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The use of lichen biomonitoring techniques for Environmental
Justice assessment and the risk perception assessment in a typical
European city (Milan, Italy)

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Tutor: Prof. Stefano Loppi

Co-tutor: Prof. Paolo Giordani

Co-tutor: Dr. Andrea Vannini

Coordinator: Prof. Massimo Valoti

Candidate: Tania Contardo

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To my family and friends.

Abstract

Aim of this thesis is to investigate the application of lichen biomonitoring techniques in the study of environmental justice and risk perception of air pollution at urban scale. Lichen biomonitoring can, in fact, fill the void that several authors claim when talking about environmental justice assessment, i.e., the lack of high spatial resolution data of air quality, that is crucial when the goal is to match the socio-economic status with air quality. The area selected for the study is Milan municipality (N Italy) as an example of a typical European big city. In this area, a lichen biomonitoring survey was firstly carried out through transplants of lichen bags of the species *Evernia prunastri*, previously collected in a pristine area (Siena, C Italy). The exposure lasted three months during the winter period. To assess the biological effects of air pollution, analysis on the physiological status of the samples were conducted, in particular the photosynthetic efficiency of the photobiont, and damage to cell membranes and the antiradical activity of the mycobiont were measured. The bioaccumulation of trace elements Al, As, Cd, Cr, Cu, Fe, Sb, Pb and Zn was quantified and an overall contamination index (CI) was elaborated on the basis of the bioaccumulated elements. The use of a geographic information system support (QGIS 3.8) allowed to visualize the distribution of the contamination over the area, elaborated through an IDW interpolation algorithm. A deeper insight on the contamination of the area was obtained through the analysis of source apportionment, i.e., the research of the main emission sources. A theoretical approach was followed through the statistical method of Positive Matrix Factorization (PMF). Through this method, the overall contamination was de-structured in “factors” that, based on trace elements correlation, provided different “contamination profiles”, ascribable to different emission sources. The spatial distribution of the profiles, combined with their elemental composition, suggested both the type of emission sources and their magnitude in the study area. An empirical approach for source apportionment was also followed: the grain size, shape and magnetic behaviour of bioaccumulated particles was studied and compared with those of known emission sources.

The results of the biomonitoring survey showed a general stress on the lichen physiology due to air pollution, but not so severe to compromise the general vitality of samples. Bioaccumulated elements were Cr, Cu, Fe, Sb and Pb and the Contamination Index showed generally high values over the whole area, even if central areas of the city were more polluted. Source apportionment analysis highlighted three main pollution profiles, ascribable to the industrial activity, brake abrasion from railways and cars, and soil resuspension. The

magnetic behaviour of particles was in line with brake abrasion of vehicles, suggesting the vehicular traffic the most responsible of trace element contamination in Milan municipality.

For the assessment of environmental justice, the air contamination was matched with the socio-economic characteristics of the citizens. An index of socio-economic deprivation (SDI) was calculated on the basis of the socio economic information provided by the national statistics institute (ISTAT), at the smallest spatial unit available, the census unit. Results showed that peripheral areas experience less air pollution but more socio-economic deprivation, however, the census units closer to main emission sources were generally more deprived than average.

The last part of the study was dedicated to the evaluation of the risk perception of the citizens about air pollution. A questionnaire was elaborated and administrated randomly in the city of Milan to ca. 300 respondents. Main results showed a generally high attention for the air pollution issue in the city, as well as a high risk perception, regardless of gender, age or education level of the participants.

Aim and research questions

Aim of this work is to investigate the suitability of lichen biomonitoring techniques in environmental justice assessment and risk perception of air pollution. This work follows a progressive pathway that from the biological evaluation of air pollution ends with the “human” evaluation of air pollution, including economic sphere and urban dynamics.

The research steps that defined the workflow are:

- Evaluate air pollution in the city of Milan
- Disentangle the main emission sources to define peculiar patterns and characteristics of the area
- Match air pollution data with the socio-economic status of the citizens
- Evaluate how and if the air pollution and the associated risk is perceived by citizens

Structure of the thesis

Given the multidisciplinary nature of this work, a brief introduction is reported for each of the main themes: air pollution, lichen biomonitoring, environmental justice and risk perception. Then, the study area will be shortly introduced.

The work is structured in 2 sections.

The first one is dedicated to the lichen biomonitoring survey. In a first chapter will be presented the study of the biological effect of air pollution on lichen samples, while in the second chapter will be treated the bioaccumulation of trace elements, the elaboration of an overall index of contamination and the study of source apportionment.

The second section is dedicated to the interaction of air pollution with the human spheres. In the first chapter, the assessment of environmental justice will be presented, while the second chapter will show the results of the air pollution perception study.

Final considerations of the work strengths and limitations will conclude the work.

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Preface

1. Air pollution

Air pollution is defined by WHO (2016) as “the contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere.” Both natural and anthropogenic sources contribute to the release of these substances: natural sources include marine spray, volcanic activity, windblown dust as well as pollen and spores from plants, while anthropogenic sources include agriculture, which emits fertilizers and pesticides, combustion processes as vehicles and energy production and waste management, among others (Schroeder et al., 1987). The released substances can be classified for chemical nature in organic molecules, as Black Carbon (BC) and Polycyclic Aromatic Hydrocarbons (PAH), and inorganic molecules as toxic trace elements (TE) and gas as NO_x and SO_x . Pollutants can be also classified on the basis of the physical nature, for which gaseous pollutants are separated from the collection of the liquid and solid particles that remain suspended in the atmosphere, conventionally called “Particulate Matter” (PM). Primary contaminants are those emitted directly from the sources as NO and SO_x , while the secondary contaminants are produced later in the atmosphere for reactions of primary contaminants as the case of O_3 and acids as H_2SO_4 and HNO_3 .

Air pollution has a great impact on ecosystems, human health but also human environments since it can affect materials and manufactures . Especially in the past decades, the massive release of SO_x and NO_x from energy production plants and vehicle engines lead to the formation of acids as H_2SO_4 and HNO_3 , that lowered the pH of wet depositions creating the phenomenon of “acid rains”, which were responsible of many damages to aquatic and terrestrial ecosystems. A significative event occurred in '50 in UK when a particularly persistent fog entrapped the SO_2 released from the house heating systems in the city of London for many days, causing several deaths among population who inhaled the toxic gas at harmful level (Fenger, 2009). During the XXI sec. many episodes shaken the public and politic opinion, as the Seveso case in 1976, in which a great amount of dioxin and dioxin-like molecules were released in the North of Italy causing long term health injuries to people, or the Chernobyl explosion in 1986. The prolonged exposure to air pollution is well known to be related to the occurrence of many health injuries that vary from asthma, cardiovascular diseases, strokes and lung cancer and premature deaths (Brunekreef and Holgate, 2002). The World Health Organization (WHO) indicates that 91% of world population lives in air

quality conditions below the minimum standard for human health, and every year about 7 million people die for causes related to air pollution exposure, both outdoor and indoor environments (WHO, 2016) and only in Europe every year more than 350 000 people die prematurely for causes linked to bad air quality. Dramatic air pollution episodes of the last century led most countries worldwide to fight emissions and ameliorate air quality. In Europe, air quality is regulated by the European directive 2008/50/EC in which it is stated:

“In order to protect human health and the environment as a whole, it is particularly important to combat emissions of pollutants at source and to identify and implement the most effective emission reduction measures at local, national and Community level. Therefore, emissions of harmful air pollutants should be avoided, prevented or reduced and appropriate objectives set for ambient air quality taking into account relevant World Health Organization standards, guidelines and programs”

While emissions of some contaminants as SO₂ are significantly decreased worldwide from the '70s, a great concern remains especially for the so called Particulate Matter (PM).

