DEPARTMENT FOR INNOVATION IN BIOLOGICAL,
AGRO-FOOD AND FOREST SYSTEMS - DIBAF
Università degli Studi della Tuscia
Via S. Camillo de Lellis ,01100 Viterbo, Italy

MSc THESIS

MSc COURSE: "FORESTRY AND ENVIRONMENTAL SCIENCES" (CLASSE LM-73)

INTERNATIONAL CURRICULUM WITH MULTIPLE DIPLOMAS – SPECIALIZATION: «MEDITERRANEAN FORESTRY AND NATURAL RESOURCES MANAGEMENT - MEDfOR»

"LEAF MORPHOLOGY CHARACTERISTICS OF TREE SPECIES FOR PARTICULATE MATTER DEPOSITION IN AN INDUSTRIAL CITY OF CENTRAL ITALY"

SUPERVISOR: Prof. Carlo Calfapietra

CANDIDATE: Susana Dreveck
Matr. SFA/LM/212

CO-SUPERVISOR: Dr. Gregorio Sgrigna

ACADEMIC YEAR 2017/18

DOUBLE DIPLOMA ISSUED IN COLLABORATION WITH

Abstract

One of the major problems that cities are facing due to the increasing urbanization, is air pollution. Within the context of pollution, we have the particulate matter (PM), which are the solid particles of pollution that can be categorized into different sizes: PM_{10} (coarse), $PM_{2.5}$ (fine) and PM_1 (ultrafine). All are harmful to humans but exceptionally fine and ultrafine that can penetrate deeply into the lungs thus causing serious health problems. Due to this increasing problem, it is urgent to think about how to reduce air pollution and improve people's quality of life.

One of the solutions for this problematic issue lies in the urban green areas. A lot of studies investigated leaves trees capability in PM interception and accumulation, but few researches compared species among them and which characteristics influence differences in PM accumulation. In this study, we compared 12 species commonly used in the urban Mediterranean arborization. The case-study city is an industrial urban area of central Italy, the municipality of Terni, notoriously known as a polluted area, mostly for air fraction. Leaves were gathered in one sampling campaign in 2017, after a dry period. A quali-quantitative analysis was promoted through two different techniques: Sacanning electron microscopy (SEM) and washing and filtering (W/F). The SEM technique coupled with energy-dispersive x-ray (EDX) analysis also allowed to identify PM size and its composition, and to differenciate for each species the main three classes of accumulation: coarse, fine and ultrafine. Secondly peculiar charactersitcs of leaves were observed, recorded and classified: macrostructures – foliage, leaf expansion, texture and leaf type by direct observation and microstructures – roughness, trichomes and stomatal density on leaf surfaces through SEM imaging analysis. Finally, we elaborated a retention index of PM with negative and positive traits to indicate which species, and even more broadly which morphologies are most favorable to enhance PM retention, thus to improve air quality in urban areas.

The species observed as strong PM_{10} accumulators were *Platanus acerifolia, Prunus cerasifera, Acer saccharinum* that retained relatively 11.96, 11.49 and 9.16μg*cm^{-2}. The same grouping was observed also for $PM_{2.5}$ and PM_1. The study revealed that some morphological micro-macro characteristics such as roughness, trichomes, low grooves dimension, high stomatal density, complex leaf type and non-soft texture acted as positive factors for PM retention. The retention index tool is in the preliminary phase but we believe it could be useful to help policymakers, urban planners, and landscapers in choosing urban tree species that are more potential to reduce the problem of pollution of their cities.

Key words: air pollution, PM deposition, SEM - EDX, urban trees, leaf morphology

Acknowledgements

My sincere thanks to the Erasmus Mundus Program consortium directors of the Master Mediterranean Forestry and Natural Resources Management, professor José Borges from University of Lisbon, professor Felipe Bravo from University of Valladolid and professor Paolo De Angelis from University of Tuscia who kindly introduce me my advisor professor Carlo Calfapietra and made this thesis possible.

My special thanks to the secretary Catarina Tavares who has always been very kind and helpful in bureaucratic matters and student welfare.

Thank you to my colleagues for the 5th edition of this masters program for the moments of relaxation and support on this journey.

First of all, it was an immense pleasure and a source of extensive knowledge to hold this masters degree in Mediterranean forests hitherto unknown to me. Secondly, it was a challenge but joy and daily motivation to carry out the thesis in the area of urban pollution in which it affects so many people negatively. But through this journey in this master's degree, I realized that it is possible to improve the quality of life of people through the science, research, and engagement of policymakers, decision makers, stakeholders and other authors to mitigate the harmful effects that pollution causes on the health of citizens.

I am extraordinarily grateful to my mentor Carlo Calfapietra for accepting me on his team and doing this work together in special collaboration with Gregorio Sgrigna who was very important for the analyzes, support, and conversations throughout this work.

Table of contents

Abstract 1
Acknowledgements 2
List of figuers 4
List of Tables 5
Chapter 1. INTRODUCTION 6
Chapter 2. MATERIAL AND METHODS 10
2.1 Study area 10
2.2 Sampling Methodology 11
2.3 Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) spectroscopy analyses 13
2.4 Washing and filtering analysis 16
2.5 Leaves morphological characteristics measurements 17
2.6 Statistical Analysis 20
Chapter 3. Results and Discussions 20
3.1 PM deposition - SEM/EDX compared to W/F technique 20
3.2 PM deposition - SEM/EDX ANOVA results 23
3.3 PM retention index 26
3.4 Difference between groups based on micro and macrostructural leaves characteristics 27
Chapter 4. CONCLUSIONS 35
REFERENCES 37

List of figuers

Figure 1. Terni city localization..11

Figure 2. Le Grazie park located in Terni city and the localization of the tree species sampled………………………………………………………………………………………..11

Figure 3. Map showing the location of Le Grazie Park in Terni, IT pointed with the yellow dot and the stell factory area in blue. On the left side of map, the wind rose of prevailing wind in the city of Terni... 13

Figure 4. (A) Electron micrograph of leaf surface, 150 x 150 µm, detailing both particulate matter, subject to this study, and additional plant structures. (B) Using Gwyddion, contrast manipulation is used on the image. Additionally, a threshold colouring (red) is applied, to marks areas to output individual particle diameter…...…………………………………………………………………14

Figure 5. Different leaves surfaces microstructural characteristics, smooth (A) *M. granfilgora*, rough (B) *P. cesarifera*, valley/rough (C) *A. saccharinum* and valley/peak (D) *R. pseudocacia*..18

Figure 6. Comparision between EDX and W/F technique ……………………………………...21

Figure 7. SEM/EDX assess of PM deposited on the scpecies leaves…………………………...24

Figure 8. PM deposition vs index values………………………..…………………………....27

Figure 9. Adaxial (1) and abaxial (2) side of the best species and different positive factors for PM adhesion, *A. saccharinum* and low grooves dimension (A1) and trichomes (A2); *P. tremula* and some roughness area (B1) and a significant hairy density (B2); *P. cerasifera* contains a high roughness area in both side of the leaves (C1 and C2)………………………………………..30

Figure 10. Different traits on the intermediate group, smooth surface (A1) and hairy density (A2) of *M.* grandiflora; low roughness (B1) and high stomatal density (B2) on *C. australis* and low rugosity and few trichomes (C1) and a complex leaf of *Q. pubescens*……………………………………………………………………………..……….31

Figure 11. Some negative micro-macrostructure factors on the species of the worst group: low stomatal density on *P. nigra* (A); zero roughness area on *T. cordata*; soft texture on *C. bignonioides* (C) and *R. pseudoacacia* (D)……………………………………………………………………….33

Figure 12. Honeydew aspect on the leaves surface and the respective causative agents. Aphid *Eucallypterus tiliae* (A1) and honeydew on *T. cordata* (A2); aphid *Phloeomyzus passerinni* (B1) and surface leaf of *P. nigra*; pollinization by bee (C1) on *R. pseudoacacia*……………………………………………………………………………….34

List of Tables

Table 1: Tree species scientific and common names and botanical characteristics……………..12

Table 2: Washing and filtering technique and the values of depositon for the tree species……..23

Table 3: Variation in PM_{10}; $PM_{2.5}$ and PM_1 accumulation on leaves surfaces (µg*cm^{-2}), as obtained by SEM-EDX for twelve trees species sampled in Le Grazie park in the city of Terni (Italy). Homogeneous groups are calculated through Fisher LSD – test……………………………………………………………………………………………..26

Table 4: Mean PM deposition ug.cm^2 and macro-microstructural characteristics and values……28

Chapter 1. INTRODUCTION

Air pollution is among the major environmental issue in urban area (WHO et al. 2013; European Environment Agency 2015). Particulate matter (PM) in the air is one of the fastest-growing types of environmental pollution (Wang et al., 2005). Traffic related PM, consisting in fine and ultrafine dusts, nitrogen dioxide (NO_2), and ozone (O_3), are the major pollutants influencing urban air quality, with daily limits exceeded in several urban regions (European Environment Agency, 2015). These pollutants are limited by European and national policies (Decree 155/2010 implementing the European Union Air Quality Directive 2008/50/EC) and extensively investigated in epidemiological research for their adverse health effects (WHO 2005). Epidemiological studies demonstrated a strong correlation between increased air pollution, as high levels of outdoor PM, and adverse health effects (Brook et al. 2010; Dominici et al. 2006; Merbitz et al. 2012; WHO et al. 2013). Fine particles in the air cause approximately 4 millions of premature deaths worldwide (European Comission, 2012). Ultrafine dust particles (<2.5 um) are relevant in the dense urban area because they can be inhaled deeply into the respiratory system and cause health problems and affect human beings (Powe & Willis 2004). A 10 mgm^{-3} increase in fine particulate matter has been associated with an approximately 4%, 6%, and 8% increased risk in all-cause, cardio-pulmonary and lung cancer mortality, respectively (Pope III et al. 2002).

Several studies quantified PM interactions with leaf surface or measured PM fluxes captured by trees in urban or periurban areas (Manes et al. 2016; Nowak et al. 2006). Fine PM can be reduced when particles, specifically the smaller size fractions (<10 um), are adhered to the leaves (Hosker & Lindberg 1982; Ottelé et al. 2010; Sternberg et al. 2010).

According to the European Commission (European Commission, 2015), nature-based solutions (NBS), can be cost-effective solutions providing environmental, social and economic benefits. Studies conducted mainly on urban trees showed the potential effects of vegetation in improving air quality. Some studies concluded thar increasing total tree cover in West Midlands,UK from 3.7% to 16.5% is estimated to reduce average primary PM_{10} concentrations by 10% from 2.3 to 2.1^{-3} mgm (removing 110 tonnes per year); increasing tree cover from 3.6% to 8% in Glasgow, UK is estimated to reduce PM_{10} concentrations by 2%, (removing 4 tonnes per year) (McDonald et al. 2007). Urban tree leaves can act as cost-effective passive air samplers for diffused monitoring and characterization of PM pollutants in distinct urban environments (Sgrigna et al. 2016).

Vegetation is an efficient sink for particulate matter (Fowler et al. 1989). It is well documented that plants can effectively adsorb and reduce particulates in the air by capturing the airborne particulate matter such as foliar dust, hydrogen fluoride, SO_2, some compounds of photochemical reactions and heavy metals such as mercury (Hg) and lead (Pb) from the air on their leaves (Freer-Smith et al. 1997; Brack 2002).

The use of trees for PM capture purposes is becoming more and more widespread. The main advantages are a higher availability of the different species, the simplicity of species identification, sampling and treatment and omnipresent of some genera, which makes it possible to cover large areas. As trees have a larger collecting surface area than other land cover types and also promote vertical transport by enhancing turbulence, there is a greater opportunity for particles to be collected on the trees surface. Trees are therefore more efficient at capturing particles from the atmosphere by dry deposition relative to short vegetation like lichens and moss (Gallagher et al. 1997; McDonald et al. 2007). Because of the great potential of trees to mitigate pollution in urban centers, new models of planting and species selection are being prioritized for urban planners (Calfapietra et al. 2013; Llausàs & Roe 2012; Morani et al. 2011).

