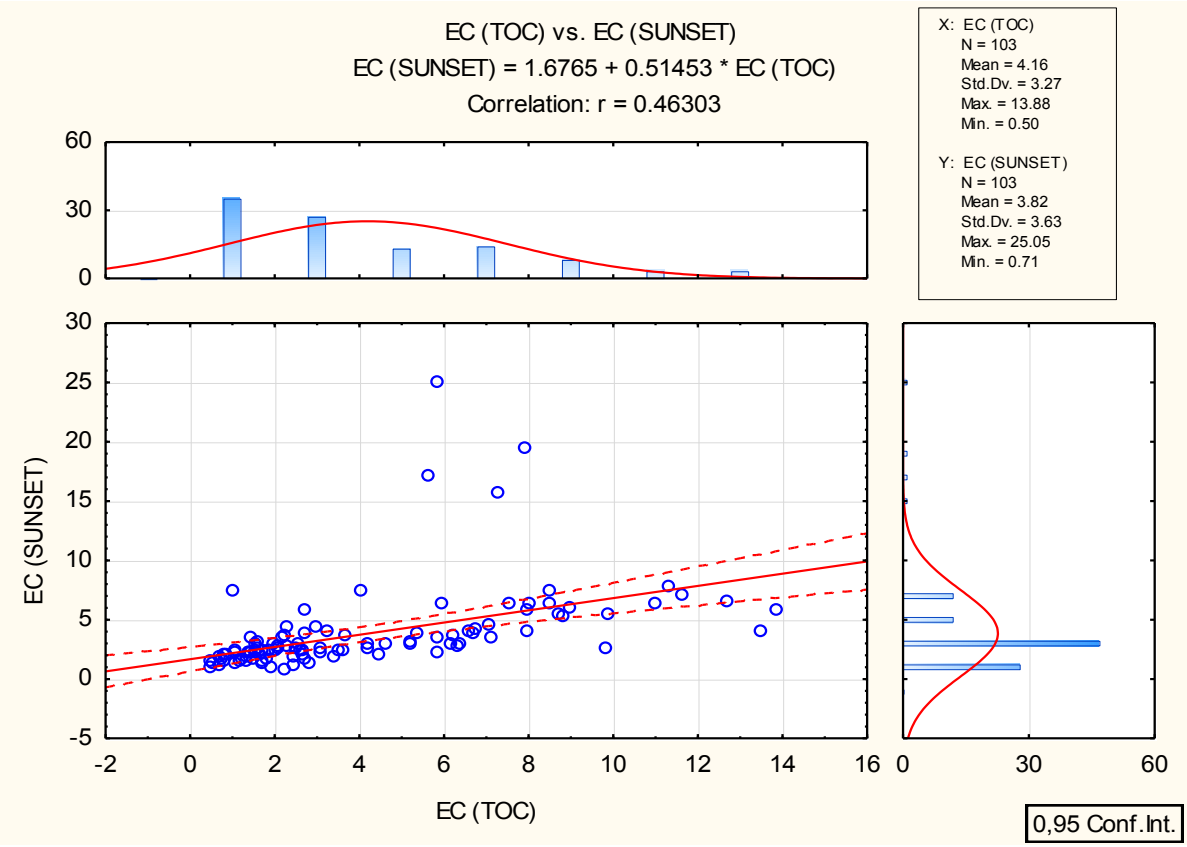


Fig. 21: Correlation of elemental carbon concentrations (µg C/m³).

These results demonstrate that the SHIMADZU-analyzer can be used also for the analyses of the carbonaceous fraction on filters. The only attention must be given to the fraction of the elemental carbon, whose correlation perhaps could be improved by using oxygen instead of ambient air in the pre-combustion process.

6.1.3 Analyses of levoglucosan

Analyses of levoglucosan as marker of the biomass combustion indicated that in the area of Venice – Mestre wood is still used as heating source, as revealed by the analyses of 119 samples collected from the end of December 2008 until the end of December 2009. During summer time, mean concentrations were 2.83 and 6.37 ng/m³ in URB and IND, respectively, while during wintertime, those values rise up to 137.81 and 140.39 ng/m³ in URB and IND, respectively (Fig. 22). During summer, 11 values obtained in the industrial area samples were <LOD.

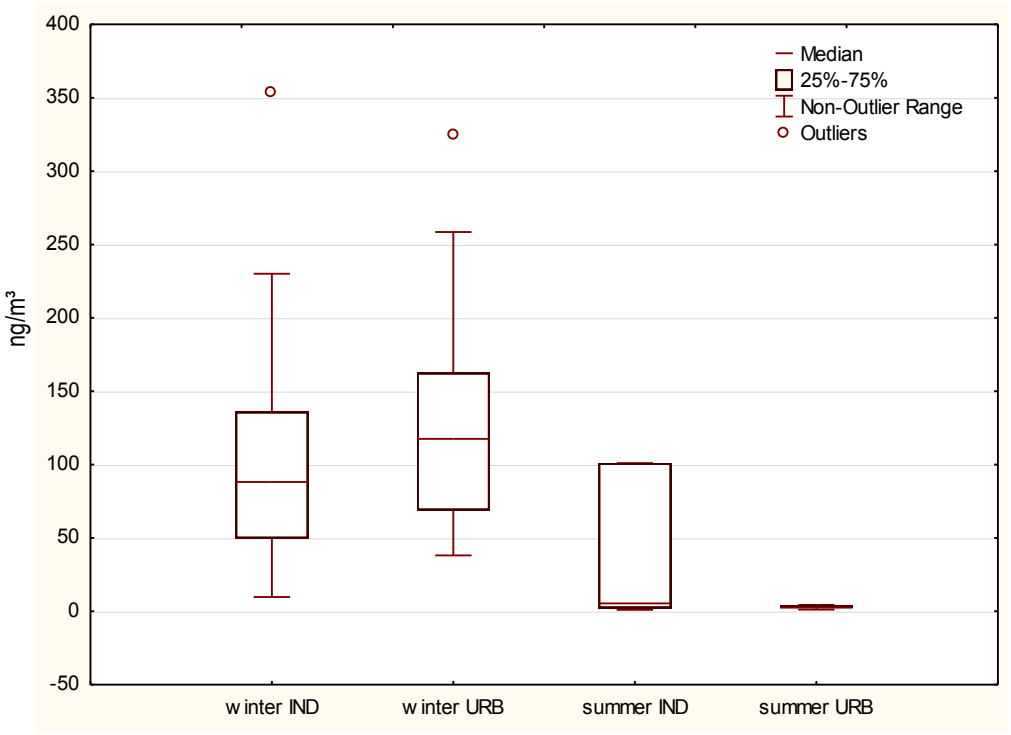


Fig. 22: Distribution of levoglucosan in the two sampling sites URB and IND during winter and summer time.

In the Venice – Mestre area the start of the winter heating on November, 1 can be detected by inspecting the trend of the Levoglucosan analytical results. Levoglucosan concentrations during winter were high in the urban area as well as in the industrial area.

In the urban area, highest concentrations of levoglucosan were detected during winter time (fig. 23), while lowest could be attributed to the summer season. As levoglucosan is the marker of biomass burning events, in this work was demonstrated by the huge differences of the detected concentrations during summer (less than 5 ng/m³) and winter time (over 300 ng/m³) in the urban area (Fig. 23), that in the area of Venice – Mestre wood is still used as heating material in domestic heating.

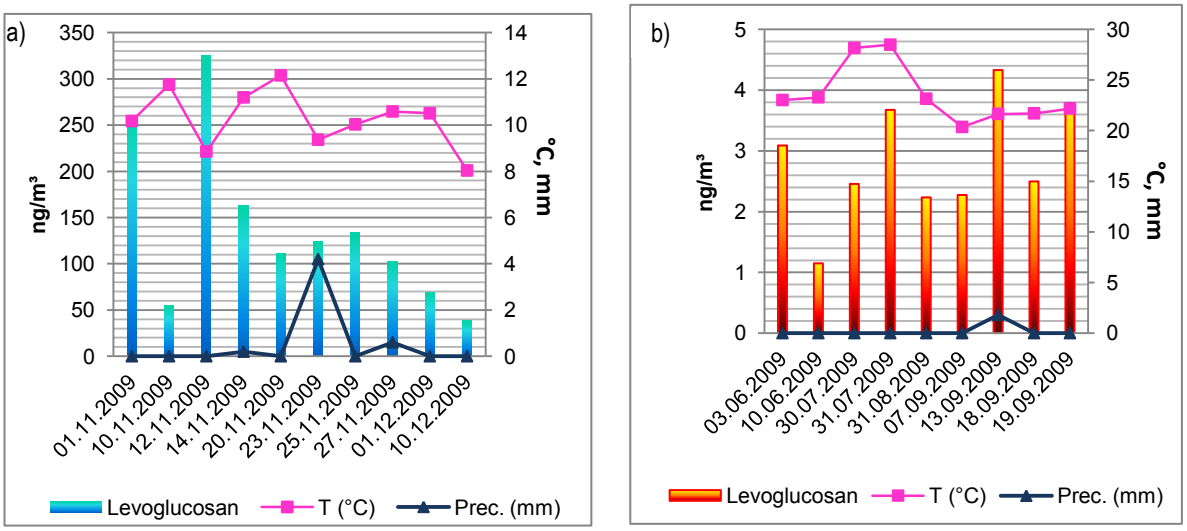


Fig. 23: Levoglucosan in URB during a) winter and b) summer, correlated to T and precipitations.

During winter, the wind blows mainly from NNE and WSW (fig. 24), therefore it can happen that the concentrations in IND were influenced by the emissions from URB.

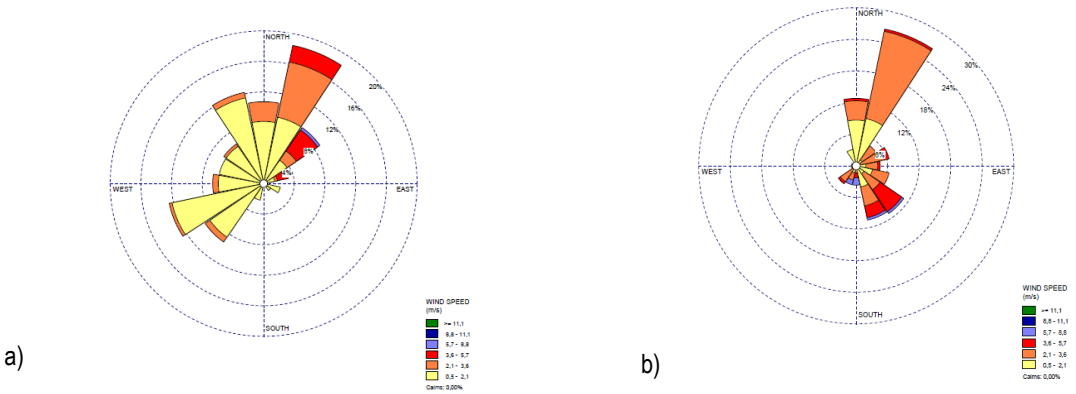


Fig. 24: Wind speed and direction in the sampling area during a) winter and b) summer.

In the industrial area, the peak on 11.11.2009 indicated the beginning of the heating season in industries and housholds located in this area. Highest values range around 1000 ng/m³ during winter time. In comparison, during summertime the value of 35 ng/m³ was not exceeded (fig. 25).

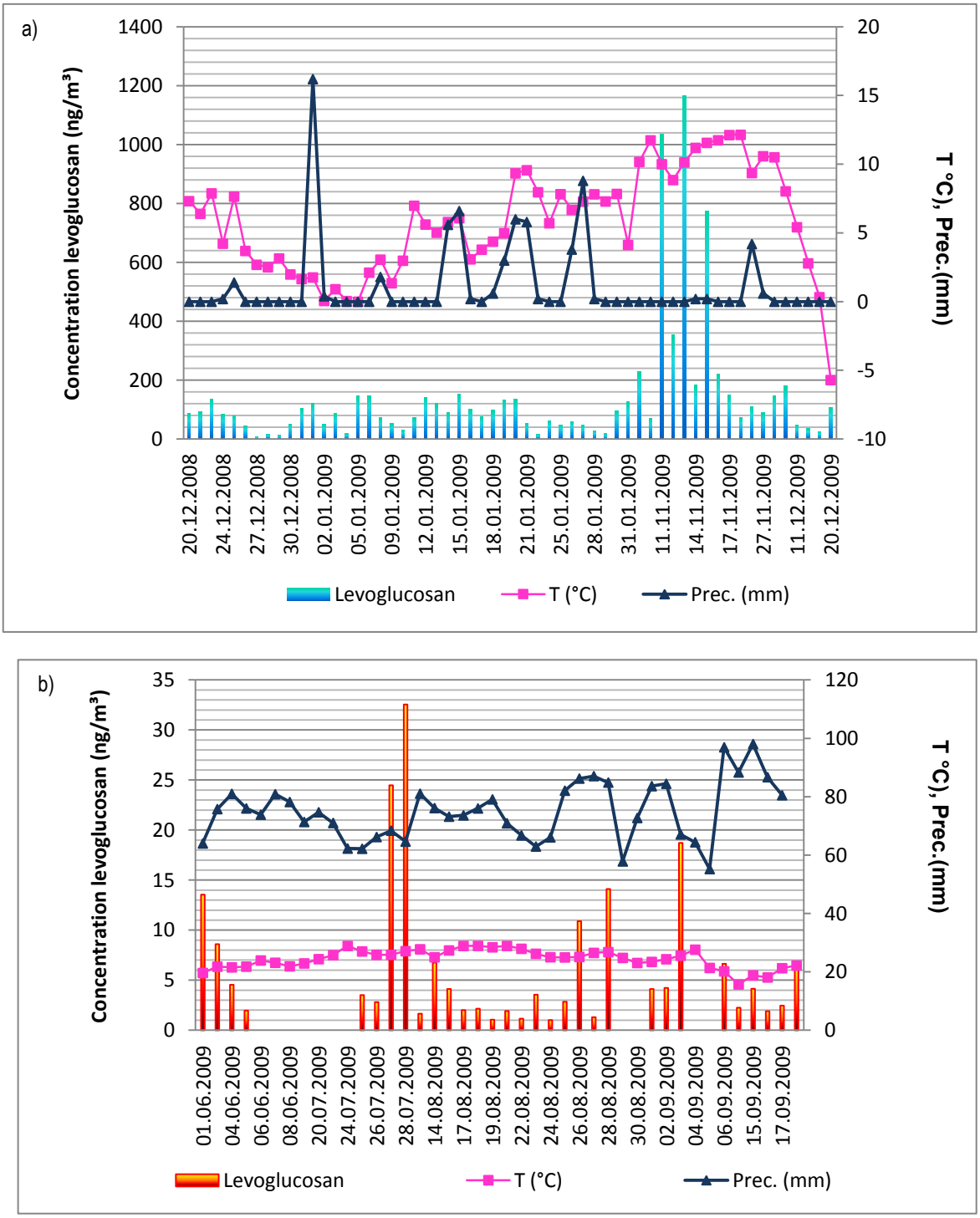


Fig. 25: Levoglucosan in IND during a) winter and b) summer, compared to temperature and precipitations.