PM is the mixture of solid and liquid droplets that remain suspended in the atmosphere for hours and even weeks or months and it has a particular relevance in epidemiological studies (Kim et al., 2015). Despite the heterogeneous composition, the main components are generally sulfates, nitrates, ammonia, sodium chloride, black carbon, mineral dust and water, but the proportion may change depending on the area and emission sources (WHO, 2016).

Conventionally, PM is classified by “aerodynamic equivalent diameter” (AED) and particles having similar diameter tend to have the same settling velocity. The coarser fraction is PM₁₀, with aerodynamic diameter lower than 10 µm and constitutes the majority of PM mass (Anderson et al., 2012). These particles can be easily inhaled by normal breathing but are generally filtered out by nose and the upper airways. The finest fractions, PM_{2.5} and PM₁, having diameter less than 2.5 and 1 µm respectively, reach the lower respiratory system where, due to the small size, can also pass the vessel barriers and enter the blood flow. These are the most relevant fractions for human health (Brown et al., 2013).

PM is responsible of a wide range of injuries. Authors report the association between exposure to PM and lung cancer, brain development impairments (Costa et al., 2019) but also depression (Signoretta et al., 2019) and cognitive disorders in children (Annarapu and Kathi, 2016), among others. The spectrum of adverse effects is widening as the research progresses, and it is becoming more and more clear that not only life quality of the single

person, but also the societal development can be compromised by high levels of air pollution. It is, thus, crucial to monitor and abate the presence of PM both in urban and rural areas.

The main contributor to PM is vehicular traffic, in particular the brake and tires wearing are gaining a dominant role (De Kok et al., 2006, Thorpe and Harrison, 2008), followed by other activities as industry and construction activities, for example.

1.1. Trace elements

Trace elements (TE) are minerals present in living tissue in small amounts, fundamental for the correct functioning of metabolism, immune system and physical development. Their deficiency is associated to a bad development, anaemia, mental delay and metabolism disfunction, while their excess can be related to organ damages and carcinogenicity (Valko et al., 2005).

Essential elements are introduced into the organism mainly via diet and respiration, however the growing contamination of these media leads to the uptake also of non-essential and toxic elements too (Samara and Voutsas, 2005). In atmosphere, TE are associated to all aerodynamic sizes of PM, being thus respirable and reaching the lower respiratory system. Ostro et al. (2007) showed that during winter, the number of TE associated with PM_{2.5} rises with excess of all-cause mortality, suggesting the high epidemiological relevance of these substances.

Trace elements derive by natural sources as soil-resuspension and anthropogenic sources, among which the main one is vehicular traffic. Both exhaust and non-exhaust emissions contribute to TE, however, after the abatement measures addressed worldwide, the exhaust source is decreasing in proportion respect to non-exhaust ones. Elements such as Sb, Cu, Ba and others are common components of brake and tyres, whose abrasion releases the elements in the atmosphere (Thorpe and Harrison, 2008).

2. Lichens

Lichens are the product of a symbiosis between an autotrophic partner (a green alga, blue-green algae or a cyanobacterium) called “photobiont” and an heterotrophic partner (a fungus, mainly *ascomycetes*) called “mycobiont”. Recent studies report the presence of a third partner, a basidiomycete yeast in the cortex of most macro-lichens (Spribille et al., 2016).

The nature of symbiosis is still debated, but some authors sustain it is a form of controlled parasitism by the fungus, that obtains the main benefits, while the photobiont grows more slowly than when free-living (Ahmadijian, 1993).

Lichens are not internally organized in tissues and organs and lack structures as roots, waxy cuticle and stomata. The gas exchange occurs through the whole surface of the thallus as well as the absorption of nutrients, which derives from dry and wet atmospheric depositions (Bargagli, 1998).

Exclusive of lichen organisms is the synthesis of a wide range of secondary metabolites called “lichen substances”, that are involved in many protective functions as antioxidant activity, but also work as anti-microbial and anti-herbivores (Lawrey, 1986; Basile et al., 2015).

These characteristics allow lichens to be ubiquitous in natural environments, covering approximately 8% of Earth’s land surface (Nash, 2008), but also human manufactures are abundantly colonised, sometimes inducing important damages to cultural stone heritage (Seaward, 1988).

Conventionally, lichens are subdivided according to the growth form and the type of colonized substratum (Nash, 2008). The main categories of the growth forms are *crustose*, *foliose* and *fruticose* (see Fig. 1). Crustose lichens are strongly interconnected with their substratum, with the hyphae that penetrate deeply inside. Sometimes only the reproductive structures are visible outside. The foliose species growth in dorsi-ventral plan in rounded forms and are characterized by having marginal lobes. Their connection to the substratum is weaker than the crustose ones, and they are more easily removable.

The fruticose growth form is characterized by having a tri-dimensional growth, thus, these species can hang from tree branches or raise from their substratum.



Figure 1: Different growth forms: **a)** crustose lichens, **b)** foliose and **c)** fruticose

Even though lichens can colonise quite every surface (see Fig. 2), they are traditionally classified in terricolous, that grow on soil, epilithic, that grow on rocks and epiphytic, that grow on tree bark. However, many sub-categories are present due to the lichen adaptivity to each surface.

The use of lichens find several applications in different fields: their ecology is used for ecosystem management (Bianchi et al., 2020), lichen substances are studied in pharmaceutical, medical and cosmesis fields (Müller, 2001; Cocchietto et al., 2002), while their use as biomonitors of air quality is discussed in the next paragraph.



Figure 2: Lichens colonising human manufactures

3. Biomonitoring through lichens

3.1 Biomonitoring

The European Standard Guidelines define the biomonitoring as “the use of biological systems (organisms and organism communities) to monitor environmental change over space and/or time”.

The biomonitoring process is carried through the use of bio-indicator organisms and bio-accumulator organisms, defined as follow:

A bio-indicator is an organism or a part of it or an organism community (biocoenosis) which documents environmental impacts.

A bio-accumulator is an organism which can indicate environmental conditions and their modifications by accumulating substances present in the environment (air, water or soil) at the surface and/or internally. (EN 16413:2014)

The biological effects of environmental pollution can be thus evaluated by the use of selected organisms.

The main characteristics a suitable biomonitor should have are:

- The sensitiveness to the contaminant or the capability to accumulate it
- Long life cycle
- Low mobility

- Active metabolism during all the year
- Low genetic variation in population
- Ubiquitous
- Not endangered species
- Reasonable size for laboratory analysis

3.2 *Lichens as biomonitors*

Lichens meet all the characteristics to be suitable biomonitors and can be used as bio-indicators as well as bio-accumulators species (Bargagli, 1988).

The sensitiveness of lichen to air pollution is documented from the XIX century when Nylander (1866) observed the disappearance of some lichen species where air pollution was more intense. The tolerance to air pollution is specie-specific and while some species find in disturbed and eutrophicated environments an ideal habitat, others undergo to a series of physiological stresses that may turn into visible damages as necrosis, bleaching and even death (Häffner et al., 2001; Munzi et al., 2013). The less tolerant species, thus, provide with their physiological status an early warning signal of air quality changes. By measuring selected vital parameters, in fact, it is possible to deduce the intensity of environmental stressors and pollutants, as shown in many studies (Paoli et al., 2011; Sujetovienè and Galynité, 2016).