Plant leaves are the primary receptors for both gaseous and PM pollutants of the atmosphere, also leaves are sensitive and highly exposed parts of a plant, may act as persistent absorbers for PM in a polluted environment (Rai 2016; Samal & Santra 2002). They act as pollution receptors and reduce dust concentration of the air. The capacity of leaves as dust receptors depends upon their surface geometry, phyllotaxy, epidermal and cuticular features, leaf pubescence, and height and canopy of trees (Mcpherson et al. 1994).

A plant's capacity to capture PM is affected by several factors including the microstructure of the leaf's surface, the macrostructure of vegetation and environmental variables like wind and temperature (Mo et al. 2015; Chen et al. 2016). The PM retention abilities of vegetation depend on several factors, such as the type of tree canopy, leaf and branch density and leaf morphology (roughness, trichomes and concave/convex, etc.), as well as prevailing meteorological conditions (Prusty et al. 2005; Qiu et al. 2009; Rai et al. 2010). Microstructural features like rough surfaces, pubescence, thick waxy epicuticles and low stomatal densities along with macrostructural features like increased plant height, whorled leaf arrangements and larger leaf areas are all individual traits that enhance PM accumulation (Popek et al. 2013; Chaturvedi et al. 2013; Prusty et al. 2005; Mo et al. 2015; Nowak et al. 2006). Many studies have demonstrated that plants can effectively retain dust due to their large leaf surface areas and sophisticated leaf microstructures (Yin et al. 2011; Nowak et al. 2013; Wang et al. 2015; Chen et al. 2017; Liu et al. 2018).

To maximize the amount of PM captured and thereby the improvement to air quality that urban green areas have, it is crucial to understand which species and micro and macrostructural traits are most effective in removing PM. While the importance of individual traits on PM deposition is well known, we are yet to appreciate how different combinations of these traits interact to influence PM accumulation. The species and planting systems that are most efficient in removing PM should be selected to maximize the benefits of urban vegetation in improving air quality. While much is known about the mechanisms involved in the deposition of PM on vegetation, fewer studies have addressed the differences in PM accumulation of various species, particularly for the smallest fractions ($PM_{2.5}$).

Due to the wide variety of plant species - trees, shrubs, climbing trees - that can be chosen for planting in urban green areas, species-specific information could help the municipalities –

policy makers, urban planners, green space managers and practitioners - select the optimal vegetation for urban and peri-urban forestry.

In the present study, leaves were collected from tree species commonly used in the urban arborization of the city of Terni, Italy. The city includes an industrial area for mettallurgical production: a steel factory pertaining to the Thyssen Krupp multinational conglomerate. The presence of such an important source of PM, togheter with the peculiar geo-morphological characteristics of the regional environment lead to important negative effects on the local air quality, and consequently affects the inhabitants health. An important epidemiological study of residents in contaminated sites made at a national scale (called "SENTIERI"), was carried out also in Terni. The study revealed that between 1995-2002 in Terni there were 365 premature deaths on the average mortality rate for air pollution-related diseases: respiratory diseases and chronic lung diseases (Pirastu et al. 2011). Due to this problem - which tends to grow due to increasing urbanization - it becomes urgent and necessary to better plan the urban green areas, as well as to select species that have a better capacity for PM retention. Therefore, the objective of this current study were: (1) to analyze the accumulation of PM in different tree species - located in the same conditions of pollution exposure, (2) through the SEM / EDX images technique observe the microstructures present on the leaf surfaces that contribute in the pollution retention and (3) indicate the most suitable tree species and their morphological charcteristics to mitigate urban pollution.

Chapter 2. MATERIAL AND METHODS

2.1 Study area

The city of Terni, a medium-sized city, is locate the southwest of the Umbria region, Italy (Fig. 1). The city has around 112,000 inhabitants, with a high density of industrial activities, which makes it the most industrialized city of central Italy. The social-economic development related to the industrial sector involves the city sprawl and urbanization density, a change on morphology and land use, a reduction of evaporative surface, a greater energy consumption and pollutants emissions.

According to the Köppen climate classification, Terni belongs to CSA (Temperate, dry Summer, hot Summer) category (i.e., to the temperate climate of the middle latitudes, with hot summer). The city experiences a typical mild Mediterranean climate during Spring and Autumn. The humid seasons are spring and autumn, mainly in November and April. The summer is hot, humid, muggy and basically has little rainfall, while winter is cold and rainy. In general, the weather is not characterized by strong winds, because the winds diminish in intensity encountering the surrounding mountains.

The geographic location, that is characterized by a peculiar landscape morphological structure, is a key factor in the climate vulnerability of the city. The surrounding mountain barriers reduce air mixing, pollutant transport. Very high PM concentrations occur throughout the year, particularly in winter. According with Sgrigna et al. (2015), this city is characterized by high average levels of PM_{10} concentration in the air. In addition to pollution from the burning of fossil fuels, the high concentration of pollution is due to the fact that there is a significant factory in the metallurgical sector covering an area of 1,500,000 sq.m in which it produces the entire stainless steel flat product manufacturing cycle, including over one hundred different types of products stainless steel plans. Also, it manufactures hot rolled titanium plates, coils, and tubes for industrial use. Terni is one of the most important industries in Italy and a world leader in steel production.

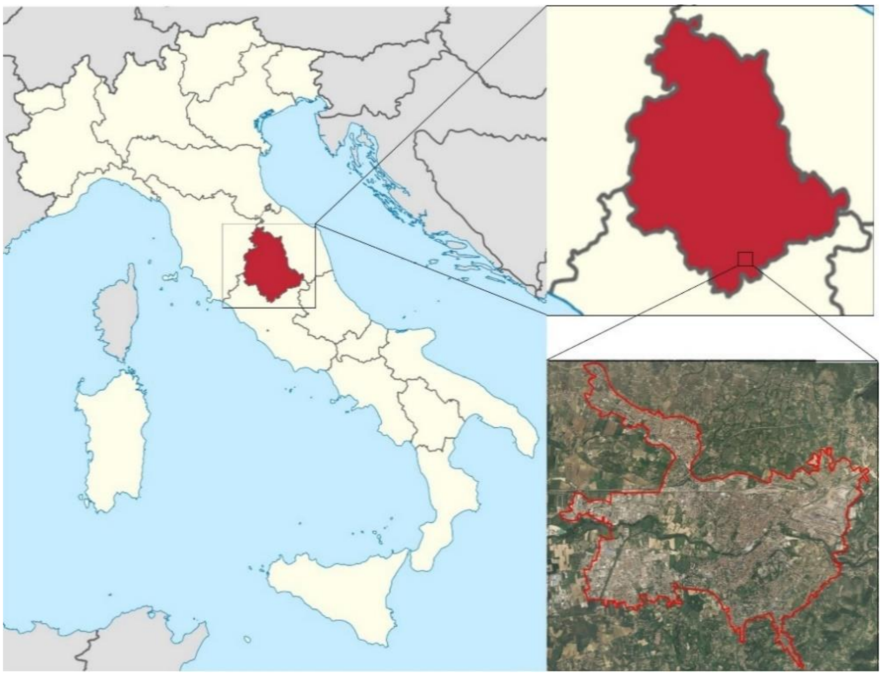

Figure 1: Terni city localization.

2.2 Sampling Methodology

During mid of June of 2017, 12 tree species commonly used in urban arborization were sampled and 36 leaf samples collected in Terni city in Le Grazie park (Fig. 2). Trees were sampled at the height of 6 m, approximately in the middle-lower region of the crown. All sampled branches were oriented in the same direction: east-north-east side of crown.

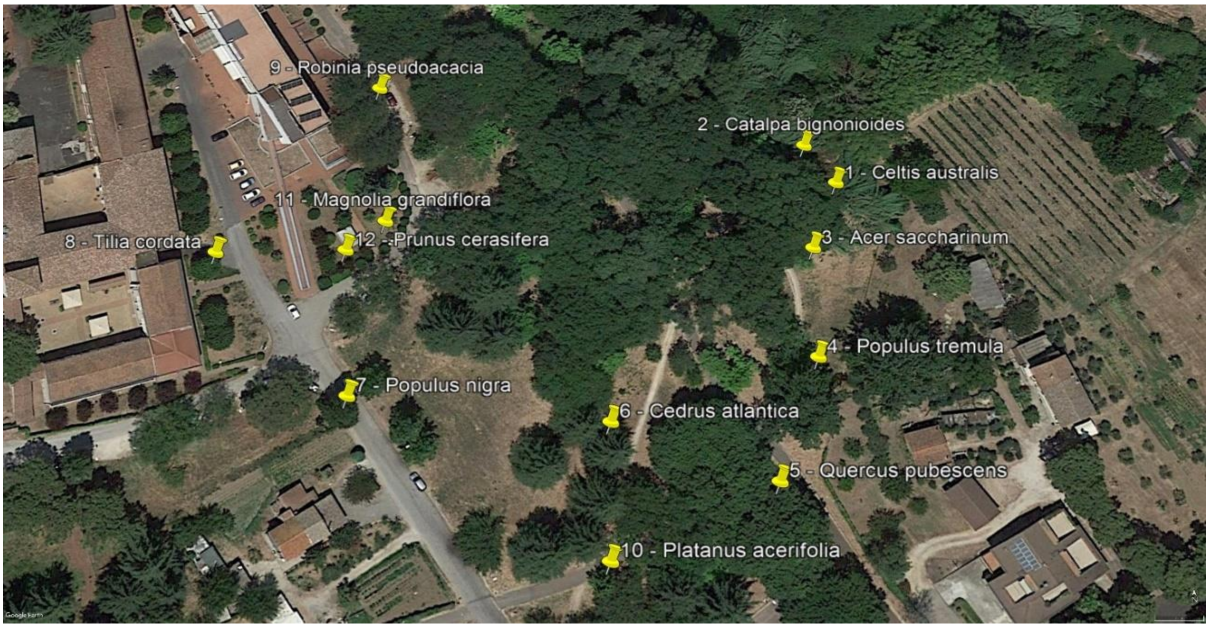

Figure 2: Le Grazie park located in Terni city and the localization of the tree species sampled.

The species were (Tab. 1): *Acer saccharinum* L., *Catalpa bignonioides* Walter, *Cedrus atlantica* (Endl.) Manetti ex Carrière, *Celtis australis* L., *Magnolia grandiflora* L., *Platanus acerifolia* (Aiton) Willd., *Populus nigra* L., *Populus tremula* L., *Prunus cerasifera* Ehrh., *Quercus pubescens* Willd., *Robinia pseudoacacia* L. and *Tilia cordata* Mill..

Table 1: Tree species scientific and common names and botanical characteristics.

Tree species	Botanical characteristics		
	Common name	**Family**	**Foliage**
Platanus acerifolia	planetree	platanaceae	deciduous
Prunus cerasifera	cherry plum	rosaceae	deciduous
Acer saccharinum	silver maple	sapindaceae	deciduous
Populus tremula	aspen	salicaceae	deciduous
Magnolia grandiflora	southern magnolia	magnoliaceae	evergreen
Celtis australis	mediterranean hackberry	cannabaceae	deciduous
Quercus pubescens	downy oak	fagaceae	deciduous
Cedrus atlantica	atlas cedar	pinaceae	evergreen
Catalpa bignonioides	indian-bean-tree	bignoniaceae	deciduous
Populus nigra	black poplar	salicaceae	deciduous
Robinia pseudoacacia	black locust	fabaceae	deciduous
Tilia cordata	small-leaved lime	malvaceae	deciduous

The choice of this study area was due to the proximity of the park Le Grazie - approximately 2km - about the steel factory. And the determination of the orientation of the leaves collection was due to the direction of the wind that ends up carrying the polluting particles in that direction (Fig. 3).