This results demonstrated that in the area of Venice and Mestre, wood is still used for domestic and/or industrial heating.

In comparison to other studies carried out in Europe (Puxbaum et al., 2007; Piazzalunga et al., 2010), values of levoglucosan in the Venice – Mestre area are comparable to those achived in the Milan area. In Venice, winter values range between 9.73 and 1165.97 ng/m³, where minimum values were detected the days after the Christmas eve (27. – 30.12.2008) and maximum values during november 2009, when the official heating season had started. In Milan, levoglucosan levels during winter time range between 173 – 963 ng/m³ (Piazzalunga et al., 2010), while in background sites within Europe, Puxbaum et al. (2007) detected values ranging from 6.6 – 1290 ng/m³. For the summer season, values detected by Puxbaum et al. (2007) range from 2.0 – 31.5 ng/m³, those carried out within this work 1.02 – 32.55 ng/m³.

6.2 Biomonitoring

6.2.1 Meteorological conditions

Before starting with the discussion of the results of the organic and inorganic fraction detected in *T. aeranthos* with the use of different methods, I would like to introduce shortly the meteorological data which prevailed within the sampling period in the sampling area. As some of the plants were exposed to indoor environments (greenhouse in the botanical garden, office and garage), the meteorological conditions of temperature (T), relative humidity (R.H.), insolation, (G.Rad.) were quite constant:

- *Greenhouse*: T = 15 – 25 °C; R.H. > 85%; watering once a day; ± constant insolation because of sunshades during summer;
- *Office:* T = 20±3 °C; R.H. < 50%; watering once a week; exposed next to a window with half-closed sunshades during summer;
- *Garage*: open doors – therefore, the exchange between outside and inside air masses was moderate. As T and R.H. variations compared to the outside values could be were low, the meteorological data achieved on the outside (MF) were considered in the further analyses. Plants were watered once a week.

For watering, water from the drinking water supply was used. It was also tested the use of distilled water for the frequent watering, but plants tended to dry more leaves compared to those watered with normal water. As it was supposed that dry leaves take up less or no particles anymore, normal water was used.

On the other hand, plants exposed to the outside environment did not need any further watering because of their metabolism which allows the opening of the stomata only during night time when it is cooler and more humid. So, the plants exposed outdoors took the water they needed from the atmosphere.

As Brighigna et al. (2002) had exposed other species of Tillandsia in similar climatic conditions to those in which this study was conducted and his study had to be interrupted because of the sensitivity of the plants to low temperature, the concern of the present study was that it would had

happened the same. But, *T. aeranthos* plants were resistant also during some daily episodes of temperatures around and below 0 °C.

The meteorological conditions the plants were exposed in the different outdoor environments are demonstrated in table 12, representative for all the outdoor sampling sites. In this table, the mean of each meteorological parameter was calculated for the four seasons. Therefore, the daily minima and maxima of the different parameters the plant had to support within the whole sampling period were not considered in the further analyses.

Table 12: Mean values of meteorological data in the outdoor sampling stations (data furnished by EZI Marghera). (T = temperature, prec. = rainfall, R.H. = relative humidity, G. Rad. = global radiation).

	T (°C)	Prec. (mm)	R.H. (%)	G. Rad. (MJ/m²)
Summer (June 21 - September 22, 2012)	24.7	1.7	66	22.3
Autumn (September 22 - December 21, 2012)	12.7	3.88	72	6.8
Winter (Dezember 21, 2012 - March 20, 2013)	4.6	3.03	63	2.9
Spring (March 20 - June 21, 2013)	15.1	4.1	78	18.02

Solar radiation and temperature showed proportional values, with lowest data during winter time. During summertime 2012, precipitations were lacking. On the other hand, during autumn and spring precipitations and relative humidity were detected to be the highest. During summer time, average T varied between 16 and 26 °C, the insolation exceeded the value of 20 MJ/m², precipitation means show a maximum of 5 mm and the relative humidity ranges below 75%. During autumn, temperatures and global radiation decreased significantly, relative humidity and precipitations first increased and then decreased again. The winter season started with high humidity periods, while the low values of T, G.Rad. and Prec. were quite stable. In spring, the values of all meteorological parameters increased, except those of the precipitation. The corresponding samples to each season are listed in table 13.

Table 13: Samples' exposure seasons within the three sampling periods.

Sampling Period	Plant number	Date: exposed from - until	Season
1	1	25/06/2012 - 19/07/2012	Summer
	2	25/06/2012 - 09/08/2012	
	3	25/06/2012 - 31/08/2012	
	4	25/06/2012 - 21/09/2012	
	5	25/06/2012 - 12/10/2012	Autumn
	6	25/06/2012 - 31/10/2012	
2	1	31/10/2012 - 22/11/2012	
	2	31/10/2012 - 13/12/2012	
	3	31/10/2012 - 03/01/2013	Winter
	4	31/10/2012 - 24/01/2013	
	5	31/10/2012 - 14/02/2013	
	6	31/10/2012 - 07/03/2013	
3	1	07/03/2013 - 28/03/2013	Spring
	2	07/03/2013 - 18/04/2013	
	3	07/03/2013 - 09/05/2013	
	4	07/03/2013 - 30/05/2013	
	5	07/03/2013 - 20/06/2013	

In a second step were elaborated wind roses to know the influence of the local transport of air masses on the concentrations of PAH and metals detected in *T. aeranthos'* leaves. For this purpose, the sampling station of MC (industrial area) was identified as the most appropriate one to represent the situation in the whole outdoor sampling area (Masiol et al., 2012a; Pecorari et al., 2013). In Fig. 26 are shown the wind roses for the four seasons.

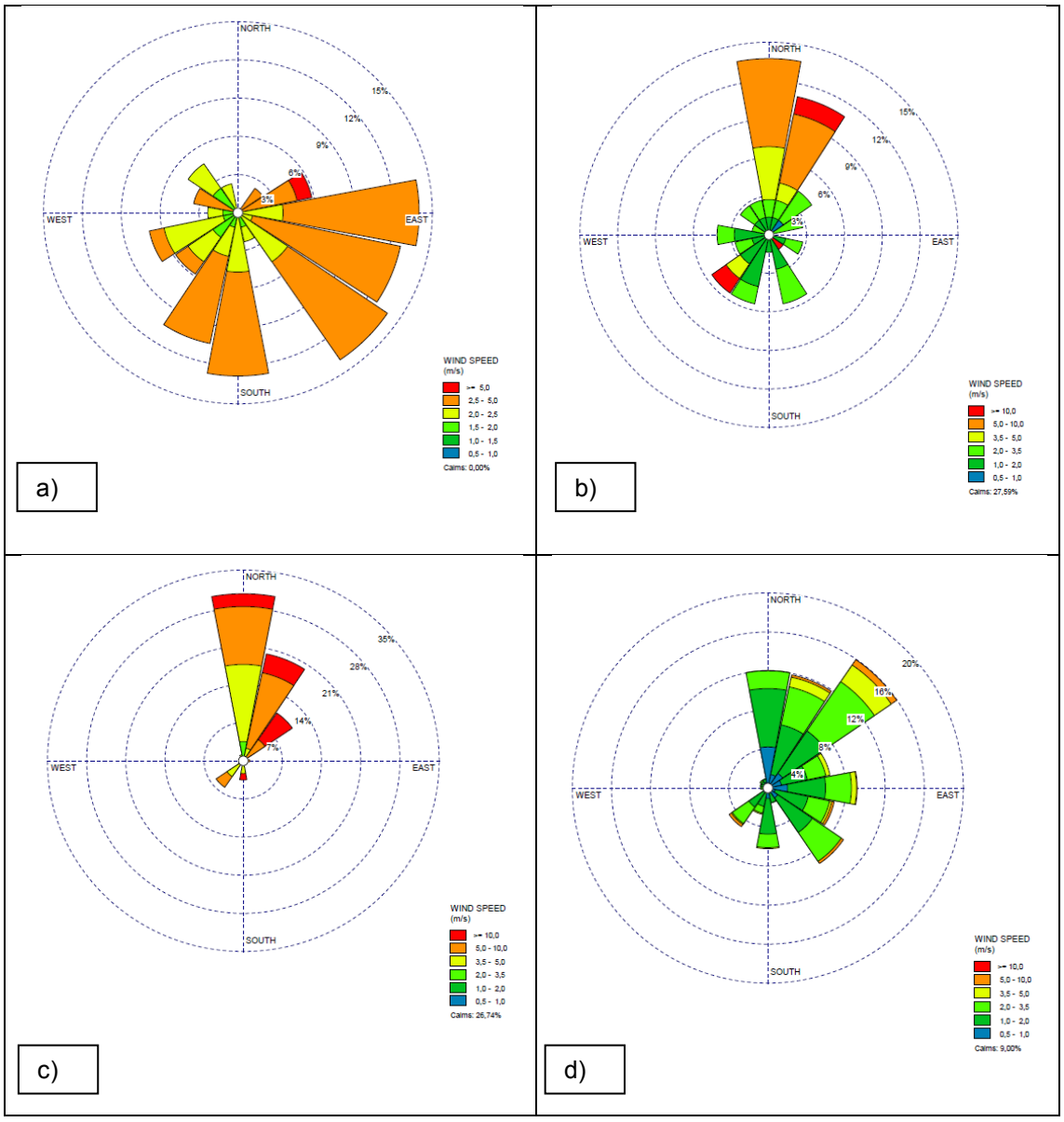


Fig. 26: Wind roses for the four seasons summer (a), autumn (b), winter (c), and spring (d) in MC.

From these wind roses the variability of the wind within the different seasons is evident. Nevertheless, the prevailing directions were N and NE during winter time which identifies the surrounding industrial and the a little farer agricultural area as main sources. During summer time as well as during spring and autumn, the wind brought air masses from N, NE but also from S and SE, which indicates the influence of sea breezes.

Furthermore, as also air masses deriving from an interregional scale calculated at 200 m a.s.l., can carry some pollutants which could influence the concentrations detected in the plant leaves, back-trajectories were carried out. In Fig. 27 are reported the back-trajectories representing the long range transport of air masses within the four seasons reaching the sampling area.

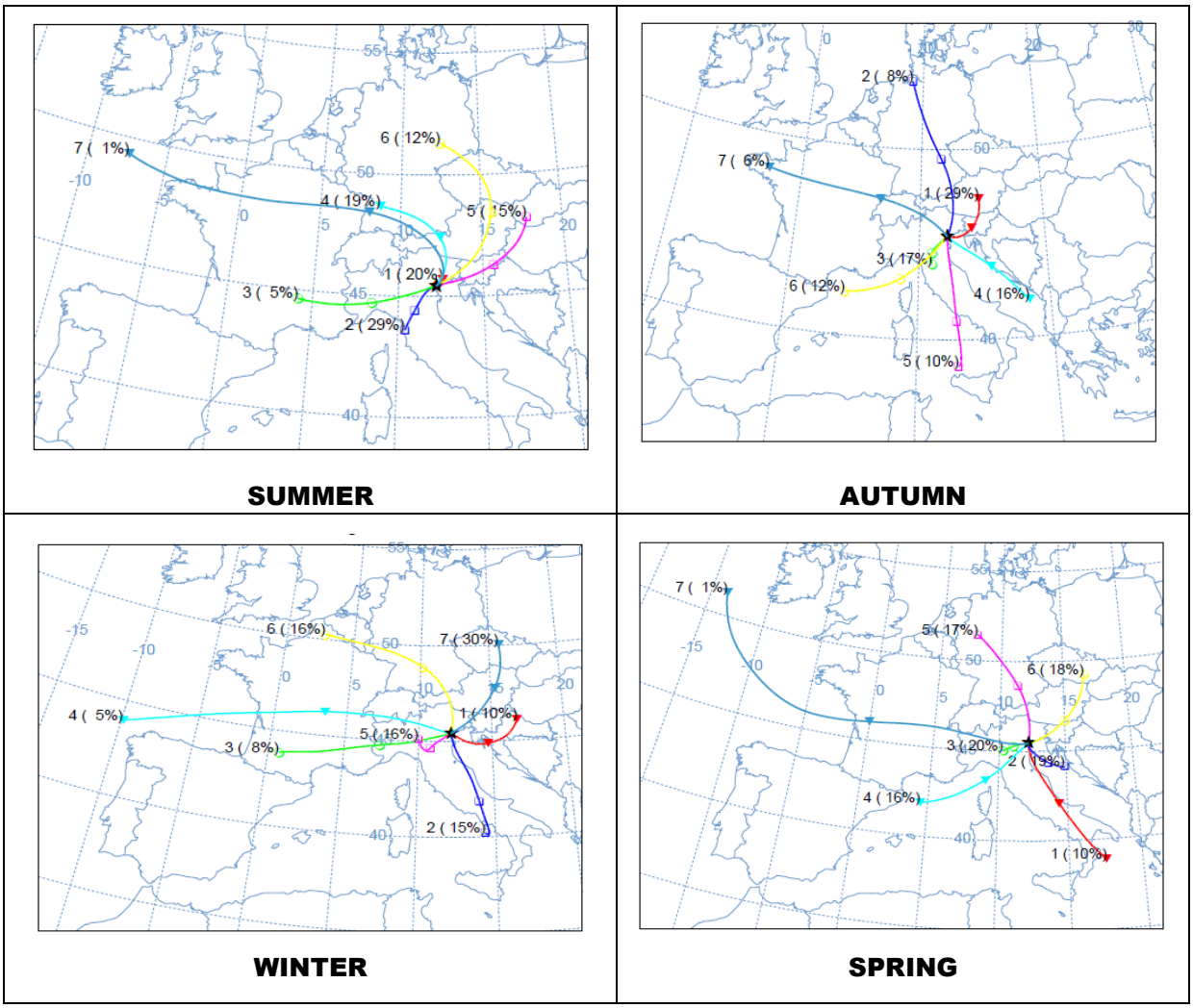


Fig. 27: Back-trajectories for the sampling periods 1(a), 2 (b) and 3 (c).