On the other side, the progressive disappearance of the less tolerant species turns into a mutate lichen community assemblage, which becomes a diagnostic tool of changed ecological conditions. The biodiversity of epiphytic lichens has been widely used to assess air quality (Giordani et al., 2002; Loppi et al., 2002).

Lichens can be also used as bioaccumulators, since they accumulate trace elements far above their nutritional requirements, and proportional to bulk deposition (Bačkor and Loppi, 2009, Loppi and Paoli, 2015). Lichens accumulate trace elements through 3 pathways: particulate trapping, for which elements are entrapped within intercellular spaces; the extracellular ion-exchange process, for which metals are bound to ionic exchange sites, mostly on the cell walls (Puckett et al., 1973) and intracellular accumulation (Vannini et al., 2017).

The use of lichens as biomonitors of air quality finds application in a wide range of studies as the impact assessment of specific sources, for example landfills (Paoli et al., 2012), municipal waste incinerators (Loppi et al., 2000), geothermal plants (Loppi et al., 2006), but

also indoor air quality (Paoli et al., 2019a and b), epidemiological studies (Cislaghi and Nimis, 1997), and environmental forensics (Loppi, 2019).

4. Environmental Justice

The US Environmental Protection Agency (USEPA) defines Environmental Justice as:

“...the fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income, with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies. EPA has this goal for all communities and persons across this Nation. It will be achieved when everyone enjoys the same degree of protection from environmental and health hazards and equal access to the decision-making process to have a healthy environment in which to live, learn, and work”

This commitment taken from the US government in 1994 with the Executive order 12898, responded to the pressure of the new-born movement for environmental justice, led by citizens, mostly black, that claimed the disproportionate burden of environmental hazards in their territories. The environmental justice activism was already present for more than a century (Taylor, 2000), however in 1978 the strong protest that took place in Warren County (North Carolina) elicited the beginning of a global movement. People of Warren County (the place with the highest rate of Afro-American inhabitants of the State) struggled against the creation of a toxic waste landfill in their territory, and for the first time they put on the map the great disproportion of toxic plants and environmental hazards present in “white” and “black” territories in all the USA states (Brulle and Pellow, 2006).

A few years later, also Europe declared with the Aarhus Convention (UNECE, 25th June 1998) the primary importance of environmental justice.

Currently, environmental justice is a discipline studied from several viewpoints, such as sociology, psychology, environmental sciences, but also economy and urban studies, among others. The intrinsic multidisciplinary nature of the matter, requires an interaction between fields with the aim to assess which are the driving forces behind environmental inequalities between population groups.

What is clear is that environmental justice is differently declined in each country, and different parameters to assess the inequalities have to be considered. In Europe, Laurent (2011) highlighted that more disadvantaged groups are more likely to experience a worse air

quality, while in USA the presence of industrial plants is related to ethnic composition of a territory. Scaling the level, other parameters emerge as important: environmental disparities appear to be related to gender and households composition in Italian provinces (Germani et al., 2014), while house market dynamics are relevant in environmental justice assessment at urban level (Banzhaf and Walsh, 2006). Given the youth of this discipline, a methodological consensus is not achieved yet, also because of the necessary interaction between disciplines that often is not the rule.

5. Perception of air pollution and related risk

The final part of this thesis investigates the perception of air pollution and its risk for humans and environmental health reported by Milan citizens. The risk perception is defined as involving

“people’s beliefs, attitude, judgements, and feelings as well as the wider cultural and social dispositions they adopt towards hazards and their benefits” (Pidgeon et al., 1992)

and it is crucial in the definition of pro-environmental behaviours, that are fundamental for a sustainable development (Bickerstaff and Walker, 2001). If in one hand air pollution has a significant negative impact on the life satisfaction (Ferreira et al., 2013), it is the *perceived* air pollution that drives to annoyance, health symptoms and habits change in population, even when the actual contamination is considered by experts as not hazardous (Cleason et al., 2013). Studying and closing the gap between laypeople perception and experts opinion is, thus, of fundamental importance in addressing politics and environmental protection. Factors that shape the perception can be cultural, demographic, “personal” as marital status and age but also socio economic status has a primary relevance (Milfont and Schultz, 2016; Milfont et al., 2010). It is no coincidence, in fact, that risk perception is often associated with environmental justice assessment. Some authors showed that economically disadvantaged groups can report a lower satisfaction of their neighbourhood even though was not objectively different from the wealthier one (Mouratidis, 2020). On the other side, for the phenomenon called “the halo effect” people tend to define their own neighbourhood less affected by air pollution than others in the same area. The discrepancy may derive from information media and personal culture or concern (Bickerstaff and Walker, 2001). This is in line with the discrepancy between the air pollution perception and the risk perception of air pollution. In many studies it is reported, in fact, that many of those that perceived air

pollution not always declared worries about it, neither were conscious about a potential health risks. The lack of information about the risk can lead to hazardous behaviours and reduce dramatically the life quality of the community.

6. State of the art

Lichen biomonitoring techniques are worldwide applied in the science of air pollution effects on biota (see the review of Conti and Cecchetti, 2001). First observation of the lichen sensitivity to air pollution came from 1866 by the studies of W. Nylander, a botanist who reported that near busy roads the lichen flora tends to change, favouring more tolerant species (see Nylander, 1866). Since that moment forward, lichens have been used both for their sensitivity to gaseous pollutants (Hawksworth and Rose, 1979; Loppi and Nascimbene 2010) but also for their capacity to bioaccumulate trace elements far above their nutritional requirements (Bačkor and Loppi, 2009). The versatility of this approach allowed its application in a great number of situations, proving to be effective in the monitoring of urban, industrial, rural areas as well as indoor environments. Of particular interest is, however, the trend that is developing in the last decades, by which the lichen biomonitoring is conjugated to other strictly-anthropic sciences: Cislighi and Nimis (1997) compared the map of lichen biodiversity with the map of lung cancer incidence in Veneto Region (N Italy), showing the quasi-perfect overlapping of the two maps and, thus, proving the reliability of lichen biomonitoring for epidemiological studies. A contribution in the environmental forensics discipline is provided by Loppi (2019) who proposed a new method for the lichen biomonitoring data interpretation in order to reduce uncertainty linked to lichen measure but keeping the biological information. In the last years, the interest about Environmental Justice discipline pushed many authors to relate biological data to socio-economic dynamics, in particular the work of Schell et al., (2020) appeared in Science, described how the systemic urban racism implies consequences at ecological level in urban areas. The application of lichen biomonitoring techniques in Environmental Justice discipline is moving its first steps, dating the first publications in 2016 by Ocelli and colleagues.