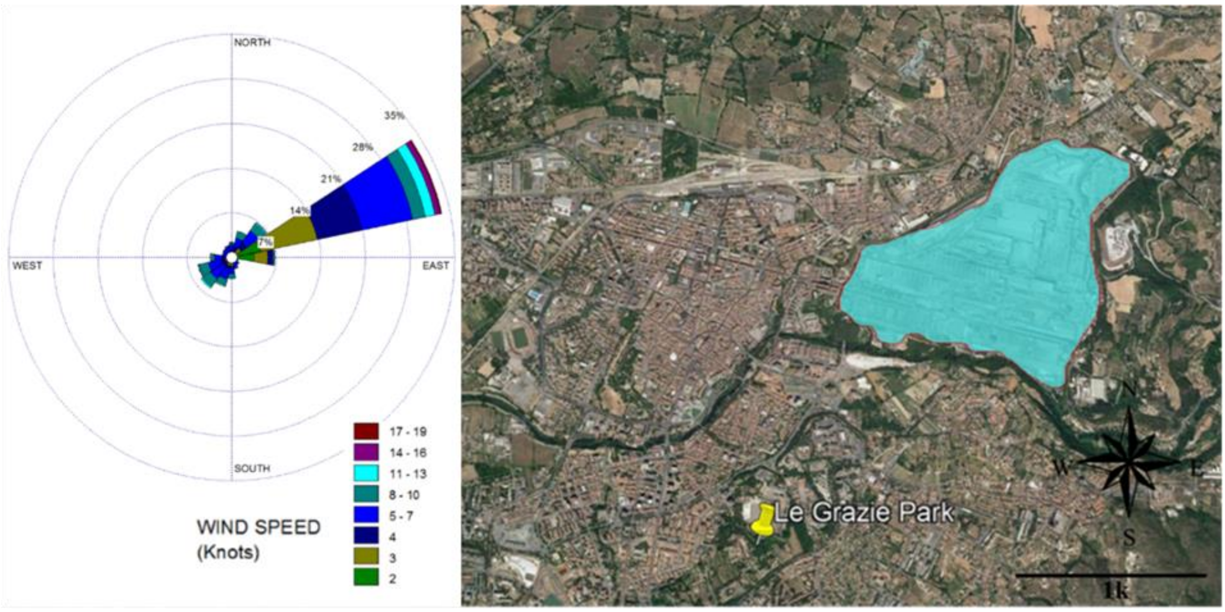

Figure 3: Map showing the location of Le Grazie Park in Terni, IT pointed with the yellow dot and the stell factory area in blue. On the left side of map, the wind rose of prevailing wind in the city of Terni.

2.3 Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) spectroscopy analyses

Particle size and density and was obtained through scanning electron microscopy (SEM) – imaging analysis, while elemental composition of PM was achieved through energy-dispersive X-ray spectroscopy (EDX) analysis, coupled with SEM. These data allowed us to estimate the total collected volume of particles and the different elements composition. On the leaf area basis thus was possible to estimate the mass of PM, expressed in weigth (µg*cm^{-2}).

To analyze the leaf microstructural morphological characterisitics, such as grooves, trichomes and stomata, and to measure the particles density/cm^2 and size for each leaf, two portions of 1 cm^2 were cut from the leaf part above the left main rib, and separately used for the analysis of the abaxial and adaxial leaf surfaces. A Phenom ProX (Phenom-World, The Netherlands) scanning electron microscope was used, equipped with X-ray analyzer and charge-reduction sample holder suited for biological samples. Leaf portions were mounted within the sample holder by using double coated carbon conductive PELCO Tabs (Ted Pella, Inc.), after having fluxed them with compressed air. SEM is set at 5Kv, found to be the most effective resolution for particulate size and number count during contrast manipulation. Ten images were taken for each sample at 1800x magnification, an approximate area of 150x150 µm, although true area was recorded after image manipulation. SEM images were analyzed with Gwyddion software (Gwyddion version 2.49: an open-source software for SPM data analysis), in order to obtain the number of particles (Nečas et al., 2012). This software was used to manipulate background contrast of each electron microscope image, by applying a colour threshold (figure 4). This allowed for rapid and repeatable selection of particulates against leaf surface background; providing a mechanism for analysis of individual particulate size and distribution of the sample. The size of particulate was estimated by equivalent sphere deq, calculated from automated minimum and maximum Ferret diameters. The total volume of particulates of the specific PM fractions (i.e., PM10, PM2.5 and PM1).

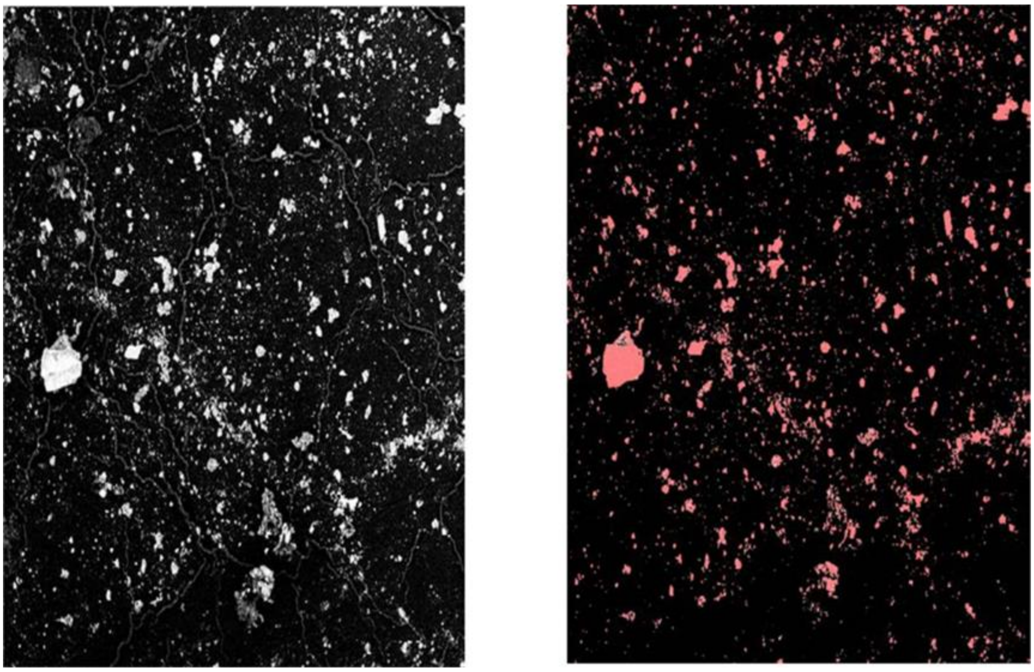

Figure 4: (A) Electron micrograph of leaf surface, 150 x 150 µm, detailing both particulate matter, subject to this study, and additional plant structures. (B) Using Gwyddion, contrast manipulation is used on the image. Additionally, a threshold colouring (red) is applied, to marks areas to output individual particle diameter.

EDX was used to identify elemental composition of leaf surface deposits. For elemental analysis using EDX, five images of the leaf surface upon each sample, at 50µm lateral size at 15KeV, a resolution and an image contrast found most effective for elemental analysis. This region is thought to cover PM size fractions 10-0.2µm. The elemental composition of 10 randomly chosen particles were measured in each image, resulting in a total of 50 particles from each leaf sample side (50 adaxial and 50 on the abaxial side) and including each replicate, a total of 200 particles per species.

A semi-quantitative estimation of the elemental composition was obtained by calculating the weighted volume percentage (W%) occupied by each element x over the N selected particles. This was obtained as a product of the composition C (as percentage) of each element x on each particle i (Cxi, as obtained by the EDX software) and the corresponding particle volume Vi, as obtained by the diameter of the equivalent sphere deq ($Vi=4/3\ \pi\ (deq/2)^3$). Then, for each element x these volumes were summed together, and the sum was normalized by using the total analyzed particle volume. The resulting W% for each element x was obtained following equation (Eq.1).

$$W_{\%_x} = \frac{\sum_{i=1}^{N} C_{x_i} \times V_i}{\sum_{i=1}^{N} V_i}$$

Eq.1

The W% obtained per each element x, for each sample s, were then multiply by the total particle volume for the corresponding sample (V_s), as obtained by the SEM images of the collected leaves, and by the corresponding elemental atomic mass per volume (am), known as solid state density (https://www.webelements.com/periodicity/density/), and normalized by the total imaged area for the sample (A_s). By following Equation 2, the amount of leaf deposited PM per unit leaf area for the sample s (M_s) is then obtained.

The M_s values were obtained also at different PM dimensional classes: PM_1; $PM_{2.5}$; and PM_{10}, as shown for the imaging analysis methodology the minimum PM dimension considered is 0.2µm.

$$M_s = \sum_{i}^{N} \frac{W_{\%x} \cdot V_s \cdot am_x}{A_s}$$

Eq.2

2.4 Washing and filtering analysis

The quantitative analysis of PM deposited were obtained through the washing of the leaves and the filtration of the solution in cellulose membranes of nitrocellulose, different degrees of known porosity. In this way the suspended portion in the micro-distilled water solution was differentiated into three-dimensional classes. The main steps of the washing and filtration process are: 1) drying filters identified with the weighing filter code and given at 70 ° C in a stove for 1h and 20 min. Extraction of the filters from the oven, closed in the special glass filter scales. Weighing after 35 min for stabilization of temperature and humidity parameters; 2) collection of samples, sealed in PVC bags, stored in the freezer at -20 ° C; 3) selection of the material of each sample: selection of leaves and branches (approximately between 200 and 700 cm2 of leaf area); 4) wash in 500 ml of microfiltered distilled water and a small brush; 5) collection of washing solution in 500 ml beaker, filtered with 1 mm PVC sieve (100 μm). Extraction of the washed and drying sample on absorbent paper; 6) filtering solution in Millipore funnel system and vacuum pump. The system consists of three vacuum flasks, two glasses for the collection of the solution and teflon grids to hold up the filters. The passage in the column filtration system takes place through three passages through the filters, with gradually decreasing porosity of 10, 2.5 and 0.2 μm; 7) repeat step "1" with the filters filled with the suspended material and dissolved in the filtered solution; 8) weighing with precision balance compared to previous measurements on virgin filters.

2.5 Leaves morphological characteristics measurements

Micro and macrostructural characteristics of leaves were considered as influencing factors for PM deposition. Microstructures, observed through SEM imaging were: trichomes density; stomata density and grooves (width and density) to determine the roughness area and different types of it on the leaf surface. A range of 100 µm was used for the density and size of the stomata, as well as for the evaluation of the trichomes distribution.

Images were saved by SEM imaging tool at the resolution of 1024x1024 pixels. Different energies (5 or 15 KeV) were used to acquire the files, in order to obtain a better resolution for finer structures. The magnification varied approximately between 400x and 1000x, to obtain images of 400x400 µm and 150x150 µm respectively. Finally images were analyzed through the open source software ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2012). ImageJ allowed to precisely measure and count the different microstructures above cited.

Further considerations about leaves shape and morphology were also included: we observed leaf smoothness (Fig. 5a), the presence of wrinkles, such as: rough (Fig. 5b), valley/rough (Fig. 5c) or valley/peak (Fig. 5d).