Back-trajectories vary significantly from one season to another. While during summer time air masses derive mainly from the area below the Po Valley and Central Europe, in autumn and winter prevail air masses deriving from the eastern part of Europe. In spring, no predominant direction could be figured out.

6.2.2 Analyses of plants' properties

1) Weight difference before and after exposure, DW/FW ratio and RWC

To establish the ability of *T. aeranthos* to take up water from the atmosphere and with it also particles from the air, the weight difference of the plants ($\Delta W = W_{after\ exposure} / W_{initial}$) was calculated. In the outdoor environment LF, MC and MF, plants have lost in mean 1.1, 0.28 and 1.4 g, respectively. In comparison, in the indoor environment GBP, where plants had been watered each day and T and R.H. values were almost constant, they weighted averagely 2.02 g more after exposure than before.

On the other hand, plants exposed in LD and Wk and watered once a week showed a loose in weight of -1.66 and -1.61 g, respectively, in average over the total sampling period.

It was found out that during summer time, plants exposed to the outdoor environment lost more weight and so did also plants in GBP, whereas in the indoor sampling station LD plants showed the highest loss, perhaps caused by the property of the heating to dry out the air in indoors. In Wk, an almost stable weight loose of 4±0.7 g was detected in all four seasons.

The DW/FW ratio was calculated for each plant and the results of the annual means range between 0.29 g/g (FW) in GBP and 0.38 g/g (FW) in LF and Wk. For samples exposed in MC and MF, an annual average value of 0.34 g/g (FW) was measured, while in LD the value of 0.37 g/g (FW) was calculated. These values are in accordance with those calculated by Pignata et al. (2002) for *T. capillaris*.

The relative water content (RWC) was calculated according to eq (6), chapter 3.2.1 in this work. Resulting values ranged between 59% (Wk) and 74% in GBP. Data are summarized in Table 14.

Table 14: Δ W, DW/FW, RWC.

	Δ W summer	Δ W autumn	Δ W winter	Δ W spring	**Yearly average**	DW/FW (g/g (FW))	RWC (%)
GBP	2.67	2.39	0.91	2.10	**2.02**	0.29	74
LD	-0.95	-1.44	-2.16	-2.00	**-1.66**	0.37	60
LF	-2.66	-0.21	-1.02	-0.66	**-1.11**	0.38	61
MC	-1.72	0.57	-0.51	0.83	**-0.28**	0.34	65
MF	-3.05	-1.06	-1.01	-0.71	**-1.41**	0.34	63
Wk	-1.17	-1.02	-1.16	-0.82	**-1.03**	0.38	59

2) Interpretation of organic pollutants

a) Lipid content in *T. aeranthos*

The lipid fraction detected in *T. aeranthos* plants vary between 0.82 and 9.6 mg/g (DW), where the lowest amount was determined in GBP samples and the highest one in plants exposed in Wk. Average values for each season in each sampling station are reported in Table 15.

Table 15: Lipids (mg/g DW) in *T. aeranthos* plants (highest values are highlighted).

	summer	**autumn**	**winter**	**spring**
GBP	1.62	4.85	5.602	5.44
LD	1.54	5.91	5.73	5.58
Wk	1.77	6.18	8.08	5.604
mean indoor	**1.64**	**5.65**	**6.47**	**5.54**
LF	2.44	6.62	7.85	4.54
MC	2.44	5.67	6.97	5.79
MF	2.09	5.57	8.14	5.53
mean outdoor	**2.32**	**5.96**	**7.65**	**5.29**

These results show that during summer time, the lipid content is the lowest in both, indoor and outdoor exposed plants. For the outside exposed plants the increase of lipids during the colder seasons autumn and winter can be explained by the normal metabolism of the plants and their self-defence mechanism, as water can freeze what could cause the death of the plants. In the indoor exposed plants the difference in the lipid amount between the different seasons was still given, which can be explained by the daily air exchange activities which allowed the entering of cool air.

b) Kv of T. aeranthos

Kv determined by Simonich and Hites (1994) was used to establish a correlation between the values of the PAH congeners B(a)A, Chry, B(a)P, IP, and B(ghi)P detected with active monitoring methods (ARPAV) and those obtained by the biomonitoring project with *T. aeranthos* exposed outdoors. The results are shown in fig. 28.

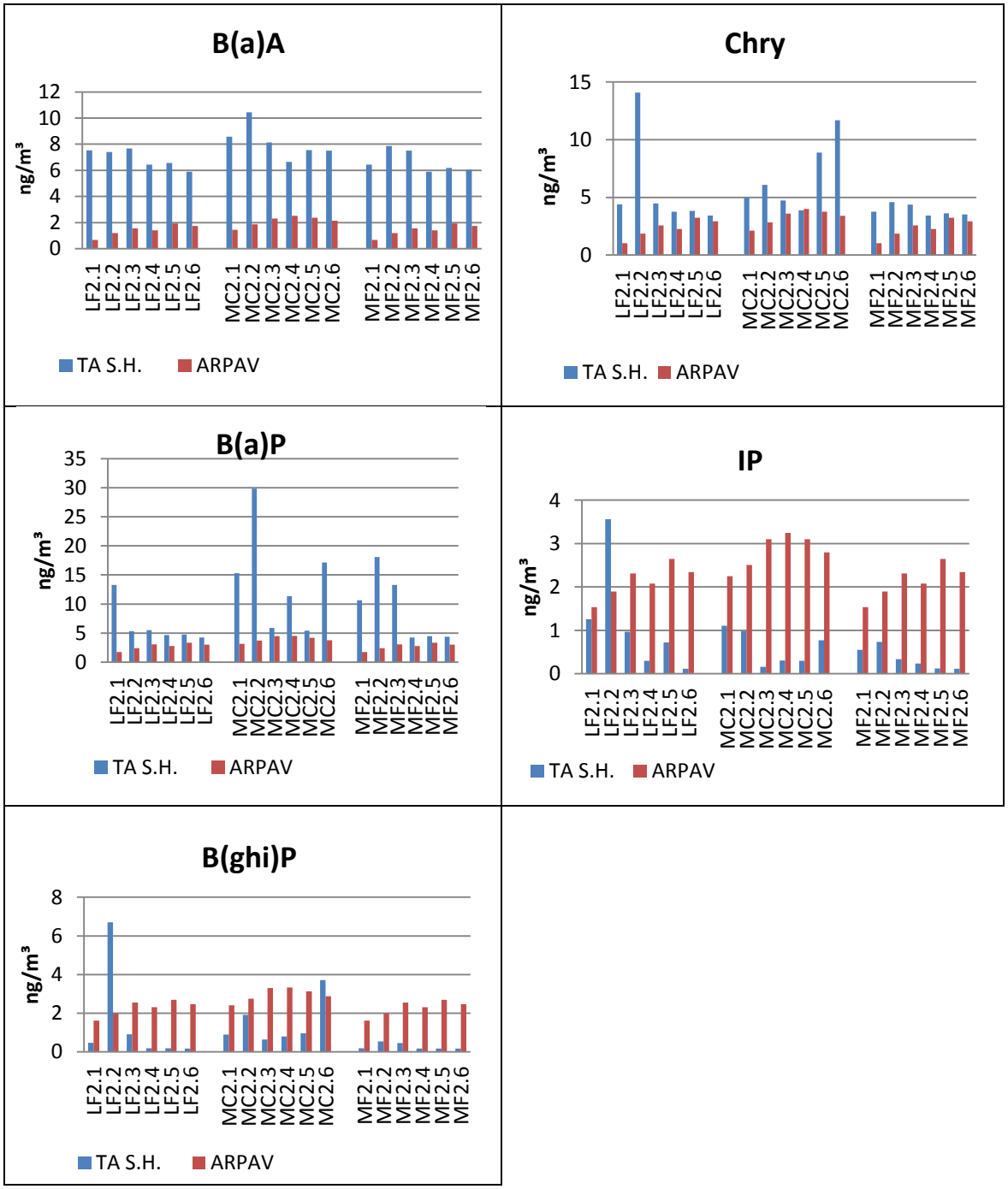


Fig. 28: Comparison of validated data of ARPAV (red) with the estimates calculated with Kv taken from Simonich and Hites (1994) (blue). Values are reported in ng/m³.

Data in fig. 28 refer only to the second sampling period. From the histograms in fig. 28 can be clearly demonstrated that values of B(a)A and Chry were overestimated when calculated, while compounds with a higher molecular weight as IP and B(ghi)P were underestimated in most of the cases.

As B(a)P is the PAH congener of major interest because of its demonstrated carcinogenicity and mutagenicity, the estimation of the concentration of $B(a)P_{atm}$ (ng/m³) for the outdoor sampling stations showed the best correlation with respect to the other compounds. In the semi – harbour site (LF) and the urban site (MF), a good estimation was found for winter samples, while in the industrial site (MC), values were too variable as a trend could be detected. However, values of ARPAV were lower than the estimates, except in MC.

Because of these results, in this work was tried to establish a new constant Kv only for T. aeranthos (table 16). As values obtained for B(a)A and Chry in *T. aeranthos* were detected to be <LOQ in more than 85% of the cases, the so calculated Kv needs to be handled with caution.

Nevertheless, as in the literature could be found only the values of Simonich and Hites (1994) as correlation factors, this was the first attempt to obtain a correlation factor for PAH - congeners detected in a Tillandsia species. Therefore, further investigations to obtain better results are absolutely necessary.

Table 16: Proposal of a Kv for *T. aeranthos*.

PAH congener	B(a)A	Chry	B(a)P	IP	B(ghi)P
Simonich and Hites (1994):	1.42	1.29	2.47	2.79	3.18
This work's proposal:	4.37	3.75	3.26	1.02	3.34

6.2.3 PAH in T. aeranthos

When analyzing the concentrations of the 18 PAH congeners listed in Fig. 3 in chapter 1.1.2.1.a) which were found in *T. aeranthos* leaves, many gas – and gas-particle phase PAH showed values <LOQ. Therefore, the value of LOQ/2 substituted the values <LOQ in order to get a complete dataset. The concentrations of the annual means, maximum and minimum values of single PAH - congeners within the different seasons are reported in ng/g DW in table 17.

Table 17: Annual mean, maximum and minimum concentrations (ng/g DW) of the single PAH - congeners in the indoor and outdoor sampling stations.

indoor	Naph	Acen	Ace	Flu	Phen	Anth	Fluor	Pyr	B(a)A	Chry	B(b)F	B(k)F	B(e)P	B(a)P	Per	IP	Db(ah)A	B(ghi)P
GBP mean	66.3	144	6.5	34.8	129	89.3	116	149	69.7	81.4	285	72.5	80.9	365	877	12.1	62.3	34.3
GBP max	368	1283	26.2	245	135	94.2	122	157	73.4	445	2716	642	699	3537	5586	48.4	269	246
GBP min	5.6	9.4	1.1	2.4	113	78.2	102	131	61.3	35.7	41.7	7.9	12.4	52.9	166	1.5	3.1	1.8
LD mean	224	259	6.6	47.7	129	97.9	127	149	592	416	661	145	452	1118	485	11.3	56.2	8.7
LD max	3338	1586	47.5	640	135	240	309	157	8944	4373	4674	813	5086	6791	1089	83.7	236	38.1
LD min	5.6	2.6	0.9	2.3	113	78.2	102	131	61.3	35.7	41.7	6.4	7.4	31.6	167	1.4	8.1	1.8
Wk mean	45.9	86.3	4.7	9	129	89.3	116	149	69.7	61.1	96.6	24.4	30.4	132	355	7.4	93.3	14.6
Wk max	378	453	17.3	33.6	135	94.2	122	157	73.4	390	194	58.6	80.3	232	1001	23	1043	83.7
Wk min	5.6	2.6	1.1	2.3	113	78.2	102	131	61.3	35.7	41.7	7.9	8.5	15.9	167	1.4	3.1	2.1
outdoor																		
LF mean	42.5	228	99.2	48.6	129	89.3	116	149	69.7	216	1483	450	325	1779	1215	12.6	82.1	14.4
LF max	145	1103	1219	482	135	94.2	122	157	73.4	2848	21671	6843	4413	25857	6422	35.3	341	83.1
LF min	5.6	2.6	1.4	2.3	113	78.2	102	131	61.3	35.7	41.7	7.9	3.2	52.9	167	1.4	11.1	1.8
MC mean	13.9	56.2	4.5	19.9	142	89.3	141	163	69.7	47.4	91.9	24.6	54.9	132	435	8.5	35.6	13.8
MC max	33	431	11.8	158	371	94.2	373	396	73.4	114	167	50.9	195	248	1127	50.9	96.2	46.1
MC min	5.6	2.6	0.9	2.3	113	78.2	102	131	61.3	35.7	13.6	6.4	3.2	15.9	55.0	1.4	3.1	1.8
MF mean	16.8	125	5.1	36.1	129	89.3	116	149	109	40.6	132	29.2	39.1	169	509	11.4	54.3	7.2
MF max	59.9	633	14.5	350	135	94.2	122	157	736	42.8	301	50.3	141	414	1390	69	156	42.8
MF min	5.6	2.6	1.4	2.3	113	78.2	102	131	61.3	35.7	41.7	6.4	8.5	15.9	167	1.4	3.1	1.8

From these results can be deduced that the highest values of the single congeners were found for more than 70% in the semi - harbor site (LF outside, LD inside), which initially seemed strange as PAHs derive mainly from the incomplete combustion of fossil fuels.

Therefore, a possible explanation could be given by i) the transport of air masses from the mainland with its industrial and urban emissions, to the island Venice; ii) the ship and cruise traffic in this area; iii) the influence of glass-factories on Murano. Nevertheless, the obtained results are in agreement with those found by Rodriguez et al.(2010), as they detected that the three, four and five – ring PAH congeners established the major part of PAH found in *T. capillaris*.

The detected annual mean PAH values were compared with those found in literature for other species of Tillandsia as no work was found with analyses of PAH in *T. aeranthos* (Table 18).

Brighigna et al. (2002) calculated the congener B(djk)F whose value is compared to the one of B(k)F from this work, while de Souza Pereira et al. (2007) combined B(b)F and B(k)F in their work. Further, Brighigna et al. (2002) identified the compound B(jhi)P, which was compared to B(ghi)P of this work. As only Brighigna et al. (2002) used the same value scale of ng/g as it was adopted within this work, it can be said that values show a difference of one order of magnitude for Acen, Anth, Chry, B(k)F, B(a)P, whereas values Ace, Flu, IP, DB(ah)A, and B(ghi)P show a good correlation.