In that study, the researchers evaluated the content of selected trace element in native samples of *Xanthoria parietina* collected in 60 sampling sites in the Dunkerque agglomeration (N France), in order to draw a map of the biological effects of the trace element contamination. The detailed map resulted was then used as the basis of the socio-economic analysis. A “Localised Disadvantage Index” (LDI) was elaborated on the basis of census data, involving items as “average school degree” and “GDP per capita” for example,

and it was calculated for each census area (102) of the territory in exam. Overlapping the spatial distribution of trace element impregnation in lichens and the distribution of the disadvantaged index, it resulted that the more disadvantaged neighbourhoods experienced a worse air quality situation. That study not only remarked the versatility of the lichen biomonitoring approach, but also proved that the biological data obtained by lichens can have further implications in the study of the human environment. In facts, some years later, Lanier et al. (2019) improved that approach by incorporating the biological data of lichen biomonitoring and the trace element content in poplar leaves in a more complex system. The researchers elaborated a three-dimensional matrix called “vulnerability matrix” by combining the biological data with the socio-economic deprivation index and a third index describing the rate of the most susceptible population in a given area, namely the rate of old (>60 years old) and young (<15 years old) people. The matrix aims to highlight the most vulnerable areas in the whole territory and, thus, it provides a strategical point of view of the area for management purposes.

At the best of my knowledge, the last work published on the use of lichen biomonitoring for Environmental Justice purposes is of Contardo et al. (2018). The researchers evaluated the bioaccumulation of trace elements in transplanted samples of the lichen *Evernia prunastri* in an area interested by a municipal solid waste incinerator (MSWI). In this case, the aim was to find out if the plant releases could harm the biota and if there was correlation between the plant position, the air pollution pattern and the socio economic deprivation in the area. Also in this case, the more disadvantaged groups experienced a worse air quality, even though the main contamination was not due to the MSWI.

7. Study area: Milan

Milan municipality was selected for this study as an example of a typical European big city, that went through important urban and social changes during the past century.

Milan is located in N Italy, Lombardy Region, in the geographic area of the Po Plain. This geographic area is characterized by a humid-temperate climate with hot summers and cold winters, persistent fog, air masses stagnation, and frequent thermal inversions. The whole area occupies most of N Italy, with a surface of 47 820 km² and it is the most industrialized and densely populated area of Italy, hosting about 20 million people (ca. 1/3 of the Italian population). The high density of industries, residential buildings, and human

infrastructures as well as agricultural activity, combined with the adverse climate factors, makes this area among the most polluted by PM_{2.5} in Europe (EEA, 2020; Fig.3).

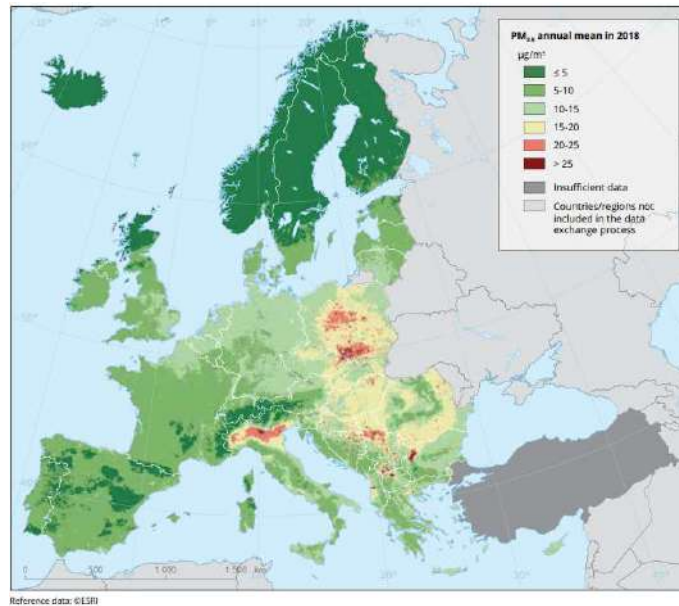


Figure 3: PM_{2.5} concentration in Europe. EEA 2020 report

Milan is among the most important Italian cities (Fig.4) due to its centrality in the Italian economy and its important industrial past. The municipality hosts about 1.4 million people in 181 km² of surface area, with a density of 7 699,21 inhabitants/km² that makes Milan among the most crowded city in Italy.

From the last decades of the 19th century, Milan has shown a particular propensity for industrial activities, primarily the export of silk, that characterized those years. During the 20th century, important mechanical industries (Pirelli, Breda, Marelli, Alfa Romeo) found in Milan their right place to be, contributing to change the urban dynamics and shape. In the last part of 1900 Milan transformed its vocation into a more de-materialized one, investing in the tertiary sector, services, and economic development. Nowadays Milan is the Italian capital of design, fashion, and the Italian center of economy, and it is the center of the metropolitan area of Milan, which counts about 7 400 000 inhabitants including 11 different cities.

The problem of air pollution in the city has been present for a long time and during the years the local administrations have tried to abate and monitor air pollutants in the city. Up to date, the administration is promoting car and bike sharing, pedestrian areas, and vehicular traffic limitations. With the definition of “Area C” in 2012, a traffic-limited area in the center

of Milan, a first attempt to lighten the air pollution in the core of the city was made. In 2019 a second, broader area was instituted, the “Area B” with many traffic limitations (Fig. 5)

The air quality is constantly monitored by 9 stable stations, and about 10-15 mobile detectors, which measure ammonia, NOx, black carbon (BC), benzene, CO, and total PM. The stable devices are unevenly distributed in the city, covering mainly the city center, and they don’t measure all the contaminants. The mobile detectors cover mainly the peripheries. Measures are taken hourly and archives of data are available on the website of the regional agency for environmental protection (ARPA Lombardia, Agenzia Regionale Protezione Ambientale Lombardia). The air quality in Lombardy is greatly improved since ’90 and a constant decline is visible as shown in Fig. 6.



Figure 4: Milan city and its internal organization in district



Figure 5: Traffic limitations delimited by Area C, more central, and Area B, the newer and broader

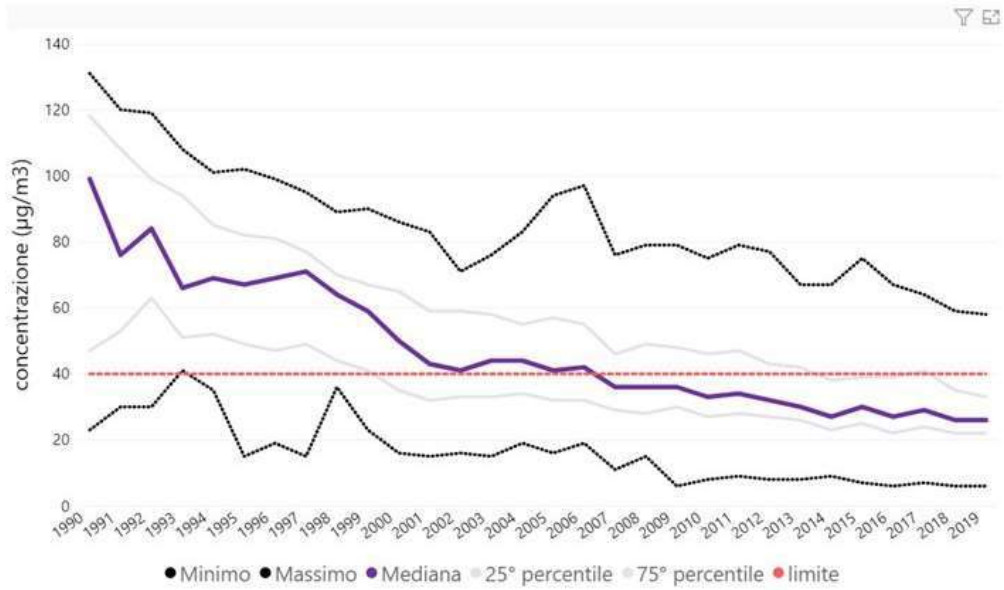
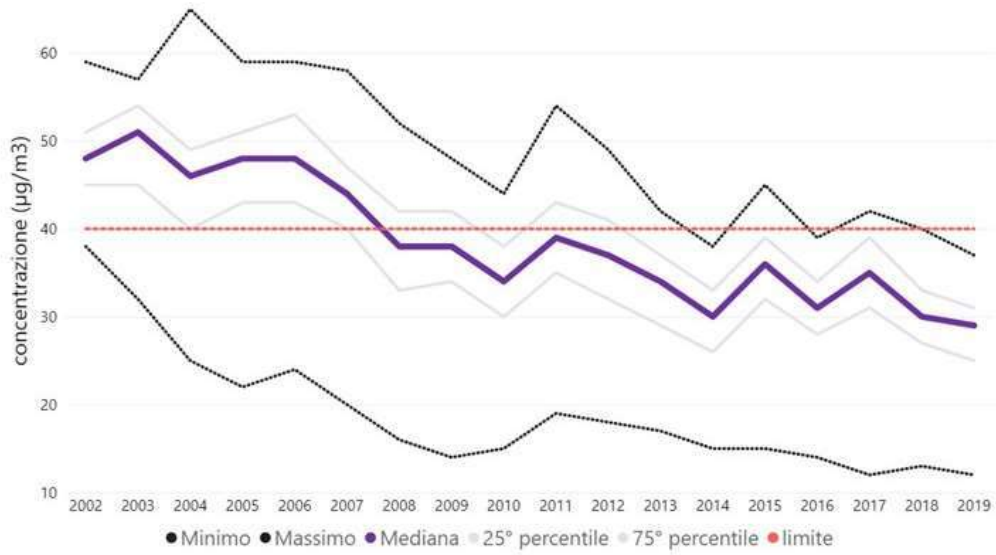
A**Trend NO_x****B****Trend PM₁₀**