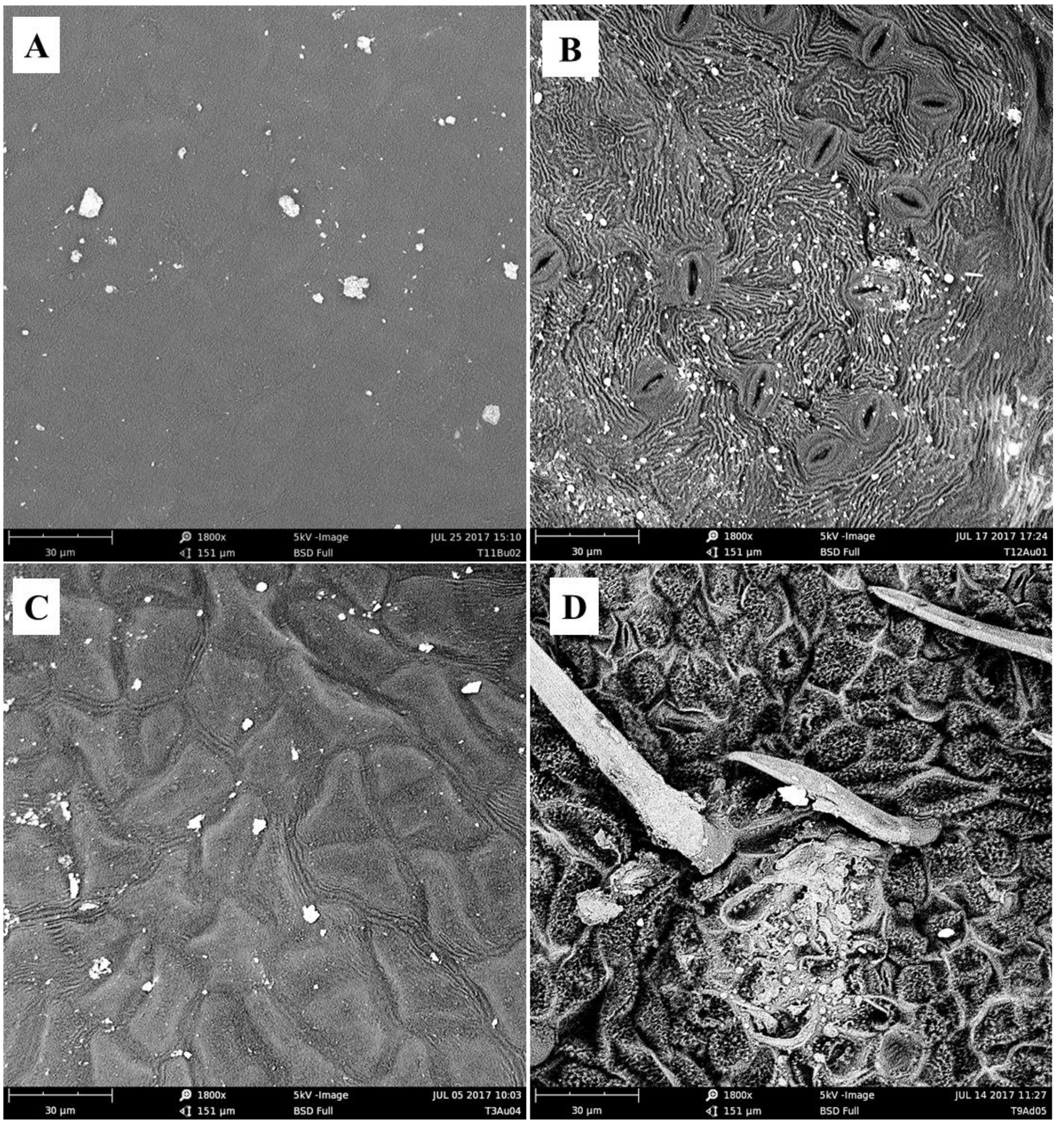

Figure 5: Different leaves surfaces microstructural characteristics, smooth (A) *M. granfilgora* , rough (B) *P. cesarifera*, valley/rough (C) *A. saccharinum* and valley/peak (D) *R. pseudocacia*.

Macrostructures included in the analysis are leaf-expansion period, foliage, leaf texture, and leaf shape. For the accomplishment of the leaf-expansion period, ten leaves of each tree species were collected in 3 different months (June, May and April), being categorized as total (60 days), partial (30 days) and initial (<30 days). For the foliage, we have two different types: deciduous or evergreen. The leaf texture characteristic was observed during the field collection of the species and categorized as soft or non-soft and regarding the differences between shape type we have: lobed, rhomboid, ovate, cordate and acicular.

To better understand how the combination of these factors – micro and macro - act positively or negatively on the pollution retention capacity, a retention index (I) of PM was elaborated:

I = Rougness area+Rougness type+Grooves dimension+Stomata density+Hairy density+Foliage+ Leaf expansion+Leaf texture+Leaf type

All the morphology characteristics were categorized in four different classes:

- Class 0: zero percentage or not recorded (NR) or zero complexity or zero dimension/density
- Class 30: low percentage or low complexity or low dimension/density
- Class 60: intermediate percentage or intermediate complexity or intermediate dimension/density
- Class 90: high percentage or high complexity or high dimension/density

As the categories - roughness area, hairy density and leaf expansion - were measured in percentage we consider the following values: class 0 = zero percentage; class 30 = percentage between 1% and 33%; class 60 = percentage between 34% and 66%; class 90 between 67% and 99%. For roughness type we use a scale of complexity: class 0 = smooth surface or NR, class 30 = peak/valley and rough/valley, class 90 = rough. Also for leaf type we use a scale of complexity: class 30 =ovate or cordate or acicular, class 60 = rhomboid, class 90 = lobed. For grooves dimension we consider: class 0 = NR, class 30 = grooves > 1mm, class 90 = grooves < 1mm. For stomata density we consider: class 0 = NR, class 30 = density < 183 stomata per cm^2, class 60 = density >184<367 stomata per cm^2 , class 90 = density > 367 stomata per cm^2. The foliage we consider class 0 to deciduous and class 90 to evergreen. Among the texture, we consider class 0 to soft and class 90 for non-soft surfaces.

2.6 Statistical Analysis

Statistica software, version 7 (TIBCO Software Inc, 2018) was used for statistical tests in this study. Significant variations in PM weight accumulated on different species, as estimated through the SEM-EDX outputs, were analyzed using one-way ANOVA following confirmation of normality using the Shapiro-Wilk test. Significant differences, thus the homogeneous groups were identified using the Fisher's LSD post-hoc test. The same test was run for the three PM categories (PM_{10}; $PM_{2.5}$ and PM_1), as shown on Table 3. The comparison between the two different techniques, SEM-EDX and washing-filtering, was perfomed on accumulation values ($\mu g*cm^{-2}$) of both techniques through regression analysis.

Chapter 3. Results and Discussions

3.1 PM deposition - SEM/EDX compared to W/F technique

Although the washing and filtering technique is widely used in PM measurement studies, it has some disadvantages. First, this technique allows just a quantitative analysis. Even if it allows to separate different dimensional classes of PM (PM included in the ranges 0.2-2.5 and 2.5-10µm) it is not possible to distinguish the chemical element from the particles. Therefore, due to these factors we used the SEM/EDX technique to make a comparison with the W/F technique and check for differences between the techniques. SEM/EDX presents some advantages over W/F, besides being more precise, it presents a greater wealth of details of the PM, as for example it is possible to measure the individual size of each one, on the other hand, the W/F technique is a quicker technique than SEM/EDX analysis and of course with evident costs advantages. Nevertheless SEM/EDX technique is widely recognized as a very important tool for PM characterization. Both techniques are commonly used for leaves PM deposition studies in urban environments, for PM characterization and quantification. The comparision between the two approaches is not frequent. Thus we decided to directly compare the two results (Fig. 6).

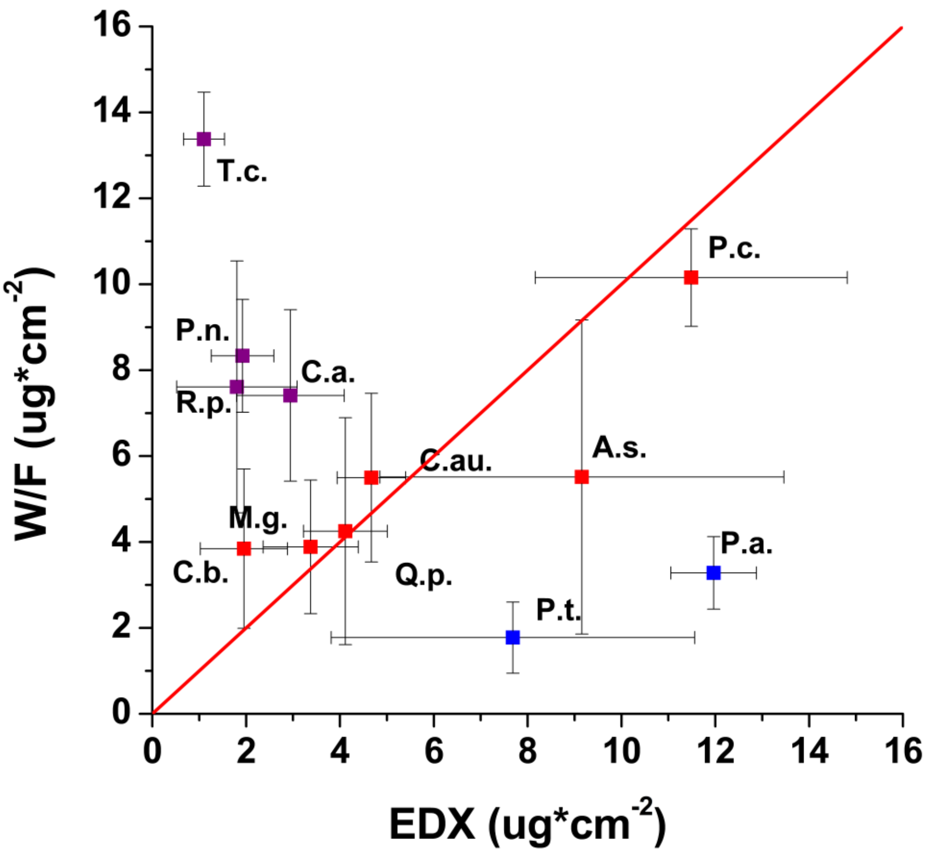

Figure 6: Comparision between EDX and W/F technique.

In a first moment when we compare the two techniques in a direct way, through a regression analysis we can not obtain a relation between them. However, if we project a 45 ° line, it is possible to verify that some species are correlated: *P. cerasifera*, *C. australis*, *A. saccharinum*, *M. grandiflora*, *Q. pubescens* and *C. bignonioides*. As these species showed a relation in both techniques, they can be good species for biomonitoring in urban areas.

On the other hand we can also observe a better performance of four species in the W/F technique (Tab. 2): *T.cordata* (13.38 ± 1.54) , *C.atlantica* (11.12 ± 2.82), *P.nigra* (8.33 ± 1.86) and *R.pseudoacacia* (7.61 ± 4.14), and two species show a better performance with SEM/EDX technique (Tab. 3): *P.acerifolia* (11.96 ± 0.53) and *P.tremula* (7.69 ± 2.24).

The difference between the techniques can be explained because one of the four species in the W/F technique is a conifer *C.atlantica* and it may present a different behavior from the broadleaves. To better understand this species, the ideal is to carry out a further study with other coniferous tree species. The other three species contained honeydew on their surface - *T. cordata*, *R. pseudoacacia* and *P.nigra* - and when these species were observed through the method of SEM/EDX it was not possible to observe the PM and consequently measure them because possibly the PM were under that sticky substance. On the order hand when the W/F technique was used, the water washes the honeydew and in this way, it is possible to measure the PM.

However, we can not be sure how much of this sticky substance is washed out of the particles or how much gets adhered in the particles and if it can influence their weight. However, within the project in which the thesis is inserted, there is a part dedicated to the analysis of the water used in the technique of W/F and that therefore may elucidate some questions about the honeydew.

For a better understanding of Figure 6, table 2 presents the W/F technique complete data of the species. *T. cordata* (13.38 ± 1.54) is the best species on this technique but on SEM/EDX technique (Tab.3), this species has the worst result (1.10 ±0.25).

What draws attention to this result is that this species is widely used in urban green areas around Europe and therefore there are other studies that point this species as one of the best in PM capture through the W/F technique. Once again we reinforce that honeydew can influence the results in a negative way: preventing SEM/EDX visualization and measurement of the particles or even overestimating the values in the W/F technique.

Table 2: Washing and filtering technique and the values of depositon for the tree species.