Even though data listed by de Souza Pereira were kept in ng/kg DW, they show a good correlation to those detected in this work, with the exception of Acen, Anth, B(b)F and B(k)F, and B(a)P.

Table 18: Comparison of results with data found in the literature.

	This work* (outdoor; ng/g DW)	Brighigna et al. (2002)* (ng/g DW)	de Souza Pereira et al. (2007)* (ng/kg DW)
Naph	24.41		54.5
Acen	136.38	20.16	7.8
Ace	36.24	54.55	12.3
Flu	34.91	30.00	20.5
Phen	133.14		149.2
Anth	89.33	8.68	17.4
Fluor	124.46		159
Pyr	153.61		109
B(a)A	82.73	18.52	31.5
Chry	101.48	22.40	89.97
B(b)F	569.05		85.5
B(k)F	167.91	18.92	
B(e)P	139.63		
B(a)P	693.12	20.13	54
Per	719.72		
IP	10.80	8.24	22.4
Db(ah)A	57.33	23.85	13.7
B(ghi)P	11.80	8.88	24

*** calculated annual mean values.**

To analyze in more detail the distribution of the single PAH compounds, boxplots (Fig. 29) were carried out for a) the total data obtained, b) the data from outdoor sampling stations and c) the data from indoor sampling sites. From Fig. 29 are clearly visible the compounds having all values <LOQ, substituted with those of LOQ/2 as they do not show any variance nor outliers, namely Phen, Anth, Fluor, Pyr, B(a)A and Chry. On the contrary, Acen, B(b)F, B(a)P and DB(ah)A are demonstrated those compounds with the largest variance within all the sampling sites.

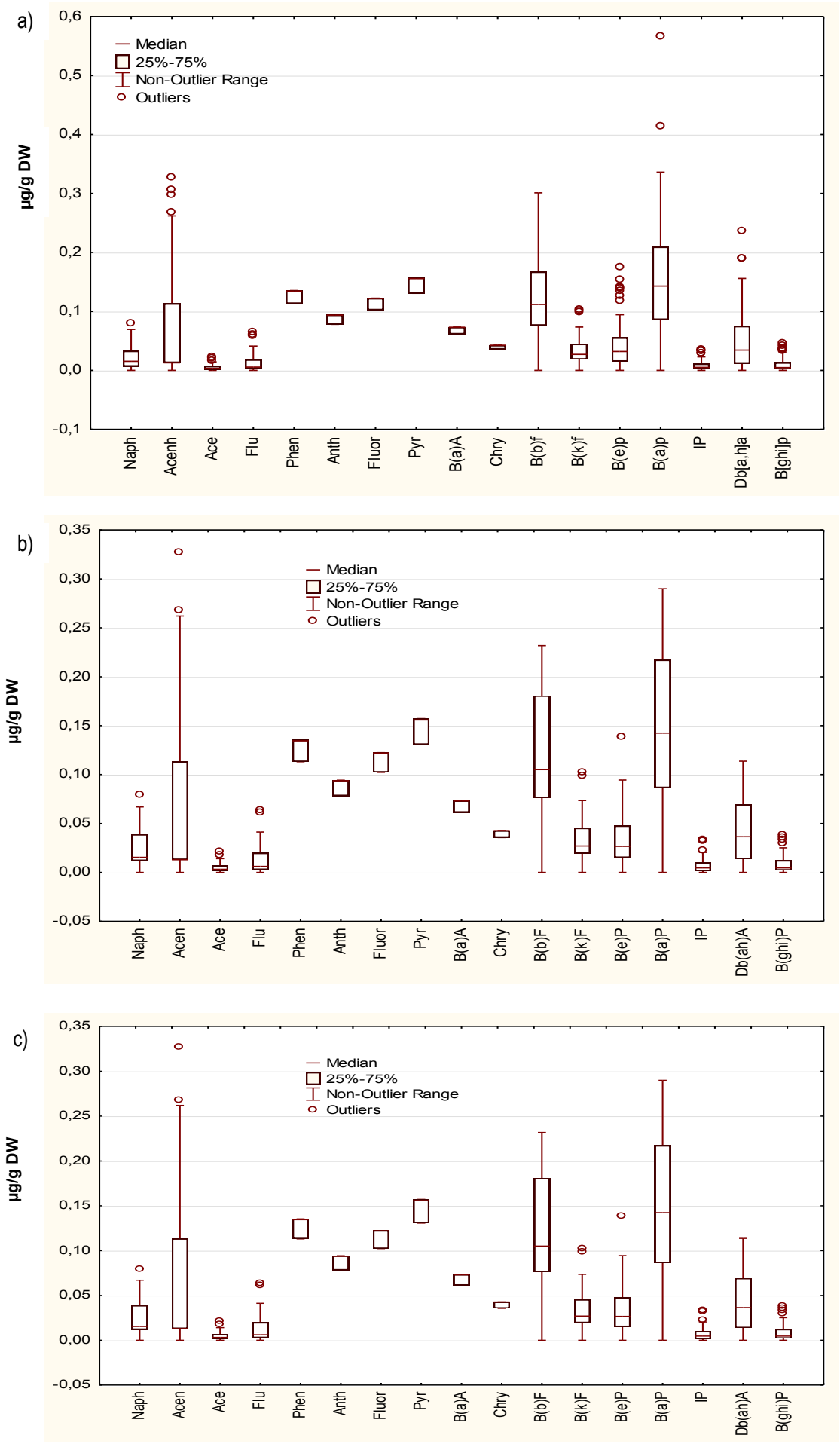


Fig. 29: Distribution of PAH congeners (µg/g DW) in a) the total data obtained, b) the data from outdoor sampling stations and c) the data from indoor sampling sites

To testify the ability of *T. aeranthos* to accumulate PAH - compounds, the mean of each sampling period was compared to the mean of the three blanks treated in the same way like the samples themselves. Results are listed in table 19, where the six compounds Phen, Anth, Fluor, Pyr, B(a)A, Chry are colored in orange because the values listed are in more than 85% of the cases values of LOQ/2. Values colored in red show a loss in the PAH congener's concentration. These results demonstrated that in most of the indoor (GBP, LD, Wk) and outdoor (LF, MC, MF) exposed plants concentrations of the compounds with 5 and 6 benzene rings (B(b)F - B(ghi)P) increased within all three sampling periods. During the first sampling period, also the concentrations of gas - phase PAH congeners in indoor exposed *T. aeranthos* plants were higher than in the blanks. The observation of the inverse proportional behaviour between Per and IP was quite interesting.

Table 19: Comparison of the mean values of each PAH congener of blanks with those of the samples of each sampling period (*see chapter 5.3 p. 53 or table 13 p. 79*).

	Naph	Acen	Ace	Flu	Phe	Anth	Fluor	Pyr	B(a)A	Chry	B(b)F	B(k)F	B(e)P	B(a)P	Per	IP	Db(ah)A	B(ghi)P
B 1	**22.9**	**5.8**	**3.6**	**3.5**	**129**	**89.5**	**116**	**149**	**69.6**	**41**	**75.9**	**27.6**	**19**	**91**	**1.4**	**14.3**	**2.99**	**3.06**
GBP 1	131	315	13.3	34	135	94	122	157	73	110	153	51.8	57	179	1.6	1620	17.7	37
LD 1	569	269	11.5	111	135	118	154	157	1552	900	307	156	866	958	0.5	530	16.2	57
Wk 1	99.8	114	7.4	13	135	94	122	157	73	101	96.6	29.1	38	116	0.35	350	7.5	188
LF 1	24.4	81	263	85	135	94	122	157	73	510	3702	1164	772	4436	1722	7	21	16
MC 1	7.3	14	3.02	5.2	175	94	122	157	73	43	62.6	25.6	63	95	408	3.3	10.7	14
MF 1	10	96	3.97	9.7	135	94	122	157	184	43	120	28.7	36	149	591	5.7	32	10
B 2	**24.5**	**20.9**	**2.09**	**2.66**	**128**	**89**	**116**	**148**	**69.8**	**40.6**	**55.7**	**15.4**	**14.7**	**90.5**	**1.36**	**20.5**	**2.0**	**2.1**
GBP 2	44	17.9	2.4	9.9	135	94	122	156	73	91	552	134	160	716	0.53	531	5.8	41
LD 2	39.9	12.95	1.8	6.7	135	94	122	156	73	43	74	40	22	92.7	0.23	226	5.5	33
Wk 2	15.4	12.95	1.99	6.2	135	94	122	156	73	43	49	11.3	24	102	0.17	167	2.95	26
LF 2	37	112	2.3	2.76	135	94	122	156	73	59	77	18	34	66	274	11.6	34	14.3
MC 2	15.4	15.9	4.2	9.3	135	94	191	196	73	62	85	22.7	52	123	341	5.3	44	13.6
MF 2	15.4	12.95	1.5	2.76	135	94	122	156	73	43	67	23	26	97	204	3. 7	31	2.99
B 3	**26.95**	**9.7**	**3.29**	**5.4**	**128**	**88.9**	**115**	**147**	**68.9**	**40**	**123.3**	**19.9**	**61**	**142**	**16.1**	**45.7**	**11.8**	**9.4**
GBP 3	14.9	91	3.13	66	113	78	102	131	61.3	36	122.3	24	15	167	0.4	401	12.96	118
LD 3	32	543	6.57	20	113	78	102	131	61.3	283	1791	256	470	2542	0.7	742	12.42	83.6
Wk 3	18	142	4.7	7.1	113	78	102	131	61.3	36	154	34	29	188	0.59	586	117	50.4
LF 3	71	544	18	59.8	113	78	102	131	61.3	53	510	112	138	646	1738	20.5	213	12.8
MC 3	20	155	6.60	50	113	78	102	131	61.3	36	135	26	48	186	581	18.5	55	13.5
MF 3	26.6	293	11	108	113	78	102	131	61.3	36	224	37	58	280	775	27.3	110	8.7

When considering the seasonal means next to the minima and maxima of the sum of the 18 PAH congeners, it was clearly visible that in the botanical garden and in the garage, values were circa stable, while in the public office mean values ranged between 0.9 and 9.07 µg/g (DW). In the outdoor samples, values were more stable (Table 20). As LD is the indoor complement to LF (Venice, semi – harbour site), it can be hypothesized that the emission of all the cruises passing in that area during summer time influence the high values of PAH within that period.

Table 20: Seasonal means, minimum and maximum values of the ∑18 PAH (µg/g DW) in indoor and outdoor samples.

season	GBP			LD			W			*Indoor*		
	mean	min	max	mean	min	max	mean	min	max	*mean*	*min*	*max*
summer	1.99	1.06	2.8	**9.07**	1.32	27.3	**1.67**	0.98	2.3	***4.24***	*0.98*	*27.3*
autumn	1.27	1.05	1.35	0.91	0.82	1.00	0.88	0.78	0.94	*1.02*	*0.78*	*1.4*
winter	**2.86**	0.9	8.6	0.98	0.81	1.21	0.90	0.78	1.04	*1.58*	*0.78*	*8.6*
spring	1.21	1.01	1.48	6.53	1.31	14.8	1.18	1.09	1.5	*2.98*	*1.01*	*14.8*
	LF			MC			MF			*Outdoor*		
	mean	min	max	mean	min	max	mean	min	max	*mean*	*min*	*max*
summer	**17**	0.89	64	1.02	0.68	1.35	1.2	0.94	1.6	***6.4***	*0.68*	*64*
autumn	1.07	0.99	1.2	1.14	0.69	1.6	1.1	0.94	1.4	*1.1*	*0.69*	*1.6*
winter	1.01	0.80	1.4	1.00	0.83	1.33	0.9	0.8	0.98	*0.96*	*0.80*	*1.4*
spring	2.9	1.01	4.3	**1.24**	1.04	1.5	**1.7**	1.4	2.4	*1.9*	*1.01*	*4.3*

During summer time, the mean concentration of the Σ_{18}PAH congeners was the highest within the whole year in the indoor sampling stations as well as in the outdoor ones. When temperatures sink and the amount of precipitations rose up, concentrations of PAHs were lowest in both indoor and outdoor sampling stations. In spring, when it became colder and precipitations were not so frequently anymore, PAH concentrations increased again. This findings show exactly the opposite scenario of the results obtained by active monitoring (Masiol et al., 2012b). Therefore it can be hypothesized that plants i) next to taking up these compounds they reduce, metabolize or transform PAH congeners in more useful compounds for the cell; ii) reduce their metabolic activities during winter time.

When considering the concentrations of single PAH - compounds in the three indoor and the three outdoor sampling stations (table 21), it were detected high values of B(a)P, the most toxic compound in the mixture (IARC, 2013), whereas the compounds with more than 85% of the obtained values were <LOQ were not considered here.

In the indoor environments the plants were exposed to, the highest values of the single PAH compounds were found mainly in summer and spring, while the lowest ones were detected mostly during autumn and winter. The exceptions were B(e)P and B(a)P in GBP which showed highest values during winter and lowest during spring and summer, respectively. B(ghi)P in Wk showed highest values during autumn, but the lowest ones during winter.

In the outdoor environments, the situation is similar: highest values were detected mainly during summer and spring, while the lowest values could be attributed to winter and autumn. This situation is totally given in LF, while in MC, Naph, B(b)F, B(a)P, IP and Db(ah)A were detected to be the lowest during summer time and with highest values during the spring season. Also in MF, lowest values of Naph and Db(ah)A were obtained from the analyses of summer samples.

When considering the meteorological seasonal variations, it can be said that the data of temperature and those of the PAH compounds were inversely proportional, while those of relative humidity and precipitations were correlated at least in LF and MF (with some exceptions) in a proportional way.