Figure 6: A) Trend of the NO_x and B) Trend of PM₁₀ in Lombardy Region since 1990.

Section 1

Lichen biomonitoring

This section is dedicated to the lichen biomonitoring, covering both the bioaccumulation of trace elements and the eco-physiological alterations in exposed samples. The bioaccumulation of selected trace elements provides a measure of the bio-availability of the contaminants, thus, it is a quantitative measure of the environmental impact of TE contamination. Firstly, the overall contamination is evaluated along with the detailed pattern of contaminant depositions; then, a deeper insight in the study area peculiarities is given through source apportionment analysis, that extracts the main emission sources of air pollution. Finally, the biological effects of the exposure are evaluated through the assessment of selected eco-physiological parameters.

A brief introduction on the sampling strategy is reported below.

1. Sampling design

In order to obtain a representative pattern of air pollution of sufficiently high detail, a stratified sampling design was selected as sampling method. A first stratification was evaluated according to proximity to the city centre: with the help of an ortho-photo of the city, three concentric areas were described as “Central”, “Semi-peripheral” and “Peripheral” (Fig. 1A). A second stratification was made considering the 9 districts of the city (8 radial and 1 central, Fig. 1B). The intersection between the concentric areas and the district borders designed the sampling units, within which two sampling sites were randomly selected (Fig. 1C), for a total of 51 sampling sites in the whole area. Since the most central sampling sites were located in pedestrian areas and urban parks, a new concentric belt was designed and called “Pedestrian area”

The sampling sites were named according to the order: number of the district (1-9), number of the concentric area (1= Central; 2=Semi-periphery; 3=Periphery) and an alphabetic sign as “a” and “b” to distinguish the 2 sites in the same area. Thus, the site, for example, “53a” means “in the district 5, periphery, site a”.

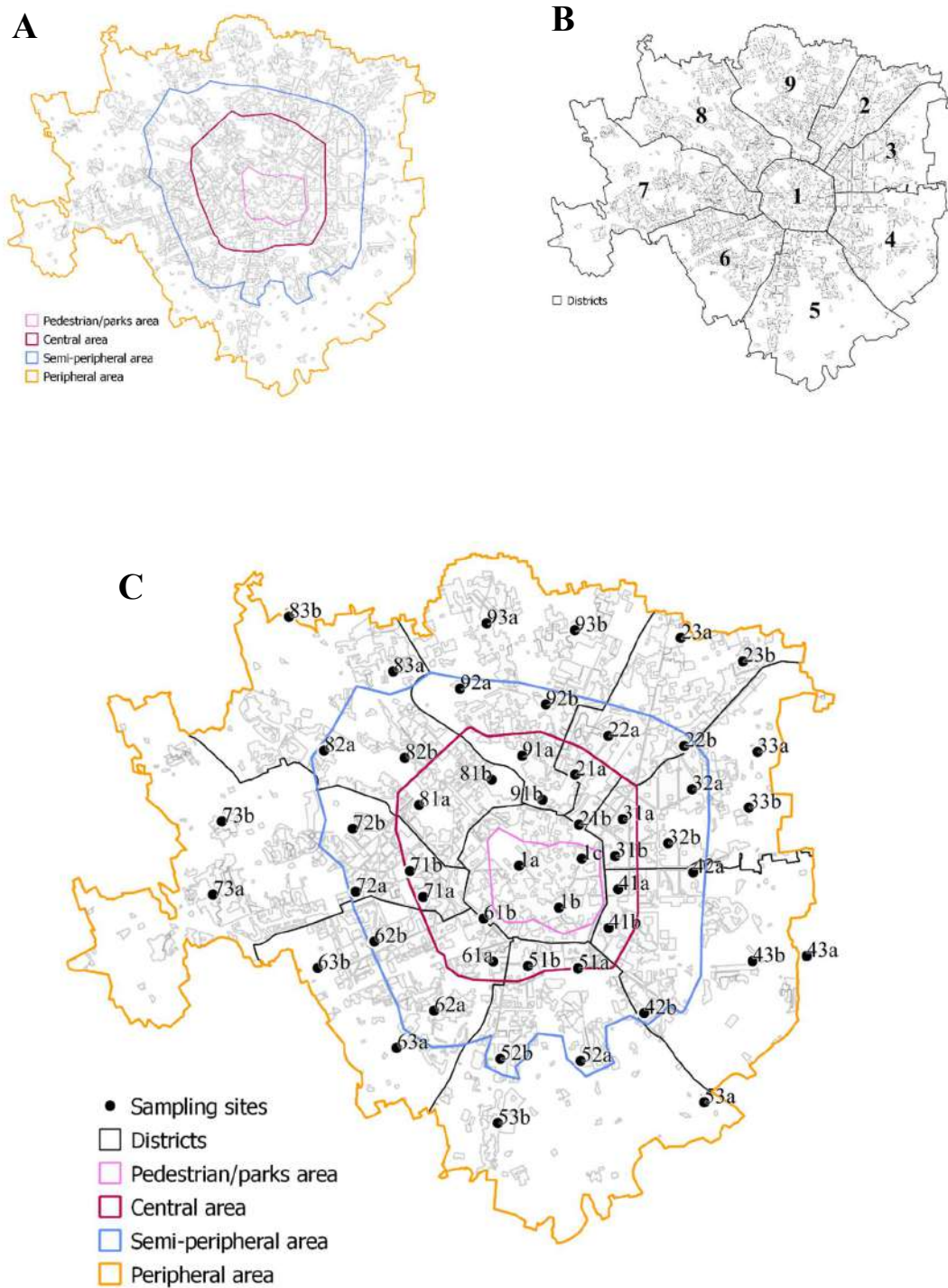


Figure 1: Sampling design. **A)** districts of Milan **B)** concentric areas and **C)** sampling sites

2. Lichen species: *Evernia prunastri*

The lichen selected for this study was *Evernia prunastri* (L.) Ach., an epiphytic (tree inhabiting) fruticose species, with a green alga belonging to the *Trebouxia* genus as photobiont. It grows hanging from branches and trunks of trees with sub-acid to neutral bark, in low or no eutrophicated environments. Thallus colour is greenish-white when dry and dark olive-green to yellow-green when wet, and the size can reach 20 cm. This species is widespread in Italy with the exception of disturbed environments as urban areas and dry environments (Nimis and Martellos, 2020)

Evernia prunastri is a widely used species in biomonitoring studies due to its great capacity to bioaccumulate trace elements (Cercasov et al., 2002; Loppi and Paoli, 2015).