Tree species	Species code	ug*cm^-2	Standard deviation
T. cordata	T.c.	13.38	1.54
C. atlantica	C.a.	11.12	2.82
P. cerasifera	P.c.	10.16	1.6
P.nigra	P.n.	8.33	1.86
R.pseudoacacia	R.p.	7.61	4.14
A. saccharinum	A.s.	5.51	5.17
M.grandiflora	M.g.	5.47	2.78
C.australis	C.au.	4.25	3.74
Q. pubescens	Q.p.	3.88	2.2
C. bignonioides	C.b.	3.84	0.2
P. acerifolia	P.a.	3.28	1.19
P. tremula	P.t.	1.775	1.17

3.2 PM deposition - SEM/EDX ANOVA results

Species differed in accumulation of all PM fractions, as observed on Figure 7. Regarding PM_{10} the highest values of accumulation were especially observed for *Platanus acerifolia*, *Prunus cerasifera*, *Acer saccharinum* that retained relatively 11.96, 11.49 and 9.16µg*cm^{-2}. The lowest accumulation (lower than 2µg*cm^{-2}) were recorded for *Tilia cordata*, *Robinia pseudoacacia*, *Populus nigra* and *Catalpa bignonoides*. Mean PM accumulation values (2 < Weight (µg*cm^{-2}) < 6) were observed for *Cedrus atlantica*, *Quercus pubescens*, *Celtis australis* and *Magnolia grandiflora*. Finally a hybrid situation was observed for *Populus tremula* with 7.69 2µg*cm^{-2}. $PM_{2.5}$ for the majority of results also followed the pattern described for PM_{10}. A slightly different behavior was observed for PM_1.

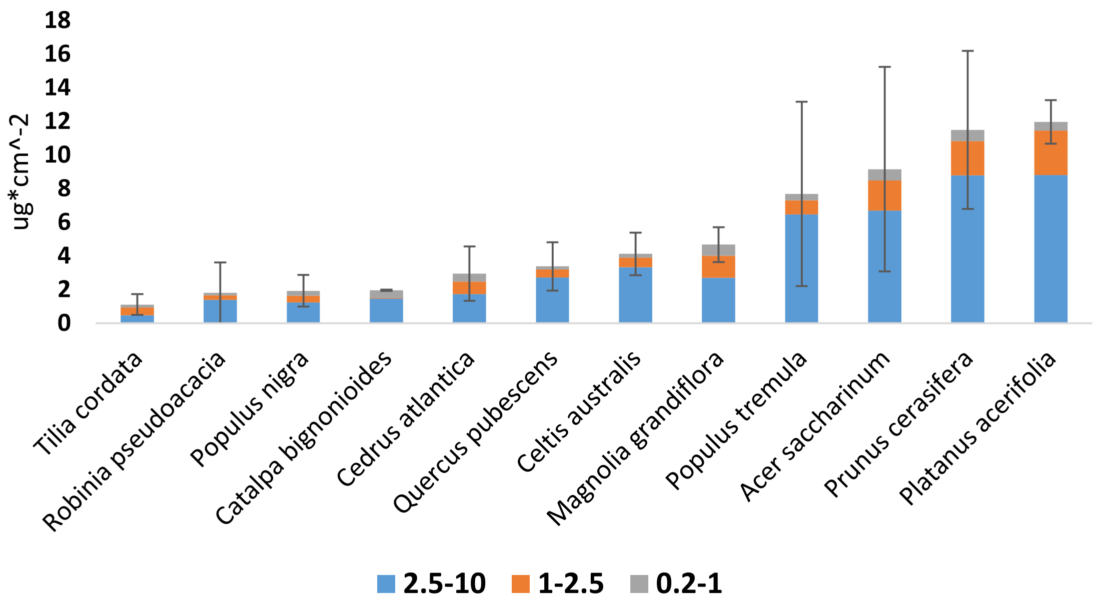

Figure 7: SEM/EDX assess of PM deposited on the species leaves.

Differences and similarities in PM accumulation among species are evidenced on Table 3. The ANOVA was performed on accumulation data for the three PM fractions, thus a post hoc test (or multiple comparison test) was used to determine the significant differences among the 12 species in an analysis of variance setting. The Fisher LSD – test was selected for this analysis.

Species that share the same letter/group do not present significantly differences according with the post hoc test, therefore we can observe in our results homogeneous groups and later hypotheses were raised through description of the micro and macrostructures presented in this thesis.

Based on Table 3, considering all three particle size fractions, it can be noticed that there was a more evident difference between the species in PM_{10}. The group with the letter "c" – *P.acerifolia* 11.96 ± 0.53 , *P.cerasifera* 11.49 ± 1.92 - presented the best performance in the PM removal capacity followed by the groups "ce,de,bd" which contains the species *A. saccharinum* 9.16 ± 2.48, *P.tremula* 7.69 ± 2.24 and *M.grandiflora* 4.67±0.42 respectively. Some hypotheses were raised for this better perfomance based on the macro and microstructures foliar of these species. It was observed that the species of this group present a high/medium percentage of roughness (Tab. 4), trichomes and a rigid leaf texture. The worst performing species in PM_{10} capture belongs to group "a", and it was *T. cordata* (1.10 ± 0.25) then followed by group "ab" *R.pseudoacacia*, *P.nigra, C.bignonioides*, *C. atlantica*. Some hypotheses pointed to this worse position is the presence of honeydew in its leaves, the absence of roughness and the soft texture of the leaves (Tab. 4).

$PM_{2.5}$ shows the same pattern than PM_{10} groups: *P.acerifolia* "d", *P.cerasifera* "cd" and *A. saccharinum* "cd" revealed the best performances in PM capture, but also *M.grandiflora* "bc" seems to have good capability. The worst groups belong to the group "a" and "ab", the species are the same as PM_{10}, excluding *P.tremula*, which belongs to the best PM retention group on PM_{10}.

PM_1 differently from PM_{10} and $PM_{2.5}$ shows a different pattern, and the Fisher test of similar groups has some limits with this data: 5 species on 12 are cumulated in 3 or more different groups: *P.nigra*, *C.bignonioides*, *C.australis* to "abc" group, *C.atlantica* to "bcd" group and the most evident case belongs to the group "abcd" - *P.tremula* – which is not differencied at all from other species. Nevertheless, like PM_{10} *T.cordata* and *R.pseudoacacia* are the worst, and similarly to $PM_{2.5}$ together with *P.acerifolia* "cd", *P.cerasifera* "d", *A. Saccharinum* "d" and *M.grandiflora* "d" are also grouped. Interesting in this class is the case of *P.acerifolia*, associated with "lower – accumulator" species on group "abc".

The different pattern showed for PM_1 and, in general the statistical difficulty in separating the species, probably lie in PM_1 behavior: the accumulation of this PM class is more homogeneous among species.

Table 3. Variation in PM10; PM2.5 and PM1 accumulation on leaves surfaces ($\mu g*cm^{-2}$), as obtained by SEM-EDX for twelve trees species sampled in Le Grazie park in the city of Terni (Italy). Homogeneous groups are calculated through Fisher LSD – test.

	PM10			**PM2.5**			**PM1**		
	Mean	**s.e.**	**group**	**Mean**	**s.e.**	**group**	**Mean**	**s.e.**	**group**
Tilia cordata	1.10	0.25	**a**	0.80	0.23	**a**	0.23	0.05	**a**
Robinia pseudoacacia	1.80	0.74	**ab**	0.49	0.09	**a**	0.23	0.06	**a**
Populus nigra	1.93	0.39	**ab**	2.47	0.71	**a**	0.67	0.23	**abc**
Catalpa bignonioides	1.95	0.02	**ab**	1.23	0.04	**a**	0.38	0.02	**abc**
Cedrus atlantica	2.94	0.66	**ab**	0.65	0.05	**ab**	0.19	0.02	**bcd**
Quercus pubescens	3.38	0.59	**ab**	1.21	0.17	**a**	0.47	0.02	**ab**
Celtis australis	4.12	0.52	**ab**	0.69	0.05	**a**	0.28	0.03	**abc**
Magnolia grandiflora	4.67	0.42	**bd**	0.48	0.09	**bc**	0.17	0.03	**d**
Populus tremula	7.69	2.24	**de**	0.42	0.17	**ab**	0.15	0.06	**abcd**
Acer saccharinum	9.16	2.48	**ce**	3.15	0.27	**cd**	0.51	0.04	**d**
Prunus cerasifera	11.49	1.92	**c**	1.97	0.38	**cd**	0.65	0.19	**d**
Platanus acerifolia	11.96	0.53	**c**	2.69	0.66	**d**	0.67	0.15	**cd**

3.3 PM retention index

Based on the results regarding the macro-microcaractheristics presented in Table 4, the classes of PM retention index were applied. After the results through the index formula, we performed a correlation analysis between PM_{10} deposition values in comparison with the values of the index. We observe a R^2=0.69 and three distinct groups: the best, the intermediate and the worse (Fig.8).

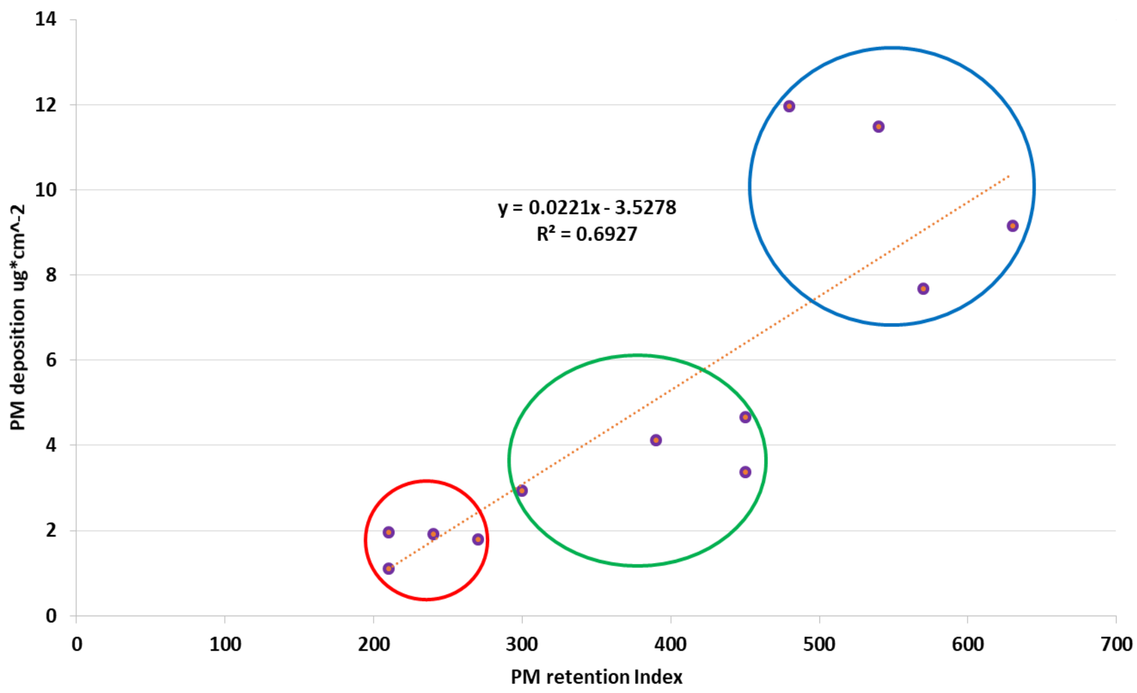

Figure 8: PM deposition vs index values.

In the blue group the best species for PM retention: *P. acerifolia*, *P. cerasifera*, *A. saccharinum* and *P. tremula*. Intermediate group is in green and the species are: *M. grandiflora*, *C. australis*, *Q. pubescens* and *C. atlantica*. And in red is the group of the worst species: *C. bignonioides*, *P. nigra*, *R. pseudococacia* and *T. cordata*.

3.4 Difference between groups based on micro and macrostructural leaves characteristics

Under identical climatic conditions, urban trees can display distinct dust-retention abilities depending on tree canopy structure, branch density, leaf inclination angle, as well as factors such as leaf morphologic structure characteristics and leaf area (Qiu et al. 2009). The difference of dust capacity between plant species is also determined by foliar morphology characteristics (including the degree of roughness, shape and amount of trichome in upper and lower epidermis of leaves) (Chai et al. 2002, (Mo et al. 2015)).

Micro and macrostructures (Tab. 4) have been shown to be relevant characteristics in the adsorption of particles. However, these characteristics act individually for each species.