Table 21: Seasonal variations (ng/g DW) of the single PAH - congeners (except Phen, Anth, Fluor, Pyr, B(a)A and Chry whose values were <LOQ for more than 85%).

indoor	Naph	Acen	Ace	Flu	B(b)F	B(k)F	B(e)P	B(a)P	Per	IP	Db(ah)A	B(ghi)P
GBP												
summer	167	464	17	45	154	58	77.96	166.5	2189	16.8	31	69
autumn	42	21.9	4.6	8.3	178	49	27	235	555	14.1	65	5.3
winter	54	12.95	1.87	12.7	725	172	221	941	484	4.3	21	3.4
spring	15	91	3.1	66	122	24	15	167.1	401	12.96	118	54
LD												
summer	849	402	16	166	414	228	1290	1380	627	23.6	74	12.2
autumn	12	9	1.5	2.5	88	21.5	13	104	297	2.9	29.5	1.9
winter	52	13	1.9	8.7	69.6	45.2	29	92	209	6.07	31	11.1
spring	32	543	6.6	20	1791	256	470	2542	742	12.4	84	9.6
Wk												
summer	147	167	10	16.5	105	34.9	33.1	138	388	11	273	19.7
autumn	11	10	1.8	4.8	61.1	15.8	28.5	79	221	2.1	18.5	24
winter	15	12.95	2.3	7.9	53	9.99	31.6	110	167	3.1	29	4.6
spring	18	142	4.7	7.1	154	34.4	28.7	188	586	117	50	21.2
outdoor												
LF												
summer	30	102	394	126	5491	1733	1156	6575	2410	7.5	21.6	22
autumn	14	40	2.6	3.4	115	25	19	125	357	15	40.6	19.5
winter	48	148	2.3	2.8	61	15	34	53	226	5.5	20.2	3.8
spring	71	544	18	60	510	112	138	646	1738	21	213	12.8
MC												
summer	8.1	19	3.8	6.7	59	26.4	89	96	437	3.3	11	17
autumn	11	13.2	4.7	12	98	33.5	29.9	131	420	5.7	49	9.7
winter	15	12.95	2.4	2.8	65	12.6	55	99.8	267	3.8	22	15
spring	20	155	6.6	50	135	25.5	48	186	581	18.5	55	13.5
MF												
summer	8	138	4.1	13	95	26	43	96	547	4.4	9.1	14
autumn	14.7	12.9	2.5	2.5	127	32	19	198	479	7.4	74	3.3
winter	15.4	12.95	1.5	2.8	59	19.9	31	72	167	2.2	11.5	2.7
spring	27	293	10.7	108	224	37	58	280	775	27	110	8.7

Nevertheless, from all the results discussed in this section it can be deduced that *T. aeranthos* takes up PAH from the air and it can be summarized that the ability of *T. aeranthos* to act as suitable biomonitor for organic air pollutants in an environment different of its original one must be further investigated.

6.2.4 Analyses of metals in T. aeranthos

In total, 26 elements (Mg, Al, Si, P, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Br, Rb, Sr, Zr, Cs, W, Ba, Hg, Pb) were detected in the 102 *T. aeranthos* samples exposed to the six sampling stations.

Elements were divided in primary elements present in over 60% of the total samples, and secondary elements which were detected in less than 60% of the total samples. Primary elements were Mg, Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Br, and Sr, whereas secondary elements were V, Cr, As, Rb, Zr, Cs, W, Ba, Hg, and Pb.

The total dataset was divided in indoor and outdoor sampling stations, in order to detect the differences in metal concentrations over the time period of one year in both environments (table 22). Primary elements did not vary significantly when compared between indoor and outdoor, while the distribution of secondary elements was detected to be more variable. Further, K and Ca constituted the major part of primary elements in both environments indoor and outdoor, whereas Cr was demonstrated predominant within the secondary elements.

Table 22: Outdoor and indoor annual mean concentrations (µg/g DW) of the 26 elements.

	outdoor mean	std.dev. (±)	*Indoor mean*	std.dev. (±)
Mg	***564***	451	***507***	349
Al	***2006***	2738	***1517***	1801
Si	***3161***	931	***3117***	1039
P	***2218***	771	***2531***	1125
S	***1435***	734	***1355***	724
Cl	***2633***	1239	***2056***	1131
K	***14795***	8964	***16957***	13305
Ca	***7380***	3913	***8097***	4106
Ti	***108***	43	***103***	42
Mn	***471***	517	***447***	478
Fe	***574***	229	***651***	250
Ni	***12.3***	17.2	***14***	21
Cu	***18.3***	7	***17***	8
Zn	***107***	47	***133***	60
Br	***18***	18	***15.6***	22.3
Sr	***11.8***	3.5	***12.7***	5.1
V	***3.2***	1.8	***3.8***	5.2
Cr	***51***	86	***42***	65
As	***1.5***	3	***3.3***	6.5
Rb	***10***	13	***4***	3
Zr	***0.6***	0.6	***1.8***	2.5
Cs	***5***	10	***39.8***	39.6
W	***12***	20	***0.29***	0.35
Ba	***16***	14	***10***	9
Hg	***1.1***	0.9	***0.4***	0.5
Pb	***3.3***	2.7	***1.46***	1.50

Annual mean values of the primary elements detected in the samples exposed to outdoor environments ranged from 11.8 µg/g DW up to 14.8 mg/g DW, while those exposed to indoors varied between 12.7 µg/g DW and 17 mg/g DW. The secondary elements ranged in *T. aeranthos* leaves from outdoors between the 0.6 and 51 µg/g DW, while in indoor exposed plants the obtained values were in the range 0.29 - 39.8 µg/g DW. The distribution around the median value as well as the outliers of single elements were figured out more in detail and they are reported in Fig. 30. As the scales of the elements were so different, they were divided in four groups to explain better their distributions. Values of all elements were considered in the scale of ng/g DW, while K and Ca are reported in mg/g DW.

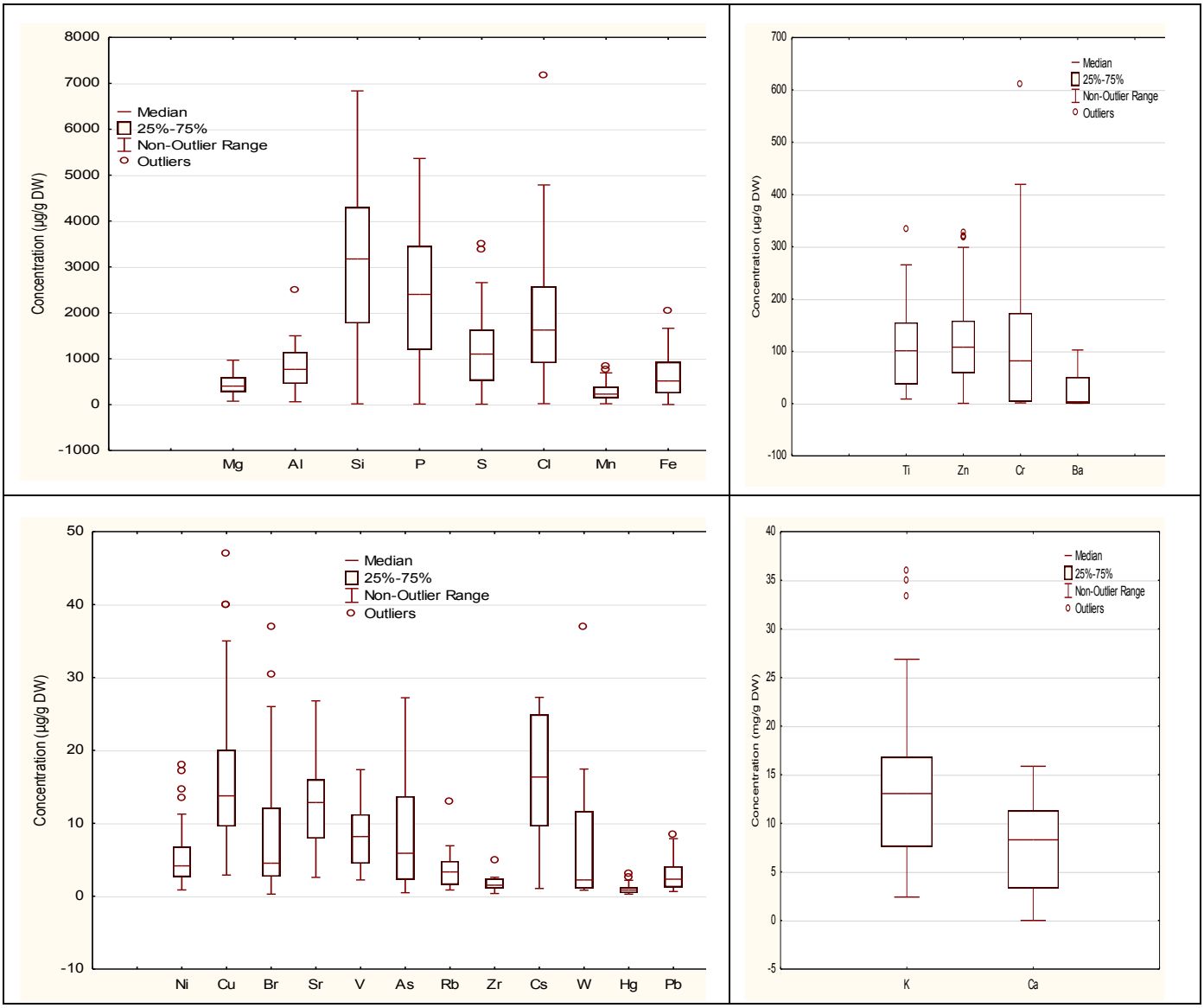


Fig. 30: Annual distribution of single elements (µg/g DW, K and Ca: mg/g DW)

When comparing the results of the PIXE analyses with the results found in the literature, a good correlation could be found for the results obtained by Rodriguez et al. (2010) for the elements Mn, Fe, Ni, Cu, Zn, Br and Pb in T. capillaris, while the results obtained within this work for Cu, Pb, Zn, Ni, Mn and Fe compared to those obtained by Pignata et al. (2002) are higher.

Brighigna et al. (2009) studied the concentrations of Pb, Cu and Cd in *T. caput – medusae morren*. Cu showed similar concentrations to those detected with *T. aeranthos* in this work, while Pb concentrations were lower in *T. aeranthos* and Cd was not detected at all. The Hg values obtained by

Fonseca et al. (2007) and Malm et al. (1998) match the range of Hg concentrations found within this work.

The concentrations carried out by Martínez – Carillo et al. (2010) showed values on a scale of mg/g. In this work, only the elements Si, P, Cl, K and Ca were detected within this scale.

On the other hand, concentrations published by Wannaz and Pignata (2006) for Mn, Fe, Ni, Cu, Zn and Pb match or slightly underestimate the values detected in this work.

The values Vianna et al. (2011) reported for Cr, Pb and Zn were lower than those found in average in this work.

Zambrano García et al. (2009) found similar values of Al, Mg, Ni and Zn to those obtained for spring within this work, while Ca and Ti matched the range of the winter concentrations. Cr, K and Mn values as well as Ba and Cu once were comparable to the summer values and average annual values of the Venice – Mestre area, respectively. Values for Fe, Pb, Sr and V published in that work were higher than those found in *T. aeranthos*, while those for P were lower.

In the work of Figueiredo et al. (2001), values of As and Zn were higher than those detected here, while Cl and Cr concentrations were lower. Ba, Br, Rb and Fe data obtained from *T. aeranthos* were in the same range than those carried out by Figueiredo.

Benzing and Bermudes (1991) detected Mn, Fe, Cu, Zn and Al in three Tillandsia species, but he found much lower values than those reported here.

The difference in concentrations found within the literature could be given by the different sampling periods, starting from the 1990's up to 2013, from the different climatic conditions prevailing in the sampling areas biomonitorings were carried out as well as from the species used for this purpose. Nevertheless, finding some concentrations matching with those found in the literature demonstrate that *T. aeranthos* works as biomonitor for metals.

In table 23, annual mean results obtained for the outdoor exposed plants in this work are compared with those figured out by Figueiredo et al. (2004), Zambrano García et al. (2009), Carreras et al. (2009), Martínez-Carrillo et al. (2010), Wannaz et al. (2012), Goix et al. (2013).

Table 23: Comparison between outdoor annual mean values obtained in this work with previous works.

	outdoor mean	std.dev. (±)	Figueiredo et al. (2004)[#] (mean)	Zambrano García et al. (2009)[◊]	Carreras et al. (2009) [≈]	Martínez-Carrillo et al. (2010) [+]	Wannaz et al. (2012)[*]	Goix et al. (2013) ◊▪
Mg	***564***	451	2052	2906				1776±526
Al	***2006***	2738	1092.6	4155				7384±2587
Si	***3161***	931						
P	***2218***	771		501				
S	***1435***	734						
Cl	***2633***	1239	1263.3					
K	***14795***	8964	4856.5	7568				8314±2193
Ca	***7380***	3913	3526.3	12556				5586±1411
Ti	***108***	43		196		33		510±178
Mn	***471***	517	180.4	80.3	77.17	106.8	310.1	154±46
Fe	***574***	229	908	1579	3051	1155	502.8	5274±1639
Ni	***12.3***	17.2		15.9	3.15	9.2	1.06	
Cu	***18.3***	7	17.8	7.0	37.9	11.8	11.5	20±19
Zn	***107***	47	73.9	41.9	111.9	62	23	186±508
Br	***18***	18						
Sr	***11.8***	3.5		30.5		30.4		45±12
V	***3.2***	1.8	2.6	43.4		21.7		11.3±3.8
Cr	***51***	86	2.1	6.1		4.4		6.8±2.3
As	***1.5***	3	0.16					31±45
Rb	***10***	13	27.2					11.3±4.1
Zr	***0.6***	0.6						9±3.7
Cs	***5***	10						2.5±1
W	***12***	20						1.5±2.2
Ba	***16***	14	23.7	38.1				77±29
Hg	***1.1***	0.9						
Pb	***3.3***	2.7		33.1	13.18	15	0.89	53±74

[*]Atomic Absorption Spectrometry (AAS), [#] Instrumental Neutron Activation Analysis (INAA), [≈] Total Reflection X-Ray Fluorescence (TXRF), [◊] Inductively Coupled Plasma Optical Emission Spectometry (ICP-OES), ▪ High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS), [+] PIXE

Further, seasonal footprints of the single elements were carried out for the

a) outdoor and

b) indoor sampling stations

in order to find some difference in the composition within the four seasons summer, autumn, winter and spring.

a) OUTDOOR:

In the outdoor sampling stations, the principal elements did not vary so far (Fig. 31a). Therefore, the focus laid on the concentrations of secondary elements (Fig. 31b, c, d, e) which varied within the four seasons.