Moreover, the easy recognizing, handling and cleaning are additional useful features of this species.



Figure 2: The lichen species *Evernia prunastri*

3. Lichen biomonitoring

Lichen biomonitoring was carried through the transplants technique. Samples of the selected species were collected in a pristine area in Siena (C Italy) from three branches at minimum 1.5 m above ground, to avoid the soil contribution to element content of the samples. In order to reduce as much as possible the natural variability (Wolterbeek and Bode, 1995), thalli of

similar size, and thus, similar age, were selected. Apparently healthy thalli were collected, by observing colour and integrity of thallus. No analytical measure were performed.

In laboratory, samples were cleaned with plastic tweezers removing extraneous material as bark, mosses and insects. The vitality of samples was randomly checked, assuring an optimal health status of the lichen material. Lichen bags were arranged loosely wrapping samples in a plastic net closed at the extremities with plastic wire. The material was stored in a climatic chamber at 16 C°, RH = 65%, 40 $\mu\text{mol m}^2 \text{s}^{-1}$ photons PAR with photoperiod of 12 h until transplantation, which occurred within 1 week.

Transplantation occurred binding the extremities of the lichen bags to tree branches at minimum 2 m above the ground in order to avoid both soil contribution and vandalisms. Samples were tied in the external part of tree branches in order to be maximally exposed to atmosphere. In each sampling sites, 3 lichen bags were transplanted.

The exposure lasted from December 2018 to February 2019, since three months is the recommended time for species *Evernia prunastri* to reach an equilibrium with the surrounding environment. The winter period is recommended because of the high metabolic rate of lichens in this period, that allows an active bioaccumulation and physiological rate (Loppi et al., 2019).

A control area was also selected 50 km from Milan, in a hilly and pristine area. Three lichen thalli were there transplanted in this area and used as control samples.

After the exposure, lichen samples were retrieved and air-dried in a climatic chamber, then stored at -20°C until analysis as suggested by Paoli et al. (2013).

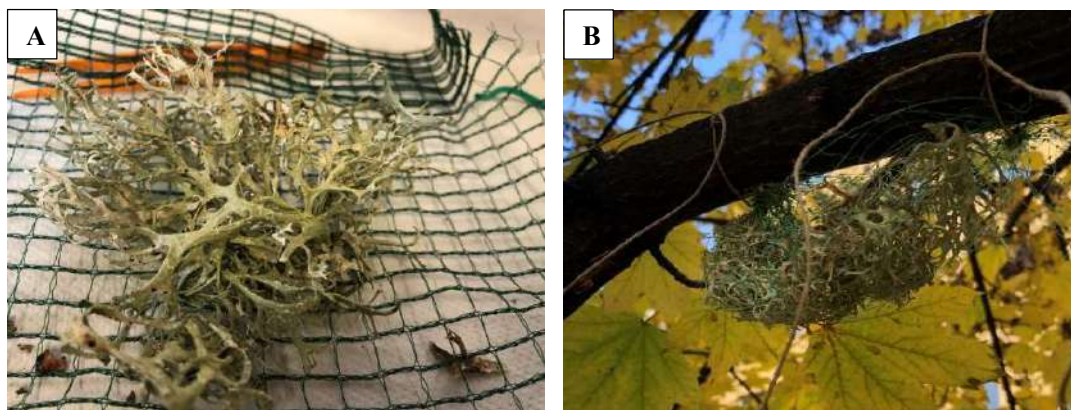


Figure 3: A) preparation of a lichen bag of *Evernia prunastri* and B) a bag exposed at a sampling site

Chapter 1

Bioaccumulation of trace elements and the *source apportionment* analysis

1. Introduction

Lichen biomonitoring techniques are widely used in the evaluation of air quality and are generally considered as complementary to the traditional monitoring devices: these are, in fact, expensive to install and maintain, thus, scarcely spread in cities. Moreover, they provide concentration measures of some contaminants but fail to provide the biological effects and the bio-availability of pollutants in the environment. Lichens, instead, are cost effective and, due to their independence from specific structures and substratum, they can be located in a high number of sites following a more detailed sampling design. This turns into a detailed pollution pattern of the biological effects due to air contamination (Loppi and Paoli, 2015).

The source apportionment analysis has been carried out both using a theoretical approach through positive matrix factorization (PMF), and empirically, through the study of the magnetic behaviour of accumulated particles.

2. Materials and Methods

2.1 Toxic element content

The bioaccumulation was assessed for Al, As, Cd, Cr, Cu, Fe, Pb, Sb and Zn following the method of Bettinelli et al (2002): an aliquot of about 200 mg for each sample was digested through a mixture of acids composed by 3 mL of 70% HNO₃, 0.2 mL of 60% HF and 0.5 mL of H₂O₂ in a microwave system (Ethos 900, Milestone). The concentration of toxic elements was then quantified by ICP-MS (Sciex Elan 6100, Perkin Elmer). The quality of the digestion process and the possible contamination during the preparation were controlled including one sample of the certified material IAEA 336 “lichen” and one procedural blank in each batch of analysis. The range of recoveries was 90%-113%; the precision of the analysis, checked with the coefficient of variation of five replicates, was >90%. The samples

were not washed, since the washing could alter the chemical composition of samples (Bettinelli et al., 1996).

2.2 Overall Contamination

The intrinsic strength of lichen biomonitoring is the information about the effects of the whole environment on the organisms as a proxy of the whole ecosystem. It is however true that natural differences between individuals lead to different bioaccumulation degree of trace elements, as well as the natural factors can differently act on biological samples. The natural variability, thus, plays a role that translates into data variability, i.e. uncertainty of the measures. In view of an increasing importance of lichen biomonitoring, also for environmental forensics issues (Loppi et al., 2019; Contardo et al., 2018) ignoring the uncertainty extent leads to wrong data interpretation and thus, wrong decision making (Milner-Gulland and Shea, 2017). The overestimation of contaminants (Type I error, false positive) lead the attention to some factors that actually may be less important, while relevant contaminants could be masked and not considered. To avoid Type I error, Couto et al. (2004) introduced the use of the Limit of Detection (LOD) and Limit of Quantification (LOQ) in biological monitoring. They used the control samples as an analytical blank for which they calculated both LOD and LOQ as the mean value of a given contaminant + 3 times and +10 times its standard deviation respectively. In this way they included the fund noise associated to transplant techniques in the data analysis.