Table 4: Mean PM deposition ug.cm^2 and macro-microstructural characteristics and values. NR: not recorded

Tree species	Mean PM deposition ug.cm2	Microstructures					Macrostructure		
		Roughness area %	Roughness type	Grooves dimension	Stomata density. cm2	Trichomes density %	Leaf expansion %	Leaf texture	Leaf type
P.acerifolia	11.96	46	rough	1.05	146.88	NR	13.86	non-soft	lobed
P. cerasifera	11.49	84	rough	0.79	351.88	0.94	71.93	non-soft	ovate
A.saccharinum	9.16	35	rough	0.66	552	12.65	77.88	non-soft	lobed
P.tremula	7.69	52	rough	0.93	211.3	83.26	28.02	non-soft	rhomboid
M. grandiflora	4.67	0	smooth	NR	288.5	99.5	89.42	non-soft	ovate
C.australis	4.12	5	rough	1.72	334.38	0.4	37.54	non-soft	ovate
Q. pubescens	3.38	6	rough	NR	260.21	20.42	35.26	non-soft	lobed
C. atlantica	2.94	0	smooth	NR	NR	NR	75.71	non-soft	acicular
C. bignonioides	1.95	65	rough/valley	1.17	168.28	6.59	0.64	soft	cordate
P. nigra	1.93	0	NR	NR	97.19	0.94	33.15	non-soft	rhomboid
R. pseudoacacia	1.80	97	peak/valley	1.42	50	4.51	26.04	soft	ovate
T. cordata	1.10	0	NR	NR	141	19.29	34.94	non-soft	cordate

Based on our results macro and microstructural characteristics are related with positive relation in PM adsorption, and the combination between two or more traits are responsible for the higher amount of PM. Combinations, rather than single traits may be critical in influencing PM deposition and accumulation (Leonard et al. 2016).

In the blue group – the best -, three of the four species have the same roughness type – rough – and a significant percentage of roughness on the leaf surface: 46%, 84%, 35% and 52%, small dimension of the grooves and medium/high stomatal density (Tab. 4).

The foliage of plants filtre numerous solid particles due to roughness and large contact area and thus can reduce the damaging effect of particulate pollution (Meusel et al. 1999). Morphological parameters like cuticular ornamentations, raised epidermal cell boundaries, stomatal ledges, trichomes and overall, the epicuticular and cuticular waxes may be responsible for roughness of leaf surface (Pal et al. 2002).

Although *A. saccharinum* presents a different type and 35% of roughness, the stomatal density is the highest and has the lowest grooves dimension among all species (Fig. 9a1) also contains 13% of trichomes density (Fig. 9a2). *P. tremula* did not present such a high leaf expansion, in contrast contains a high percentage of trichomes - 80% (Fig. 9b2). Trichomes on the leaf not only increase the surface area that can intercept PM but, may also make it harder for PM to dislodge when leaves are moving (Neinhuis, C; Barthlott 1998; Qiu et al. 2009; Prusty et al. 2005). Observation under SEM showed that the leaves with deep grooves, trichomes and wrinkled form of cuticle had strong dust-capturing; on the contrary, that had strumose projections on the leaf surface had poor dust-capturing (Wang et al. 2017). Leaf surfaces with grooves or trichomes have a higher capability of dust retention, while leaves with a smooth surface have a lower capacity for dust retention (Liu et al. 2012; Thakar & Mishra 2010). In addition three of these species have complex leaves (Tab. 4). Regarding *P. cesarifera* which has a low complexity about the leaf, it has the highest roughness area among all tree species (Fig. 9c1/9c2), so we can observe that one negative characteristic can be compensated with a positive one.

According to (Weerakkody et al. 2017) lobed shapes, trichomes and rough leaf surfaces may have a positive impact on PM capture and retention. These complex shape of the leaves may generate more turbulance in the boundary air layer (Weerakkody et al. 2018). Our positive factors for PM dust-capability are in agreement with other simular ones in the scientific literature.

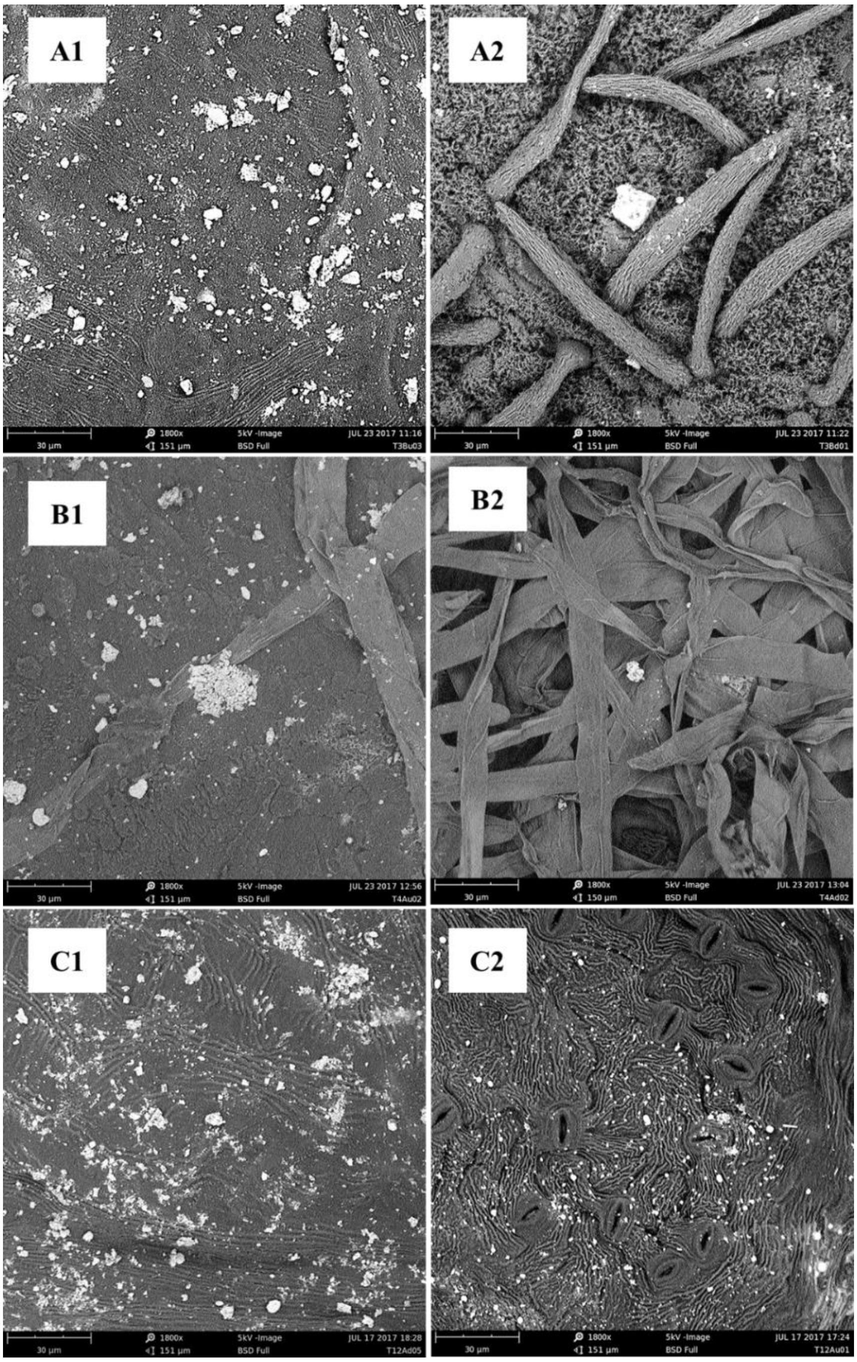

Figure 9: Adaxial (1) and abaxial (2) side of the best species and different positive factors for PM adhesion, *A. saccharinum* and low grooves dimension (A1) and trichomes (A2); *P. tremula* and some roughness area (B1) and a significant hairy density (B2); *P. cerasifera* contains a high roughness area in both side of the leaves (C1 and C2).

In the intermediate group – green - the species present a low or zero percentage of roughness, size of grooves above 1 µm, negative traits. On the other hand the species present a medium/high stomatal density, non-soft texture and intermediate complex leaf type, all positive factor. Again it is possible to observe that one micro-macrostructure compensates the other. As for example *M. grandiflora*, has a smooth surface (Fig. 10A1) – a negative factor -, on its abaxial side of the leaf and on the adaxial side 99% is cover by a net trichomes ((Fig. 10A2), a positive key factor. And the opposite also occurs, *C. australis* presents low roughness area and 0.4% hairy density (Fig.10B1) but a high stomatal density (Fig. 10B2). In addition to these traits, *M. grandiflora* and *C. atlantica* are evergreen species, and the other ten species are deciduous, this element should also be considered as a positive factor against deciduous species, because these species can adhere PM at all seasons of the year. Although *Q. pubescens* presents negative factors like low rugosity (Fig. 10C1), it has a medium density of trichomes and is the unique in this group that has a complex leaf (Fig. 10C2) that favors it for PM uptake.

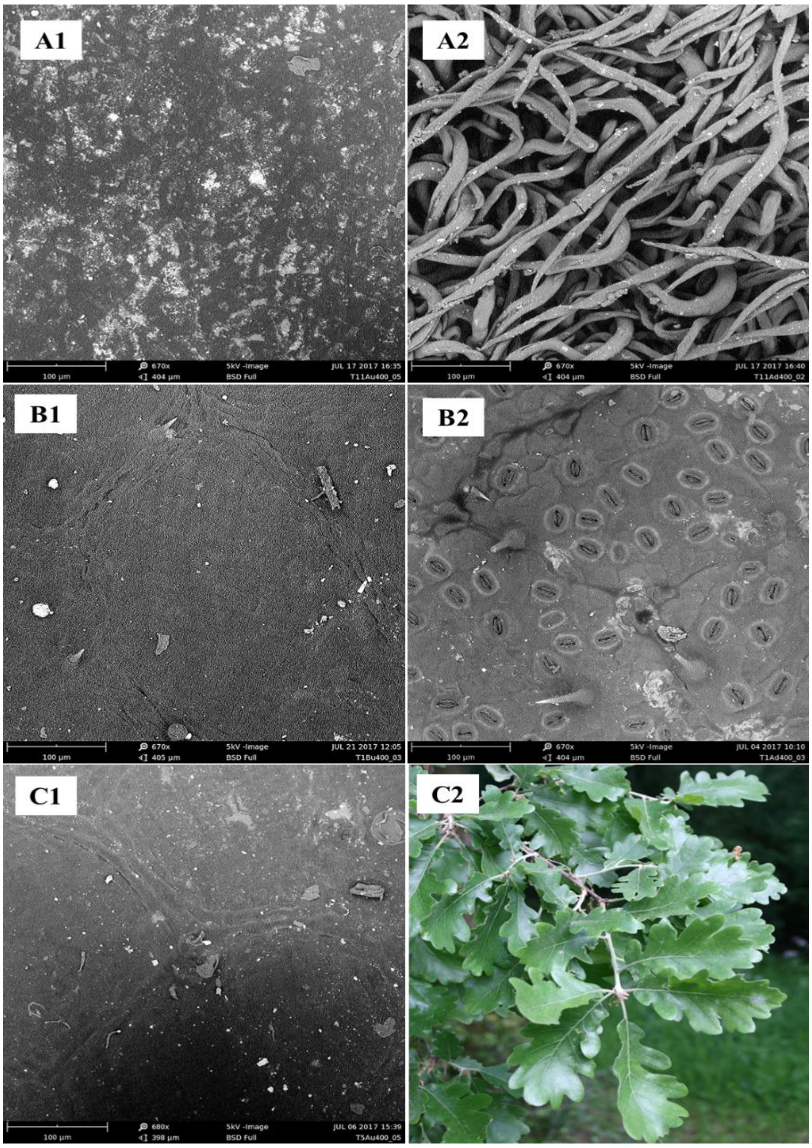

Figure 10: Different traits on the intermediate group, smooth surface (A1) and hairy density (A2) of *M.* grandiflora; low roughness (B1); and high stomatal density (B2) on *C. australis* and low rugosity and few trichomes (C1) and a complex leaf of *Q. pubescens* (C2).

The species in the worst group present some similarities *P.nigra* and *T. cordata* do not present any roughness on the surface of the leaf and therefore no grooves, also show low stomatal density and low percentage or no trichomas (Fig. 11a/b), all these elements are negative factor for PM adherence. And the other two species of this group – *C. bignonioides* and *R. pseudoacia* - despite having a high roughness in the leaf, both have soft texture leaves (Fig. 11c/d), a negative factor also found by other authors and already mentioned above.