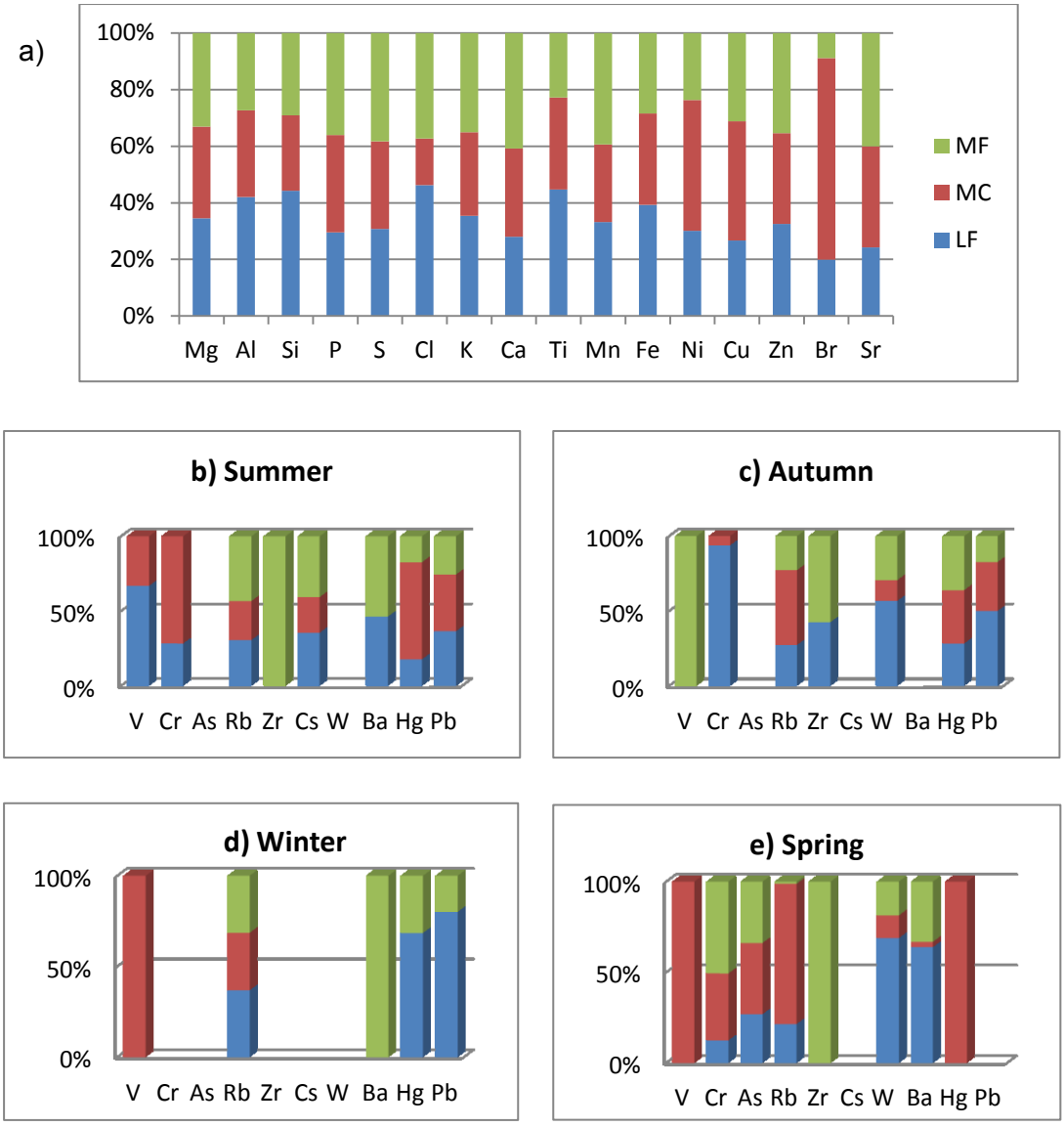


Fig. 31: Seasonal variations of elements in outdoor sampling stations.

As those elements can be transported by the action of the wind and/or by air masses carried on a long range scale, the attribution of them to a specific source is quite difficult. Nevertheless, it can be established that plants exposed to the industrial and the urban area took up more V, Zr, Ba and Hg than those of LF.

Concentrations of primary and secondary elements (µg/g DW) within all the outdoor sampling stations in the four seasons are listed in Table 24. Within all the four seasons, values for Si, P, Cl, K, Ca are detected in the range of some mg/g DW, while As, Rb and W were detected only in traces in the outdoor sampling stations.

Table 24: Mean concentrations of all elements detected outdoors in the four seasons (µg/g DW).

	Summer			Autumn			Winter			Spring		
	LF	MC	MF	LF	MC	MF	LF	MC	MF	LF	MC	MF
Mg	234.12	219.98	224.23	439.75	356.24	393.73	419.62	364.71	423.74	1177.95	1182.64	1326.26
Al	552.84	402.15	360.23	701.04	821.95	411.78	928.17	789.89	781.14	6630.56	5641.41	6048.28
Si	2466.78	1487.88	1623.68	4002.79	4443.59	2394.51	4256.82	4392.11	3351.64	3263.33	2997.78	3252.63
P	1177.97	1366.49	1433.58	3099.36	2532.39	2010.57	3332.95	2727.18	3239.76	1543.17	2229.64	1926.11
S	472.29	476.49	587.62	1282.32	1472.40	882.45	2264.74	1646.02	1600.52	1029.18	3905.78	1597.58
Cl	1405.54	500.25	1132.57	2586.74	2461.62	2483.94	4064.33	2805.39	2223.34	2895.83	4620.71	4415.05
K	6457.66	5391.99	6400.37	13811.49	11551.58	10879.36	14657.41	11584.08	14884.28	27714.48	26446.85	27764.69
Ca	4142.58	4603.53	6036.97	10367.61	9934.71	10087.28	11163.46	10723.72	11864.36	2975.04	3020.16	3641.98
Ti	68.49	49.88	34.90	133.89	173.79	87.06	163.47	143.57	142.08	120.42	94.47	80.00
Mn	154.58	127.25	183.54	256.22	199.58	220.32	289.47	255.40	236.80	1207.41	1152.77	1372.66
Fe	399.43	328.97	289.20	680.32	833.94	406.36	981.39	878.04	729.82	400.64	440.76	523.47
Ni	2.41	3.69	1.90	4.40	4.78	3.05	5.21	3.91	3.89	8.18	49.51	56.55
Cu	10.84	17.12	12.67	11.83	12.50	40.84	18.78	15.44	44.73	7.51	9.40	17.30
Zn	64.68	63.75	70.31	104.27	189.77	91.84	182.31	152.21	157.50	66.43	86.68	60.33
Br	7.95	28.46	3.53	5.77	13.89	4.92	7.30	4.88	3.80	53.18	43.56	38.45
Sr	5.68	8.38	9.39	12.42	15.79	15.95	14.65	15.54	13.89	8.35	10.82	10.42
V	4.49	2.23				5.82		8.89			17.36	
Cr	3.08	7.80		61.25	4.05					67.14	199.45	273.58
As										4.84	7.08	6.10
Rb	1.30	1.11	1.84	3.37	6.18	2.81	4.80	4.13	4.09	18.96	68.43	1.11
Zr			1.26	1.92		2.60						1.82
Cs	21.09	14.13	24.18									
W				71.20	17.44	36.90				11.64	2.11	3.16
Ba	46.04		53.43						37.41	36.57	1.68	19.04
Hg	0.44	1.62	0.43	0.97	1.22	1.24	4.71		2.17		0.82	
Pb	2.36	2.46	1.65	8.98	5.87	3.11	9.36		2.33			

b) INDOOR

In the indoor sampling stations GBP, LD and Wk, primary elements were distributed almost equally between the three sampling stations, with the exception of Ni which was found predominantly in GBP. Al, Fe and Cu were the elements prevailing in Wk while Cl showed highest concentrations in LD (Fig. 32).

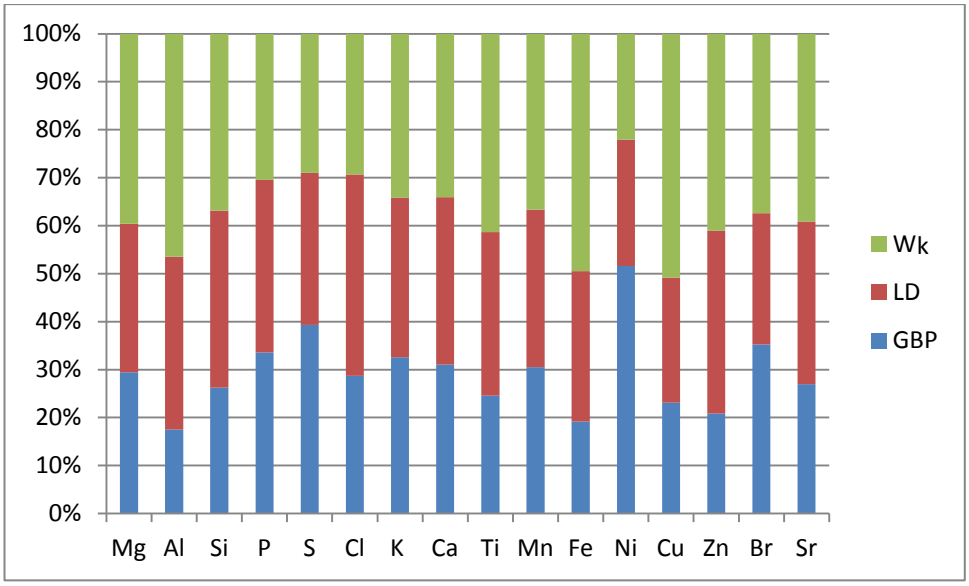


Fig. 32: Annual percentages of primary elements in indoor environments.

When considering the seasonal variations in secondary elements (Fig. 33), it was detected that during summer time, V and Ba were the main elements in Wk, Cr in LD and Hg in GBP. During autumn, the elements V, Cr and As were not detected in any sampling station, whereas traces of Zr and Cs were accumulated by the plants exposed to the ambient air of the public office (LD). The element wolfram (W) was detected only in GBP, while the presence of Ba could be verified only in the garage (Wk). Concentrations of V, Cr as well as Cs were evidenced during winter time only in GBP and Wk, respectively. In summer, Cs was detected only in LD.

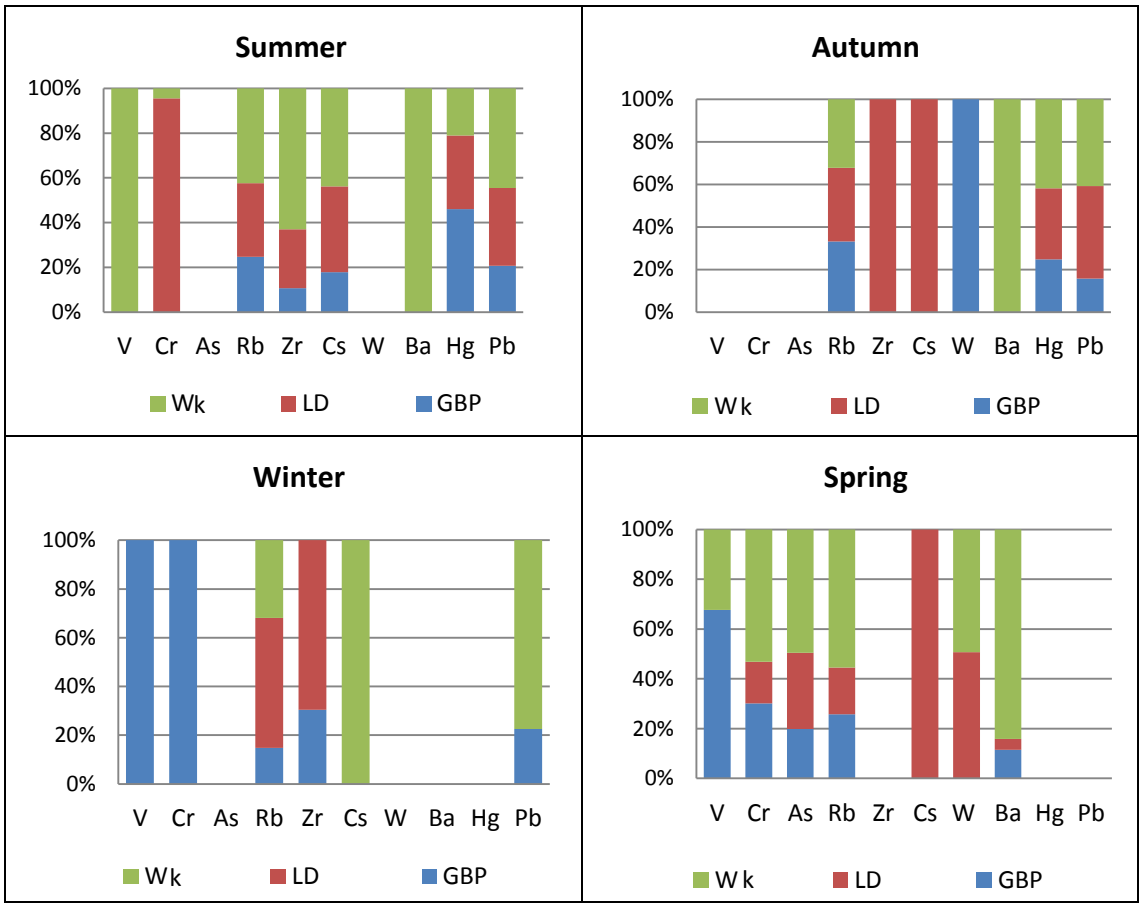


Fig. 33: Seasonal variations of secondary elements in indoor sampling stations.

The mean concentration of each element (primary and secondary) within all the seasons is listed in Table 25. For the elements K and Ca concentrations in the scale of mg/g DW were detected. From the results obtained by the analyses of the secondary elements (V – Pb) was demonstrated that these elements were present not only in the outdoor environments, but also indoors. Even at quite stable temperatures and constant watering events with drinking water, differences between the concentrations of primary and secondary elements could be carried out.

Table 25: Seasonal variations in elements' concentrations (µg/g DW) in indoor environments.