The LOQ we calculated is here indicated as Effect Detection Limit (EDL) since it is the value above which the accumulation is considered significant. Since we have a control site, the approach suggested by Frati et al. (2005) was used to evaluate the bioaccumulation of trace elements in exposed samples: the concentration of elements in exposed samples was compared to the control ones, elaborating the “Exposed-to-Control ratio” (EC). To avoid the Type I error accounting for natural variability, the elaboration of EC ratio followed the approach of Couto et al. (2004), i.e., a correction factor of 10 times standard deviation was added to each control mean of elements, as already used in Contardo et al. (2018) and shown in the formula below.

$$EC = \frac{[El_{exp}]}{([El_{ctrl}] + 10 SD)}$$

Ratios exceeding 1 indicate a robust measure of an effective bioaccumulation. Those elements that exceed 1 at least in the half of sampling sites were, thus, considered the main contaminant of the area and used for the elaboration of the overall Contamination Index.

For each sampling site an overall Contamination Index (CI) was finally calculated as the geometric mean of the EC ratios of the resulting elements.

The spatial distribution of the CI was graphically elaborated through the QGIS software by using the Inverse Distance Weighting (IDW) algorithm.

2.3 Statistical analysis

The differences in contamination index by concentric areas were tested by using ANOVA test and Tukey test for post-hoc comparison for parametric analysis. The normality of data was previously tested through the Shapiro Wilk test. Data in this way analyzed were those aggregated for 3 concentric areas (Central, Semi-periphery, and Periphery).

Disaggregating also for Pedestrian area, a non-parametric analysis was necessary, thus it was used the Kruskal-Wallis test and Pairwise Mann Whitney U-test for post-hoc comparison.

Elaborations were performed with R software (R Core Team, 2020).

2.4 Source apportionment

The critical values of air pollution in urban areas are reached because of the contribution of many different emission sources and thus, disentangling the main emission sources is fundamental for better addressing abatement measures.

To pinpoint the main emission sources in the study area, a source apportionment (SA) analysis based on receptor modelling was performed. The *receptor models* (RM) are a class of models based on the chemical mass balance (CMB) principle between the emission sources and the measured concentration at the sampling site (“receptor site”). The concentration of a pollutant in the receptor site is statistically analysed to infer the source-types and evaluate their contributions to the total concentration. The assumptions for the mass balance are:

- The *source profile* remains constant or slightly changes over time. If it does, the fractionation coefficients of contaminants must be known.

- The *receptor species* is chemically and physically stable from the source to receptor site, i.e., it does not react chemically or change physical status between the emission and the deposition.

These requirements are generally well satisfied by metals as receptor species (in particular, assumption 2).

The most used statistical method for the source apportionment analysis in USA and Europe is the Positive Matrix Factorization (PMF) proposed by Paatero and Tapper in 1994 (Belis et al., 2013). This method is a variant of Factor Analysis and PCA, providing non-negative solutions. For this reason, it is widely used in environmental and physical sciences. Briefly, this method allows to solve a bilinear matrix problem taking into account the matrix \mathbf{X} of the observed data (dimension $n \times m$) and the known matrix σ of standard deviations of elements of \mathbf{X} (dimension $n \times m$). The method solves the bilinear matrix problem $\mathbf{X} = \mathbf{GF} + \mathbf{E}$, where \mathbf{G} is the unknown left-hand factor matrix (scores) of dimensions $n \times p$; \mathbf{F} is the unknown right-hand factor matrix (loadings) of dimensions $p \times m$, and \mathbf{E} is the matrix of residuals. In our case, the data matrix \mathbf{X} has $m \times n$ dimensions where m = EC ratios while n = sampling sites. The matrix is spatially resolved into the product of \mathbf{G} , that represents the loadings (or “source profile”), of dimensions $m \times f$, where f = factors; and \mathbf{F} , a score contribution matrix, of dimensions $f \times n$. Factor contributions (%) were calculated as the sum of all matrix elements of a row in the \mathbf{F} matrix, for a given factor, over the aggregate sum for all the factors.

The method provides different pollution profile, called “factors” elaborated for each sampling site.

2.5 Spatial elaboration of data

The spatial distribution pattern of both the overall contamination and the emerged factors from the PMF analysis, were separately mapped over the study area through the deterministic Inverse Distance Weighting (IDW) interpolation algorithm using QGIS 3.8 software, and represented as quintiles for a better visualisation. While the map of CI indicates areas more or less averagely polluted, the maps produced with the PMF factors are indicative of the distribution of *pollution profiles*. The sources determination was carried coupling the analytical pollution profile with its spatial distribution over the area, highlighting those urban elements that fitted with both spatial distribution and trace element composition of the given profile. For a reliable determination of the sources, the value of the

factor assumed at increasing distance from the suitable source was taken as indicative of the effective influence of that source to the factor profile. This operation was carried with the spatial support of the Milan Census Units. These are the smallest areas in which the municipality is subdivided for census operations and are about 6000, with different size and shape. Inside each one (a vector shapefile), the mean value of every Factor (raster layer), was calculated by “Zonal Statistics” geo-processing tool, i.e., the mean of Factor pixel values within a census unit was calculated and the resulting value assigned to the centroid of the patch. The centroids were used to calculate the minimum distance from each suitable urban element for the Factor. Then, distances were sorted into deciles and the mean was calculated both for the distance and for the respective factor value. A regression analysis was finally carried between distance deciles and factor value deciles.

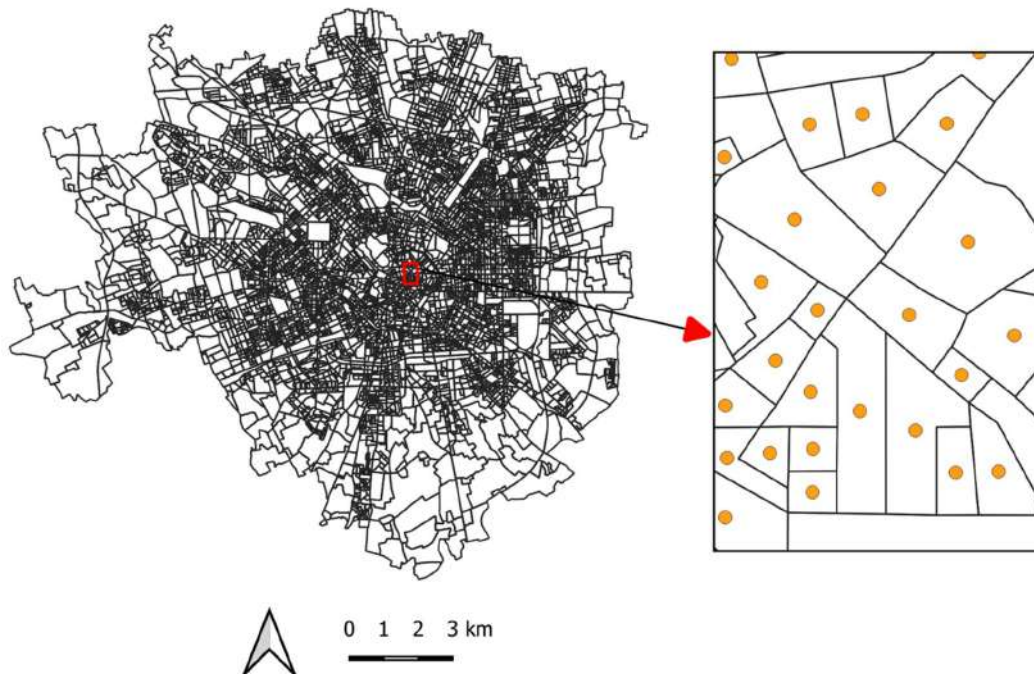


Figure 1: Census units of Milan and a detailed view. Yellow point are the centroids of the polygons, used for the distance calculation to emission sources

3. Results

Of the 51 samples exposed in Milan, 50 were successfully retrieved and only one went lost (23a) due to the tree cut in the area. The raw data of element concentrations are reported in Appendix A.