Regarding the macrostructures, leaf texture - the non-soft texture of the leaves is one of the most essential characteristics in adhering PM in combination with some microstructural elements such as roughness and trichomes. For instance, when we compare one of the best species and one of the worst species is notable that this macrocharacteristics has positive key factor: *C. bignonioides* has soft surface and 65% of roughness on the other hand, one of the best species *P. acerifolia* has a non-soft texture and 46% of roughness.

Despite the high percentage of roughness, soft surface can be a disadvantage in wind conditions, where it moves the leaves, and the PM can be detached. According with (Prusty et al. 2005), air movement easily disturbs leaves with thin lamina and smooth surfaces. Consequently, such leaves can hold lesser amounts of dust while thick leaves with rough surfaces or hairs on the surface can hold relatively large amounts of dust and hence are better collectors of dust.

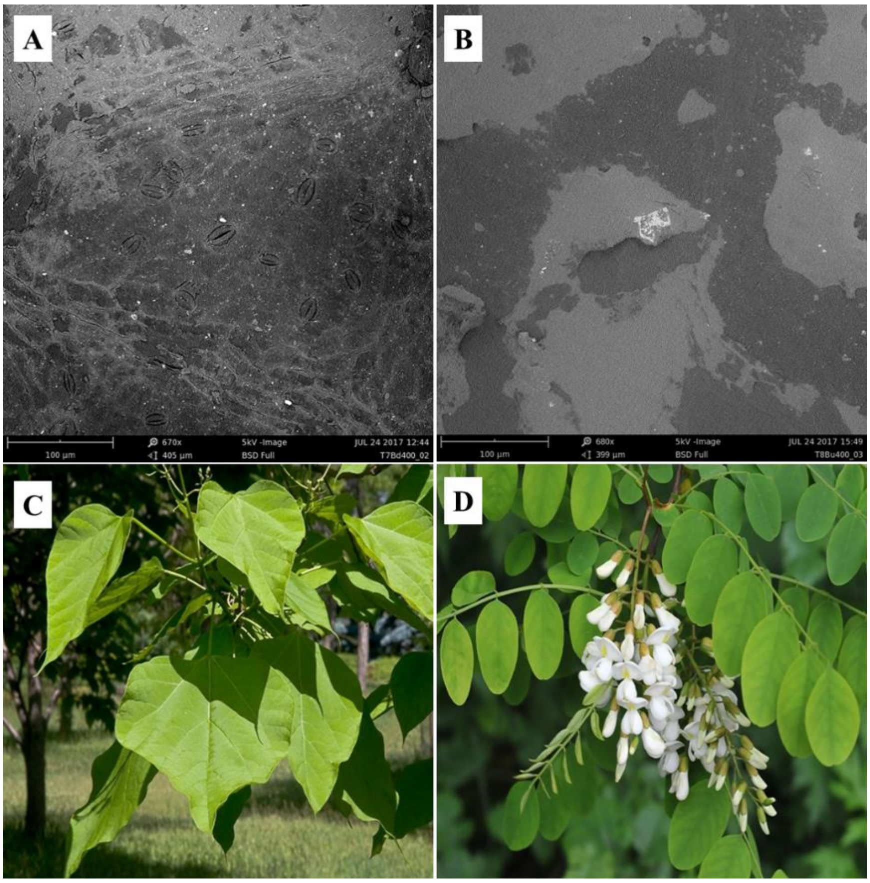

Figure 11: Some negative micro-macrostructure factors on the species of the worst group: low stomatal density on *P. nigra* (A); zero roughness area on *T. cordata*; soft texture on *C. bignonioides* (C) and *R. pseudoacacia* (D).

The three species in the worst positions - *T. cordata*, *R. pseudocacia* and *P. nigra* - have similar characteristics; the first two contain small white flowers with a strong odor that are potential for pollination and consequently the visitation of insects and *P. nigra* also has small flowers which can attract the insects by visual and olfactory cues. Also, the presence of honeydew on the leaves surface (Fig.12) of these three species was observed, in the case of the *T. cordata* (Fig. 12a_2), it is caused by the excretion of the aphid *Eucallypterus tiliae* (Fig. 12a_1) (Tourn et al. 1980) and on *P. nigra* (Fig. 12b_2) the aphid *Phloeomyzus passerinni* (Fig. 12b_1) is responsible for this aspect on the leaves surface (Allegro & Cagelli 1996). In the case of *R. pseudocacia* (Fig. 12c_2) the honeydew observed can be from the excretion of the insects that come to polinizate the flowers (Fig. 12c_1), the pollen of this species is one of the most presence in the romanian honey (Escuredo et al. 2014). In consideration of this honeydew probably the particles could not be noticed in the

SEM/EDX technique and that is a possible reason why they have lower amounts os PM, in addition the microstructures characteristics couldn`t be observed and measured.

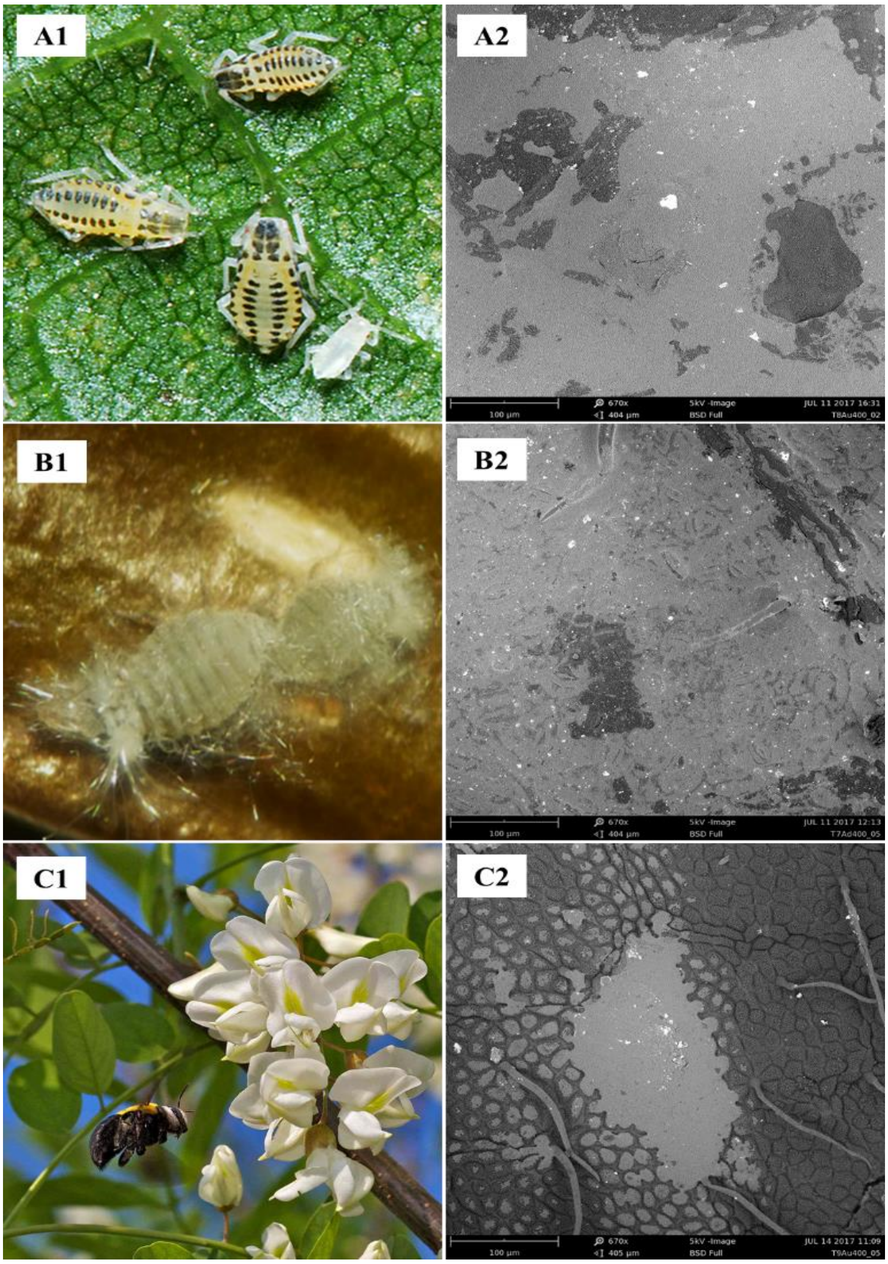

Figure 12: Honeydew aspect on the leaves surface and the respective causative agents. Aphid *Eucallypterus tiliae* (A1) and honeydew on *T. cordata* (A2); aphid *Phloeomyzus passerinni* (B1) and surface leaf of *P. nigra*; pollinization by bee (C1) on *R. pseudoacacia*.

Unfortunately the layer of honeydew in these species made it impossible to visualize some important microstructures that could contribute to the improvement of our PM retention index. For future research, we recommend collecting this material after a rainy day, as it can wash this negative aspect and then it will be possible to analyze these structures through SEM technique.

Chapter 4. CONCLUSIONS

It is notorious the that trees are capable of retaining urban pollution and therefore are important for improving the quality of life of the inhabitants. However, there is still a lack of knowledge in distinguishing which species are most likely to retain pollutants and which ones are lower. In this study we observed that the macro-microstructures can help to understand how the PM are adhered the leaves and thus we can elaborate practical tools to help the urban planners in choosing the suitable species to mitigate the problem with pollution in their cities.

Although we still need a multivariate statistical analysis to understand the synergia of how micro-macrostructures work together, how one trait can influence negatively or positively the PM uptake, through this study we managed to list several morphological structures that may be responsible for the retention of pollution.

We noticed that there is no single characteristic responsible for the higher adsorption of particles but rather the combination of more than two micro and macrostructural features. In the species with higher values of PM_{10} - *Platanus acerifolia, Prunus cerasifera*, *Accer saccharinum, Populus tremula*, we believe that some foliar morphological structures were positive factors for the retention of pollution such as: the presence of roughness and trichomes, low size of the grooves, high stomatal density, non-soft texture and foliar complexity. In contrast in the worst species - *Tilia cordata*, *Robinia pseudoacacia*, *Populus nigra* and *Catalpa bignonioides* - the following characteristics showed to being negative aspects: low roughness, high grooves size, low stomatal density, the absence of trichomes, soft texture and low leaf complexity.

Based on these positive and negative micro-macrostuctures, we performed the pollution retention index, a tool still preliminary, because we still need to better understand how each of these characteristics can influence the index. And in the next stage of this work will be made a multivariate analysis to understand the performance of these traits. In addition, we need to better understand how the honeydew influence on the total weight of PM observed in the species of this study. When we used the two techniques in this study - SEM/EDX and W/F, we observed that there are differences in the PM amount in distingue species in both techiques. Although the SEM/EDX technique is more accurate and presents more details about the PM through images, it has a disadvantage if the leaves contain honeydew. With this sticky substance is not possible to observe the particles, possibly because they are under that, and therefore we can have a lower weight of PM compared to the other technique. However, in the technique of W/F we still can not allege if the water is able to wash all the honeydew present in the leaves or if any remnants remain adhered to the particles. We are already analyzing the water used in this technique and future studies are necessary to analyze these filters through SEM images and check if there is honeydew on them, on this way we can clarify if it can influence the total weight of the PM.

Therefore, we still need studies to understand the behavior of honeydew and what would be the best strategy to measure the PM present in these kind of leaves. In addition, we still need to analyze in more depth how each foliar micro-macrostructure can influence the retention of pollution. Based on these results, we can build a more accurate retention index and check this index in practice in different species and see the feasibility of being a practical tool for urban planners in choosing which species are most feasible to mitigate the problem of urban pollution.

REFERENCES

Allegro, C. & Cagelli, L., 1996. Susceptibility of Populus nigra L. to the woolly poplar aphid (Phloeomyzus passerinii Sign.). *Forest Genetics*, 3(1), pp.23–26.

Brack, C.L., 2002. Pollution mitigation and carbon sequestration by an urban forest. *Environmental Pollution*, 116(SUPPL. 1).