	Summer			Autumn			Winter			Spring		
	GBP	LD	Wk	GBP	LD	Wk	GBP	LD	Wk	GBP	LD	Wk
Mg	137.95	187.67	172.29	386.89	335.65	395.46	483.31	521.49	503.49	801.49	855.99	1307.39
Al	128.99	470.88	546.80	356.65	746.20	788.92	528.70	800.54	1228.70	2177.58	4542.80	5889.08
Si	607.16	1883.08	3154.03	4197.70	4099.39	3968.19	2341.35	4427.18	4799.90	3261.75	3093.26	1576.70
P	887.50	1077.57	1230.10	2930.00	3552.86	3129.73	3416.80	3759.74	3594.39	3153.10	2174.04	1466.22
S	295.39	452.32	525.72	1193.61	1141.43	1256.26	1466.62	1807.29	1780.62	3925.63	1364.47	1046.57
Cl	688.37	733.52	851.59	1564.05	1816.80	3166.60	1703.97	2034.12	1635.79	2971.58	5316.33	2189.40
K	3641.96	4663.82	5239.38	10269.66	15629.25	14078.67	11449.69	16280.67	14802.77	39681.66	32068.96	35673.92
Ca	3288.97	4131.24	3783.92	8461.76	12805.01	10982.95	11941.45	12243.08	12854.76	6454.18	4731.43	5485.37
Ti	25.71	59.18	79.52	54.23	168.88	193.99	128.39	106.59	176.62	87.40	98.10	58.72
Mn	46.18	93.54	98.87	127.19	404.40	235.95	211.78	404.01	293.85	1213.62	912.00	1324.85
Fe	127.81	420.04	527.01	284.11	854.52	1374.81	555.42	590.33	1491.98	668.15	533.25	389.11
Ni	1.06	3.65	2.16	2.08	4.43	6.23	2.44	4.51	6.74	87.44	27.94	19.72
Cu	3.89	7.57	8.99	20.54	17.32	35.83	9.89	16.77	38.27	14.64	10.49	16.87
Zn	32.04	73.45	82.12	121.31	223.77	216.89	96.34	156.99	284.90	76.65	189.87	47.24
Br	1.63	1.72	1.47	9.58	4.24	9.49	3.60	4.41	4.44	61.95	31.75	52.93
Sr	3.98	6.25	5.85	10.87	18.72	17.37	13.40	14.28	21.21	14.93	11.26	14.74
V			3.72				7.46			23.14		11.04
Cr		81.73	3.84				3.05			123.83	68.43	218.70
As										7.73	11.94	19.34
Rb	1.01	1.35	1.74	3.41	3.55	3.31	1.80	6.46	3.87	5.72	4.16	12.31
Zr	0.37	0.92	2.18		1.55		4.99	11.40				
Cs	6.87	14.76	16.89		1.08				249.77		188.15	
W				1.31							1.09	1.05
Ba			30.43			66.38				2.81	1.08	20.73
Hg	0.90	0.65	0.41	0.75	1.01	1.26						
Pb	0.64	1.08	1.38	1.69	4.62	4.35	0.85		2.90			

To determine the **suitability of *T. aeranthos*** not only as biomonitor but also as **accumulator of inorganic compounds**, the data obtained from exposed samples were compared to those of blanks for the outdoor and the indoor sampling stations (tables 26, 27). In red are highlighted the concentrations below the blank value.

During the **outdoor** exposure of *T. aeranthos* plants (table 26) was figured out a decrease of the primary elements Cl, Mn, Ni, Cu, V, and Hg within all the three sampling stations in the first period (summer/autumn), while during the second one (autumn/winter), concentrations of P, Ca, Mn, Sr and Hg were lower than the reference value of the blanks. During spring (third period), a decrease of concentrations was detected for the elements Si, P, Ti, Fe, Zr, Cs, and Hg.

Table 26: Comparison of elements' concentrations in the samples with the blank values.

	Sampling period 1				*Sampling period 2*				*Sampling period 3*			
	B1	**LF 1**	**MC 1**	**MF 1**	***B2***	**LF 2**	**MC 2**	**MF 2**	***B3***	**LF 3**	**MC 3**	**MF 3**
Mg	***257***	296	271	294	***361***	434	356	401	***511***	1178	356	1326
Al	***377***	632	511	348	***819***	823	831	687	***1213***	6631	831	6049
Si	***1712***	2950	2601	1737	***3775***	4201	4281	3176	***4944***	2611	4281	1952
P	***893***	1709	1597	1353	***3374***	3364	2820	3103	***2881***	1543	2820	1541
S	***490***	716	715	645	***1559***	1963	1682	1402	***904***	823	1682	1598
Cl	***2055***	2008	952	1672	***1534***	3363	2893	2221	***2019***	2896	2893	4415
K	***3243***	8370	6573	7018	***12661***	14914	12445	14425	***14235***	27715	12445	27765
Ca	***4924***	6468	6414	7110	***11837***	10648	10428	11549	***10431***	2380	10428	2914
Ti	***46.1***	97	88.1	46.5	***152***	147	157	130	***177***	72	157	64
Mn	***196***	187	144.9	168	***279.96***	279.68	243.24	259.60	***432***	1207	243	1373
Fe	***504***	542	465.8	278	***733***	832	895	672	***1106***	401	895	314
Ni	***20.2***	3.2	4.3	2.3	***3.1***	4.8	3.3	3.6	***2.1***	6.5	3.3	56.6
Cu	***30.7***	10.8	15.6	30.1	***14.4***	16.8	14.4	35.4	***10.9***	3.0	14.4	6.9
Zn	***53.8***	78.1	120.3	72.2	***136.4***	156.1	150.2	140.9	***141***	49.8	150.2	48.3
Br	***4.6***	7.3	19.4	4.5	***3.8***	6.7	9.8	3.6	***2.5***	53.2	9.8	38.5
Sr	***10.3***	7.8	11.5	11.2	***15.14***	14.06	14.9	14.97	***14.7***	3.3	14.9	4.2
V	***2.6***	0.8	0.4	1.0			1.5				1.5	
Cr	***24.1***	0.5	3.3			10.2				40.3		219
As										0.97		3.7
Rb	***0.8***	1.0	0.6	0.9	***1.4***	1.3	3.1	2.3		7.6	3.1	0.2
Zr	***2.3***	0.5		1.3	***0.6***	0.5			***14.6***			0.4
Cs		10.5	7.1	8.1					***17.7***			
W		0.5	2.9	6.2		23.2				2.3		0.6
Ba		7.7		8.9				6.2	***25.7***	29.3		11.4
Hg	***1.5***	0.4	1.2	0.4	***13.6***	0.8		0.9	***0.3***			
Pb	***3.6***	4.7	3.6	2.5		5.2	1.1	0.8			1.09	

Within the first sampling period, an increase in the secondary elements Cs, W and Ba was detected in all the three outdoor sampling stations, while Pb showed higher values only in LF and MC.

On the contrary, during the second sampling period Pb was not detected in the blanks, therefore the values from the exposed samples were considered higher than the reference value. This is valid also for V in MC, Cr and W in LF, and Ba in MF.

During the third period, values of V and Pb increased in MC, those of Cr, As and W in LF and MF, as well as those of Rb in all three sampling stations, whereas Zr, Cs and Hg decreased in LF, MC and MF.

In the **indoor** exposed plants (table 27), during the first period the primary elements Mn, Ni, Cu, Br and Sr showed lower concentrations than the blanks in all three sampling stations GBP, LD and Wk. Within the second period, not even one primary element decreased its concentrations in all three sampling stations. On the contrary, during spring (period 3), concentrations of P, S, Ca, Ti, Fe, Cu, Zn and Sr were reduced with respect to the blanks' one.

The decrease of almost all major elements in GBP within each single sampling period was a rather interesting discovery.

Table 27: Comparison of elements' concentrations detected indoors with blanks.

	Sampling period 1				*Sampling period 2*				*Sampling period 3*			
a) Indoor	***B1***	**GBP 1**	**LD 1**	**WK 1**	***B2***	**GBP 2**	**LD 2**	**WK 2**	***B3***	**GBP 3**	**LD 3**	**WK 3**
Mg	***257***	172	222	261	***361***	500	475	454	***511***	802	856	1307
Al	***377***	167	581	557	***819***	509	764	1153	***1213***	2178	4543	5889
Si	***1712***	2528	2604	3025	***3775***	2236	4336	4924	***4944***	1957	2475	1261
P	***893***	1347	1850	1643	***3374***	3476	3743	3660	***2881***	2523	2174	1173
S	***490***	566	673	709	***1559***	1405	1595	1666	***904***	2355	1092	628
Cl	***2055***	969	1043	2152	***1534***	1669	2014	1618	***2019***	2972	5316	1752
K	***3243***	4828	8324	7968	***12661***	12079	16059	14779	***14235***	39682	32069	35674
Ca	***4924***	4479	7140	5923	***11837***	11315	12313	12491	***10431***	5163	3785	4388
Ti	***46.1***	37	112.3	102.5	***152***	104	111	198	***177***	87	79	59
Mn	***195***	53	193	146	***280***	204	409	273	***432***	1214	912	1325
Fe	***504***	165	637	726	***733***	480	606	1537	***1106***	401	427	311
Ni	***20.2***	1.1	4	3.2	***3.1***	2.6	4.4	6.9	***2.1***	70	27.9	19.7
Cu	***30.7***	6.1	10.4	19.4	***14.4***	16.8	17.4	36	***10.9***	5.9	4.2	6.8
Zn	***54***	39	134	110	***136***	127	169	279	***141***	61	114	47
Br	***4.6***	2.5	2.4	4.0	***3.8***	7.3	2.8	4.1	***2.5***	37.2	31.8	42.3
Sr	***10.3***	5.6	9.9	8.6	***15.1***	13.3	16.3	21.1	***14.7***	6	4.5	5.9
V	***2.57***			0.62		1.24				9.3		2.2
Cr	***24.1***		13.6	0.6		0.51				74.3	41.1	131
As										4.6	7.2	3.9
Rb	***0.8***	0.3	0.9	0.6	***1.4***	0.87	1.6	1.8		2.3	0.8	4.9
Zr	***2.3***	6	0.3	0.4	***0.6***	0.83	2.2		***14.6***			
Cs		1.1	2.8	8.5				41.6	***17.7***		37.6	
W		0.2									0.43	0.42
Ba				5.1				22.1	***25.7***	0.6	0.7	12.4
Hg	***1.5***	0.3	0.8		***13.6***	0.23	0.14		***0.3***			
Pb	***3.6***	0.5	2.1			0.87						

For the secondary elements, during sampling period 1 the concentrations of V, Cr, Hg and Pb decreased in all three sampling periods, while during sampling period 2 the only Hg concentration were minimized. In sampling period 3, this happened for Zr and Ba.

During sampling period 3, an increase of Cr, As and Rb was detected in all three indoor sampling stations. Further, concentrations of V and W increased in GBP/Wk and LD/Wk, respectively.

From all the results discussed in this section it can be deduced that *T. aeranthos* takes up metals from the air, but as no trend could have been detected, it can be summarized that the ability of *T. aeranthos* to act as suitable biomonitor for inorganic air pollutants must be further investigated.

7. Conclusions

7.1 Filter analyses

- ***PAH analyses***

During winter time, PAH concentrations in the Venice – Mestre area are among the highest within Europe (Puteaud et al., 2010). This can be caused by the influence of local emissions sources but also from PAH congeners carried to the sampling area by the long range transport. Concentrations of Σ_{16}PAH revealed to be proportional to the total $PM_{2.5}$ detected on the filters, but inversely proportional to the variations of precipitation and temperature.

The highest concentrations in the PAH mixture detected could have been associated in the industrial area as well as in the urban one to PAH congeners taking part of IARC's class 1 (B(a)P), class 2B (B(a)A, Chry, B(b)F, B(k)F, IP) and class 3 (Fluor, Pyr, B(e)P, Per, B(ghi)P) (IARC, 2013). The concentrations detected for these compounds were almost equal in both sampling areas, even if the distribution around the median value varies between data obtained from each sampling stations.

It can be noticed that the air quality of the study area during winter 2009 - 2010 was between the worst within Europe which could have had a negative impact on the quality of life of residents.

Values of B(a)P exceeded the limit established by the European and Italian legislation of 1 ng/m³ in more than 50% of the samples.

Therefore, the toxicity of the PAH mixture was tested by calculating the toxicity and mutagenicity equivalent (TEQ, MEQ) values for the winter samples from the industrial and the urban area. The obtained results showed higher in the industrial area than in the urban one which could be caused by i) industrial activities, ii) heavy traffic, iii) the action of the wind which transported polluted air masses from the urban area to the industrial one, iv) a stagnation of air masses containing high concentrations of PM over the sampling site.

- ***Carbonaceous fraction***

The comparison of the two methods (TOT and combustion/NDIR) showed good corresponding values for TC and OC, but less for EC. TC and OC values were correlated at 98% and 85%, respectively, while the correlation of EC was identified to be 46%.

This could be given by the fact that sample punches had been pre – treated in the muffle at 350 °C which could have caused the combustion of native carbon, decreasing so the concentration detected, but also the pyrolysis of carbon present on the filter which increased the concentration of EC could have influenced the low correlation.

Nevertheless, considering these results, the combustion/NDIR method carried out with an instrument used for the analyses of the carbonaceous fraction in different solid matters, can be a valid instrument for at least total and organic carbon analyses in air samples.

- ***Levoglucosan***

The marker of wood combustion, levoglucosan, was detected in winter and summer samples of the sampling area. During summer time, mean concentrations range between 2.83 and 6.37 ng/m³ in URB and IND, respectively, while during wintertime, those values rise up to 137.8 and 140.4 ng/m³ in URB and IND, respectively. Further, during summer, 11 values obtained in the industrial area samples were <LOD. The concentrations of levoglucosan resulting from the analyses were proportional to data achieved for the temperature, just shifted for one to two days.

However, the results obtained from these analyses demonstrated that within the sampling area many households (and perhaps also industries) use wood as combustion material for heating during the cold season.

The sporadic concentrations of levoglucosan detected during summer time could be generated by wood burning in form of open fires on agricultural lands.

7.2 Biomonitoring

- ***Analyses of plants' properties***

➢ ***Weight difference, DW/FW ratio and RWC***

Tillandsia aeranthos plants lost in indoor environments (LD, Wk), where temperature and relative humidity were quite stable and they were watered once a week, in average 1.6 g. In comparison, in GBP, where they were watered once a day, their weight *increased* of 2g on an annual scale.

In outdoor environments, where the plants get water exclusively from the atmosphere, they lost in mean 0.9 g with respect to their weight before exposure, where a distinction has to be made between the single sampling stations: in LF, MC and MF, plants have lost in mean 1.1, 0.3 and 1.4 g, respectively.

Plants exposed to the outdoor environment lost more weight during summer time and so did also plants in GBP. In the indoor sampling station LD plants showed the highest loss, whereas in Wk the weight loss was almost stable at 4±0.7 g.