Brook, R.D. et al., 2010. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the american heart association. *Circulation*, 121(21), pp.2331–2378.

Calfapietra, C. et al., 2013. Role of Biogenic Volatile Organic Compounds (BVOC) emitted by urban trees on ozone concentration in cities: A review. *Environmental Pollution*, 183(x), pp.71–80. Available at: http://dx.doi.org/10.1016/j.envpol.2013.03.012.

Chai YX, Zhu N, Han HJ., 2002. Dust removal effect of urban tree species in Harbin. *Chinese Journal Applied Ecology*, 13(9):1121–1126.

Chaturvedi, R.K. et al., 2013. Effect of dust load on the leaf attributes of the tree species growing along the roadside. *Environmental Monitoring and Assessment*, 185(1), pp.383–391.

Chen, L. et al., 2016. Experimental examination of effectiveness of vegetation as bio-filter of particulate matters in the urban environment. *Environmental Pollution*, 208, pp.198–208. Available at: http://dx.doi.org/10.1016/j.envpol.2015.09.006.

Chen, L. et al., 2017. Variation in Tree Species Ability to Capture and Retain Airborne Fine Particulate Matter (PM2.5). *Scientific Reports*, 7(1), pp.1–11.

Dominici, F. et al., 2006. Fine Particulate Air Pollution and Hospital Admission for Cardiovascular and Respiratory Diseases. *American Medical Association*, 295(10), pp.1127–1134.

Escuredo, O. et al., 2014. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry*, 149, pp.84–90. Available at: http://dx.doi.org/10.1016/j.foodchem.2013.10.097.

European Commission, 2015. Nature-Based Solutions | Environment – Research & Innovation [WWW Document], URL https://ec.europa.eu/research/environment/index.cfm?pg=nbs (Accessed 30.02.18).

European Environment Agency, 2015. *Air Quality in Europe*, Available at: papers2://publication/uuid/1D25F41B-C673-4FDA-AB71-CC5A2AD97FDD.

Fowler, D. et al., 1989. Deposition of atmospheric pollutants on forests. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 324(1223), pp.247–265. Available at:

http://rstb.royalsocietypublishing.org/content/324/1223/247.short.

Freer-Smith, P.H., Holloway, S. & Goodman, A., 1997. The uptake of particulates by an urban woodland: Site description and particulate composition. *Environmental Pollution*, 95(1), pp.27–35.

Gallagher, M.W. et al., 1997. Measurements of aerosol fluxes to speulderforest using a micrometeorological technique. *Atmospheric Environment*, 31(3), pp.359–373.

Hosker, R.P. & Lindberg, S.E., 1982. Review: Atmospheric deposition and plant assimilation of gases and particles. *Atmospheric Environment (1967)*, 16(5), pp.889–910.

Leonard, R.J., McArthur, C. & Hochuli, D.F., 2016. Particulate matter deposition on roadside plants and the importance of leaf trait combinations. *Urban Forestry and Urban Greening*, 20, pp.249–253. Available at: http://dx.doi.org/10.1016/j.ufug.2016.09.008.

Liu, J. et al., 2018. An investigation of the leaf retention capacity, efficiency and mechanism for atmospheric particulate matter of five greening tree species in Beijing, China. *Science of the Total Environment*, 616–617(March), pp.417–426. Available at: https://doi.org/10.1016/j.scitotenv.2017.10.314.

Liu, L., Guan, D. & Peart, M.R., 2012. The morphological structure of leaves and the dust-retaining capability of afforested plants in urban Guangzhou, South China. *Environmental Science and Pollution Research*, 19(8), pp.3440–3449.

Llausàs, A. & Roe, M., 2012. Green Infrastructure Planning: Cross-National Analysis between the North East of England (UK) and Catalonia (Spain). *European Planning Studies*, 20(4), pp.641–663.

Manes, F. et al., 2016. Regulating Ecosystem Services of forests in ten Italian Metropolitan Cities: Air quality improvement by PM10and O3removal. *Ecological Indicators*, 67, pp.425–440. Available at: http://dx.doi.org/10.1016/j.ecolind.2016.03.009.

McDonald, A.G. et al., 2007. Quantifying the effect of urban tree planting on concentrations and depositions of PM10in two UK conurbations. *Atmospheric Environment*, 41(38), pp.8455–8467.

Mcpherson, E.G., Nowak, D.J. & Rowntree, R. a, 1994. Chicago's Urban Forest Ecosystem: Results of the Chicago Urban Forest Climate Project. *Urban Ecosystems*, (April), p.201.

Merbitz, H., Fritz, S. & Schneider, C., 2012. Mobile measurements and regression modeling of the spatial particulate matter variability in an urban area. *Science of the Total Environment*, 438, pp.389–403. Available at: http://dx.doi.org/10.1016/j.scitotenv.2012.08.049.

Meusel, I. et al., 1999. Ultrastructure, chemical composition, and recrystallization of epicuticular waxes: transversely ridged rodlets. *Canadian Journal of Botany*, 77(5), pp.706–720.

Mo, L. et al., 2015. Assessing the Capacity of Plant Species to Accumulate Particulate Matter in Beijing , China. , (x), pp.1–18.

Morani, A. et al., 2011. How to select the best tree planting locations to enhance air pollution removal in the MillionTreesNYC initiative. *Environmental Pollution*, 159(5), pp.1040–1047. Available at: http://dx.doi.org/10.1016/j.envpol.2010.11.022.

Neinhuis, C; Barthlott, W., 1998. Seasonal changes of leaf surface contamination in beech , oak , and ginkgo in relation to leaf micromorphology and wvettabiity. *New Phytologist*, 138(1), pp.91–98.

Nowak, D.J. et al., 2013. Modeled PM2.5 removal by trees in ten U.S. cities and associated health effects. *Environmental Pollution*, 178, pp.395–402. Available at: http://dx.doi.org/10.1016/j.envpol.2013.03.050.

Nowak, D.J., Crane, D.E. & Stevens, J.C., 2006. Air pollution removal by urban trees and shrubs in the United States. *Urban Forestry and Urban Greening*, 4(3–4), pp.115–123.

Ottelé, M., van Bohemen, H.D. & Fraaij, A.L.A., 2010. Quantifying the deposition of particulate matter on climber vegetation on living walls. *Ecological Engineering*, 36(2), pp.154–162.

Pal, A. et al., 2002. Do leaf surface characters play a role in plant resistance to Auto-exhaust pollution? *Flora*, 197(1), pp.47–55.

Pirastu, R. et al., 2011. *SENTIERI - Studio epidemiologico nazionale dei territori e degli insediamenti esposti a rischio da inquinamento : RISULTATI SENTIERI Project - Mortality study of residents in Italian polluted sites : RESULTS*,

Pope III, C.A. et al., 2002. Lung Cancer, Cardiopulmonary Mortality, and Long-term Exposure to Fine Particulate Air Pollution. *The Journal of the American Medical*, 287(1), pp.132–141.

Popek, R. et al., 2013. Particulate Matter on Foliage of 13 Woody Species: Deposition on Surfaces and Phytostabilisation in Waxes - a 3-Year Study. *International Journal of Phytoremediation*, 15(3), pp.245–256.

Powe, N.A. & Willis, K.G., 2004. Mortality and morbidity benefits of air pollution (SO2and PM10) absorption attributable to woodland in Britain. *Journal of Environmental Management*, 70(2), pp.119–128.

Prusty, B.A.K., Mishra, P.C. & Azeez, P.A., 2005. Dust accumulation and leaf pigment content in vegetation near the national highway at Sambalpur, Orissa, India. *Ecotoxicology and Environmental Safety*, 60(2), pp.228–235.

Qiu, Y. et al., 2009. Capture of heavy metals and sulfur by foliar dust in urban Huizhou, Guangdong Province, China. *Chemosphere*, 75(4), pp.447–452. Available at:

http://dx.doi.org/10.1016/j.chemosphere.2008.12.061.

Rai, A. et al., 2010. Leaf surface structure alterations due to particulate pollution in some common plants. *Environmentalist*, 30(1), pp.18–23.

Rai, P.K., 2016. Impacts of particulate matter pollution on plants: Implications for environmental biomonitoring. *Ecotoxicology and Environmental Safety*, 129, pp.120–136. Available at: http://dx.doi.org/10.1016/j.ecoenv.2016.03.012.

Samal, A.C. & Santra, S.C., 2002. Air quality of Kalyani township (Nadia, West Bengal) and its impact on surrounding Vegetation. *Indian Journal of Environmental Health*, 44(1), pp.71–76.

Sgrigna, G. et al., 2016. Characterization of leaf-level particulate matter for an industrial city using electron microscopy and X-ray microanalysis. *Science of the Total Environment*, 548–549, pp.91–99. Available at: http://dx.doi.org/10.1016/j.scitotenv.2016.01.057.

Sgrigna, G. et al., 2015. Particulate Matter deposition on Quercus ilex leaves in an industrial city of central Italy. *Environmental Pollution*, 197, pp.187–194. Available at: http://dx.doi.org/10.1016/j.envpol.2014.11.030.

Sternberg, T. et al., 2010. Dust particulate absorption by ivy (Hedera helix L) on historic walls in urban environments. *Science of the Total Environment*, 409(1), pp.162–168. Available at: http://dx.doi.org/10.1016/j.scitotenv.2010.09.022.

Thakar, B.K. & Mishra, P.C., 2010. Dust collection potential and air pollution tolerance index of tree vegetation around vedanta aluminium limited, jharsuguda. *The Bioscan*, 3, pp.603–612.

Tourn, M.L. et al., 1980. Quantitative Analysis of Carbohydrates and Organic Acids in Honeydew, Honey and Royal Jelly by Enzymic Methods. *Journal of Apicultural Research*, 19(2), pp.144–146.

Wang, S.L., Zhang, Y.H., Zhong, L.J., Li, J.L., Yu, Q., 2005. Interaction of urban air pollution among cities in Zhujiang Delta. China Environ. Sci. 25, 133–137.

Wang, L. et al., 2015. Accumulation of particles on the surface of leaves during leaf expansion. *Science of the Total Environment*, 532, pp.420–434. Available at: http://dx.doi.org/10.1016/j.scitotenv.2015.06.014.

Wang, R.Y. et al., 2017. Research on the Dust-Capturing Capacities of Environmental Friendly Materials Based on the Typical Garden Trees and the Morphological Structure of Leaves in Suzhou. *Key Engineering Materials*, 737, pp.412–416. Available at: http://www.scientific.net/KEM.737.412.

Weerakkody, U. et al., 2018. Evaluating the impact of individual leaf traits on atmospheric particulate matter accumulation using natural and synthetic leaves. *Urban Forestry and*

Urban Greening, 30(September 2017), pp.98–107. Available at: https://doi.org/10.1016/j.ufug.2018.01.001.

Weerakkody, U. et al., 2017. Particulate matter pollution capture by leaves of seventeen living wall species with special reference to rail-traffic at a metropolitan station. *Urban Forestry and Urban Greening*, 27(July), pp.173–186. Available at: http://dx.doi.org/10.1016/j.ufug.2017.07.005.

WHO, W.H.O.-, 2005. *Air quality guidelines. Global update 2005. Particulate matter, ozone, nitrogen dioxide and sulfur dioxide*, Available at: http://www.euro.who.int/en/health-topics/environment-and-health/air-quality/publications/pre2009/air-quality-guidelines.-global-update-2005.-particulate-matter,-ozone,-nitrogen-dioxide-and-sulfur-dioxide.

WHO, W.H.O.-, Henschel, S. & Chan, G., 2013. Health risks of air pollution in Europe – HRAPIE project New emerging risks to health from air pollution – results from the survey of experts. *World Heath Organization (WHO)*, p.65. Available at: http://www.euro.who.int/__data/assets/pdf_file/0017/234026/e96933.pdf?ua=1.

Yin, S. et al., 2011. Quantifying air pollution attenuation within urban parks: An experimental approach in Shanghai, China. *Environmental Pollution*, 159(8–9), pp.2155–2163. Available at: http://dx.doi.org/10.1016/j.envpol.2011.03.009.