The DW/FW ratio of the annual means in indoor stations varied between 0.29 g/g (FW) and 0.38 g/g (FW). For outdoor exposed samples, an annual average value of 0.34 g/g (FW) - 0.38 g/g (FW) was measured. These values are in accordance with those calculated by Pignata et al. (2002) for *T. capillaris*.

The relative water content (RWC) ranged between 59% and 74%.

- **Lipid content in *T. aeranthos and Kv***

The lipid fraction detected in *T. aeranthos* plants vary between 0.82 and 9.6 mg/g (DW), where the lowest amount was determined in GBP samples and the highest one in plants exposed in Wk. During summer time, the lipid content is the lowest in both, indoor and outdoor exposed plants, while during winter time the amount of lipids in the plants increased.

Kv determined by Simonich and Hites (1994) and applied to the PAH congeners B(a)A, Chry, B(a)P, IP, and B(ghi)P obtained by the biomonitoring project with *T. aeranthos* exposed outdoors showed an overestimation of the values of B(a)A and Chry, and an underestimation of IP and B(ghi)P, with respect to those obtained by ARPAV with active monitoring methods. The best correlation was detected for B(a)P. Nevertheless, values were too variable as a trend could have been detected.

As in the literature could be found only the correlation values of Simonich and Hites (1994), in this work was attempted for the first time to obtain a correlation factor for PAH - congeners detected in a Tillandsia species.

- ***PAH analyses in T. aeranthos***

The annual average of the concentration of the sum of 18 PAH congeners was detected to be ≈ 2.5 µg/g (DW) in both, indoor and outdoor sampling stations, with highest values during summer time and lowest during autumn and winter. These findings showed exactly the opposite scenario of the results obtained by active monitoring in the same area (Masiol et al., 2012b).

When considering the concentrations of single PAH - compounds in the three indoor and the three outdoor sampling stations, high values of B(a)P, the most toxic compound in the mixture, were detected during winter time.

As in previous studies other species of Tillandsia were employed to monitor the air quality, the obtained results in this work could have been compared only to data obtained for other species. Nevertheless, they are in agreement with those found by Rodriguez et al.(2010), as they detected that the three, four and five – ring PAH congeners established the major part of PAH found in *T. capillaris*.

To testify the ability of *T. aeranthos* as a bioaccumulator of organic air pollutants, the mean of each sampling period was compared to the mean of the three blanks treated in the same way like the samples themselves. Results showed that in most of the indoor (GBP, LD, Wk) and outdoor (LF, MC, MF) exposed plants, concentrations of the compounds with 5 and 6 benzene rings (B(b)F - B(ghi)P) increased within all three sampling periods.

Finally, it can be concluded that *T. aeranthos* takes up PAH from the air and it can be summarized that the ability of *T. aeranthos* to act as suitable biomonitor and bioaccumulator for organic air pollutants in an environment different of its original one needs further investigations.

- ***Metal concentrations in T. aeranthos***

In total, 26 elements (Mg, Al, Si, P, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Br, Rb, Sr, Zr, Cs, W, Ba, Hg, Pb) were detected in the 102 *T. aeranthos* samples exposed to the six sampling stations (indoor and outdoor).

Elements were divided in primary (Mg, Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Br, Sr) and secondary (V, Cr, As, Rb, Zr, Cs, W, Ba, Hg, Pb) elements based on the frequency they were detected in the samples.

The primary elements' composition did not vary significantly when compared between indoor and outdoor, while the distribution of secondary elements was detected to be more variable. Further, K and Ca constituted the major part of primary elements in both environments indoor and outdoor, whereas Cr was demonstrated predominant within the secondary elements.

Annual mean values of the primary elements detected in the samples exposed to outdoor environments ranged from 11.8 µg/g DW up to 14.8 mg/g DW, while those exposed to indoors varied between 12.7 µg/g DW and 17 mg/g DW. The secondary elements ranged in *T. aeranthos* leaves from outdoors between the 0.6 and 51 µg/g DW, while in indoor exposed plants the obtained values were in the range 0.29 - 39.8 µg/g DW. These values agree with those obtained by other research projects carried out with Tillandsia species. Significant seasonal variations were detected mainly for the secondary elements' concentrations.

To determine the accumulation capacity of *T. aeranthos* plants regarding inorganic air pollutants, the data obtained from exposed samples were compared to those of blanks for the outdoor and the indoor sampling stations. As no trend could have been detected nor for plants exposed to outdoor environments nor for those exposed indoors, further investigations regarding the accumulation property of *T. aeranthos* are needed.

Nevertheless, the decrease of almost all major elements in GBP within each single sampling period was a rather interesting discovery.

In conclusion, and deduced from the results obtained within this work, can be said that *T. aeranthos* takes up organic and inorganic compounds from the air it is exposed to. Nevertheless, it's suitability as biomonitor and bioaccumulator in environments differing from its original one must be further investigated. Further research could also be carried out on the transformation of the taken up elements as well as the detoxification mechanism of *T. aeranthos*.

7.3 Difficulties identified during the biomonitoring project

The problems to solve within the biomonitoring project started from the choice of the biomonitor. Many studies had been carried out in the past with lichens and mosses, some of them at least in the neighbourhood of the sampling area considered in this work, but no studies were carried out with Tillandsia. After reading the work of Brighigna et al. (2002), the main problems which could be faced due the sampling with Tillandsia seemed to be the climate of the area. Therefore, a species which grows naturally at heights from the sea level up to the Andes, namely *T. aeranthos*, appeared to be the best choice.

After this decision, the problem of the furnisher had to be solved, because the only one able to provide the required number of these plants in Italy was uneager to supply the plants. Therefore, *T. aeranthos* plants were purchased in Germany.

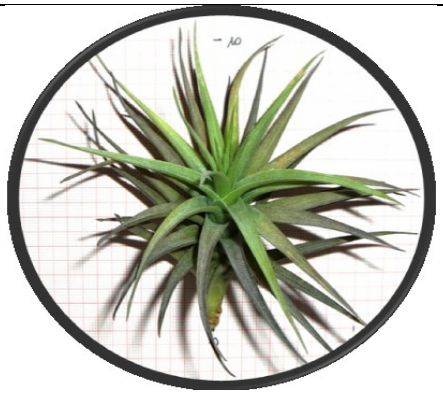
As the plants did not exceed the size of 10 cm in length and width, a support had to be thought for the outdoor sampling points to protect the plants from being transported by the action of the wind and from direct sunlight as well as from direct rainfall.

During the analyses, the fitness of the plants reduced, as were not in their usual environment. This was clearly visible from the loss of weight during the exposure periods.

Further, in the plants exposed indoors was assumed that the PAHs and the metals detected during the analyses derived only from the air. Nevertheless, there was always a doubt if the tap water they had been poured was influencing the final results.

However, the results obtained in this work stimulate further analyses in terms of i) another annual sampling campaign to compare the results, ii) identification of the tissues the plant stores the metals and the PAHs, iii) detection of differences in the metabolism of *T. aeranthos'* plants exposed to different climatic conditions, iv) identification of the DNA of *T. aeranthos*, and many other researches.

7.3 Summary of pro and cons of both, the filter sampling system and the biomonitoring

	
Glass (or quartz) fibre filter (Ø = 47 mm), sampled by active samplers for 24h at a constant flow of 2.3 m^3/h	***Tillandsia aeranthos*, passive sampler and biomonitor**
Advantages	
- Daily data	- Low budget required
- Validated method	- Energy saving method (no electricity is needed for automated samplers)
- Does not respond to meteorological variations	- Exposure possible also in remote areas
- Easy handling, extraction and analyses	- Values of B(a)P contained in the air can be estimated
- Data are comparable on a global scale	- Good response for metal detection with PIXE
Disadvantages	
- PIXE analyses is not possible with glass nor with quartz fibre filters => ICP – MS	- Only “long term” monitoring is possible => not suitable for monitoring accidental events
- Higher material costs	- Has a metabolism => can use/ modify/ destroy/ excrete the compounds under request
- Additive costs of electric supply and maintenance	- Dependent of meteorological conditions
- Only suitable for places with electricity supply	- More work before extraction => meshing and freeze drying
- Samplers are quite noisy => residential areas has to be avoid for sampling.	- In our latitudes, plants have to be grown in greenhouses before exposure.

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7. Annexes

Annex I: Complete dataset of the PAH mixture detected on filters (ng/m³).

IND	Fluor	Pyr	BaA	Chry	BbF	BkF	BeP	BaP	IP	DB(ah)A	B(ghi)P
22.12.2009	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0.01	0.14	0.13	0.10
23.12.2009	1.48	2.76	4.82	5.42	4.76	6.50	3.10	4.83	1.86	0.49	4.17
24.12.2009	0.73	0.91	0.59	1.36	2.74	3.03	2.02	1.90	0.40	0.08	2.72
25.12.2009	0.24	0.40	0.37	0.92	0.47	1.10	0.94	0.65	3.18	2.13	2.14
26.12.2009	0.56	0.68	0.49	0.93	1.38	2.38	1.57	2.00	1.62	0.21	2.11
27.12.2009	0.49	0.82	0.89	1.21	1.72	2.04	1.15	1.47	0.40	0.21	1.83
28.12.2009	2.09	3.60	6.62	4.79	5.20	6.00	3.84	5.97	2.15	1.46	7.36
29.12.2009	3.20	5.21	10.39	7.46	9.62	11.66	6.76	8.83	2.57	0.82	8.53
30.12.2009	2.40	3.84	6.27	7.22	6.14	8.83	5.94	7.82	2.54	1.24	8.08
31.12.2009	1.34	2.27	9.41	3.14	6.41	6.64	3.89	5.41	3.09	0.70	9.73
01.01.2010	1.16	1.74	3.31	2.67	3.35	4.46	2.91	3.28	2.29	0.97	7.61
02.01.2010	0.95	1.64	1.62	2.29	2.00	4.36	2.84	3.66	3.29	0.58	3.70
03.01.2010	0.06	0.19	0.38	0.47	0.68	0.65	0.41	0.46	0.28	0.04	1.24
04.01.2010	1.78	2.48	3.73	3.06	2.53	4.36	2.78	3.20	2.01	0.93	8.15
05.01.2010	0.93	1.33	2.01	2.58	3.09	4.75	2.21	2.62	1.04	0.56	3.36
06.01.2010	1.16	1.60	2.57	2.44	1.88	3.68	1.95	2.93	1.12	0.32	3.11
07.01.2010	2.22	3.05	6.03	6.43	3.33	6.95	3.78	5.04	1.80	0.57	5.28
08.01.2010	1.16	1.91	2.07	3.12	3.87	5.23	2.45	2.94	1.12	0.60	3.94
09.01.2010	0.45	0.38	0.02	0.16	0.02	0.02	1.78	1.42	0.60	3.29	2.28
10.01.2010	0.38	0.31	0.36	0.63	0.59	0.22	0.62	0.57	1.30	0.75	0.97
11.01.2010	0.14	0.08	0.06	0.22	0.12	0.69	0.03	0.07	0.42	0.11	0.24
12.01.2010	0.04	0.02	0.02	0.03	0.03	0.02	0.02	0.08	0.19	0.09	0.07
13.01.2010	0.03	0.01	0.02	0.01	0.02	0.12	0.01	0.02	0.03	0.04	0.04
URB											
13.01.2010	0.81	1.26	2.66	2.78	4.29	4.10	3.01	3.63	1.15	0.45	4.09
14.01.2010	0.90	1.53	3.08	3.15	5.20	5.23	3.37	4.96	1.05	0.40	4.07
15.01.2010	0.79	1.23	1.84	2.09	3.33	3.84	2.32	3.37	0.73	0.12	2.40
16.01.2010	0.37	0.61	1.03	0.96	1.24	1.53	0.79	0.97	0.30	0.02	0.99
17.01.2010	0.49	0.66	1.26	1.65	1.32	2.56	1.63	2.38	0.80	0.32	2.01
18.01.2010	0.55	0.85	1.67	1.97	2.00	4.16	2.00	2.89	0.79	0.15	2.78
19.01.2010	1.73	2.77	6.37	5.91	7.23	7.90	5.30	8.25	1.99	0.66	5.95
20.01.2010	0.78	1.18	1.19	1.91	1.47	2.61	1.22	1.04	0.12	0.08	1.03
21.01.2010	0.89	1.33	1.13	3.81	1.93	3.11	2.30	2.07	0.44	0.69	2.10
22.01.2010	0.88	1.69	1.41	2.74	2.22	1.71	2.37	2.28	0.68	0.55	0.47
23.01.2010	0.65	0.87	1.43	2.07	2.75	3.32	1.50	1.90	0.69	0.19	2.29
24.01.2010	2.01	2.64	1.42	1.99	1.99	2.32	1.16	1.69	0.79	0.50	2.71
25.01.2010	2.01	2.64	2.78	3.45	2.38	3.90	2.65	3.01	2.46	1.49	4.44
26.01.2010	1.11	1.40	0.97	1.60	1.22	2.15	1.30	1.45	0.50	0.21	1.47
27.01.2010	1.55	1.99	1.13	1.89	2.14	2.45	1.38	1.51	0.30	0.11	1.63
28.01.2010	0.82	1.08	0.98	1.92	0.85	1.90	0.88	1.13	0.69	0.12	1.69

Estratto per riassunto della tesi di dottorato

L'estratto (max. 1000 battute) deve essere redatto sia in lingua italiana che in lingua inglese e nella lingua straniera eventualmente indicata dal Collegio dei docenti.
L'estratto va firmato e rilegato come ultimo foglio della tesi.

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Dottorato: **Scienze Ambientali**

Ciclo: **XXVI**

Titolo della tesi:
Studio della componente organica ed inorganica del particolato atmosferico

Abstract:

Il monitoraggio della qualità dell'aria é un primo passo attraverso un futuro migliore perché ci segnala quale processo emette più sostanze dannose nell'atmosfera e dove c'è bisogno di applicare tecnologie nuove per ridurre le emissioni. In questo lavoro, la componente organica (IPA, TC-EC-OC, levoglucosano) e quella inorganica (metalli) sono state determinate mediante il monitoraggio attivo (filtri) e il biomonitoraggio (*T. aeranthos*).

In this study, the organic and inorganic fraction of the airborne particulate matter was determined on a) filter devices (active sampling), and b) by biomonitoring (passive sampling). Organic components of the atmospheric particulate such as PAH, compounds out of the carbonaceous fraction, and levoglucosan, which are demonstrated to induce adverse effects on humans' health, were detected. Further, also inorganic compounds such as As and Hg were found in some exposed samples. Monitoring the components of the air is a first step towards an increase in life quality.

Firma dello studente