3.1 Overall contamination

Tab. 1 shows the mean values and standard deviation of control samples (in $\mu\text{g/g dw}$), values of the Effect Detection Limit (EDL) and the EC ratio elaborated in each sampling site for each element.

Table 1: Mean (\pm standard deviation) values ($\mu\text{g/g dw}$) of control samples, effect detection limit (EDL), and values of Exposed-to-Control (EC) for each sampling site.

Site	Al	As	Cd	Cr	Cu	Fe	Pb	Sb	Zn
CTRL	496 \pm	0.21 \pm	0.06 \pm	1.67 \pm	3.79 \pm	442 \pm	0.11 \pm	1.96 \pm	27.1 \pm
	20.2	0.015	0.005	0.086	0.027	10	0.006	0.082	1.55
EDL	698	0.35	0.11	2.53	4.06	543	0.17	2.78	42.6
1a	0.63	0.61	0.56	1.51	3.01	1.49	1.59	2.85	0.93
1b	0.44	0.95	0.80	1.45	2.85	1.55	1.68	2.95	1.16
1c	0.75	0.85	0.57	1.40	2.59	1.60	1.51	3.00	0.92
21a	0.98	1.10	0.64	1.96	7.14	2.05	2.43	6.74	0.96
21b	1.10	0.90	0.51	2.30	6.96	2.63	1.61	4.47	0.77
22a	1.00	1.14	0.65	1.83	3.91	1.76	2.95	5.28	0.84
22b	0.44	0.81	0.51	1.17	5.07	1.10	1.62	3.38	0.70
23b	0.40	0.69	0.53	0.92	1.61	0.87	1.49	3.24	0.79
31a	0.94	0.72	0.66	1.22	2.81	1.28	1.62	3.23	0.64
31b	0.78	0.76	0.47	1.49	6.24	1.28	1.14	3.83	0.85
32a	0.53	0.94	0.72	1.34	3.76	2.10	2.47	6.56	1.00
32b	0.70	0.82	0.60	1.16	4.20	1.34	1.68	3.34	0.73
33a	0.52	0.79	0.83	1.28	3.82	1.19	1.77	5.51	1.20
33b	0.98	0.98	0.64	1.64	4.55	1.94	2.09	5.00	1.25
41a	1.28	1.12	0.57	1.73	3.41	1.88	1.44	4.47	0.72
41b	1.25	0.95	0.61	1.57	3.79	1.65	1.70	4.03	0.81
42a	0.67	1.05	0.64	1.82	10.60	1.71	2.29	6.41	1.10
42b	1.21	1.07	0.65	1.30	3.72	1.70	2.02	2.92	1.75
43a	0.84	0.74	0.63	1.08	2.22	1.30	1.50	3.22	0.60
43b	0.61	0.86	0.62	1.22	2.16	1.02	1.45	2.85	0.67
51a	1.24	1.07	0.64	1.92	7.89	2.13	1.81	6.61	0.94
51b	1.42	1.13	0.60	2.26	10.46	2.48	1.48	9.72	1.25
52a	0.66	0.68	0.64	0.87	3.02	0.91	1.76	2.28	0.68
52b	1.34	1.20	0.80	1.61	2.90	1.81	4.29	4.44	0.84
53a	0.83	0.79	0.49	1.02	2.27	1.15	1.03	1.72	0.39

53b	1.05	0.98	0.93	1.34	3.24	1.50	1.38	3.17	0.41
61a	1.52	0.94	0.56	1.28	2.04	1.52	1.71	3.24	0.64
61b	1.18	0.70	0.43	1.78	3.79	1.77	1.46	3.67	0.68
62a	0.71	0.93	0.73	1.47	5.04	1.60	2.13	6.02	0.93
62b	1.12	0.87	0.64	1.10	1.74	1.31	1.53	3.09	0.67
63a	1.05	1.49	0.76	1.90	3.96	1.66	2.08	5.63	0.97
63b	0.49	0.79	0.62	1.27	4.43	1.34	1.48	4.88	0.93
71a	1.49	1.03	0.73	1.97	6.22	2.24	2.89	7.92	1.18
71b	1.56	0.91	0.51	1.31	3.76	1.67	1.85	3.42	0.90
72a	0.75	0.77	1.08	1.08	2.17	1.21	1.70	3.27	1.38
72b	0.73	0.79	0.64	1.04	1.79	1.29	2.12	2.81	0.67
73a	0.53	0.76	0.51	0.97	1.92	1.01	1.37	3.60	0.71
73b	0.76	0.94	0.53	1.27	1.34	1.44	1.45	3.01	0.62
81a	0.63	0.73	0.56	1.26	3.55	1.31	1.92	4.47	0.90
81b	0.87	0.90	0.60	1.97	7.67	2.05	2.76	6.29	0.96
82a	0.63	0.89	0.65	1.39	4.97	1.47	3.11	5.66	1.42
82b	0.69	0.81	0.51	1.15	3.82	1.26	2.77	4.00	0.92
83a	0.84	0.88	0.82	1.17	2.04	1.21	5.39	4.29	0.53
83b	0.78	0.76	1.02	1.05	2.79	1.15	2.77	2.71	0.64
91a	0.99	0.92	0.67	1.83	6.79	1.83	3.28	5.49	0.98
91b	0.64	0.88	0.71	1.45	5.25	1.48	2.72	4.40	0.99
92a	0.96	1.23	1.16	1.89	4.83	2.00	9.95	10.17	2.01
92b	1.10	0.77	0.51	1.19	2.66	1.21	2.67	2.89	0.52
93a	0.88	0.84	0.85	1.36	5.08	1.40	7.76	9.17	0.95
93b	0.89	0.85	0.73	1.03	2.81	1.24	4.06	2.79	0.79

Elements showed different rate of EC ratios, ranging from a minimum of 0.39 registered in the sampling site 53a for Zn, and a maximum of 10.60 registered in the sampling site 42a for Cu. Cadmium was the less bioaccumulated element, with only 3 sites on 50 where bioaccumulation occurred, while the most bioaccumulated elements were Cu, Pb and Sb, for which the bioaccumulation occurred in all the sampling sites. Aluminium was bioaccumulated in 16 sites on 50 (32%), As in 11 (22%), Cr in 47 (94%), Fe in 48 (96%) and Zn in 10 (20%).

In Tab. 2 is summarized the mean concentration of control samples, the detection limit, the mean concentration of exposed samples and the median values of EC ratios elaborated for the whole area. Elements with an EC ratio higher than 1 were Cr (1.64), Cu (3.76), Fe (1.49), Sb (1.75) and Pb (4.00), while Al, As, Cd, and Zn showed no accumulation in lichen samples. Thus, a situation of contamination is registered for Cr, Fe, and Sb, while a situation of severe contamination is registered for $EC > 1.75$, here reported for Cu (3.76) and Sb (4.00), and thus considered as the main air pollutants in the area.

Table 2: Mean (\pm standard deviation) values ($\mu\text{g/g dw}$) of control samples, mean (\pm standard deviation) values ($\mu\text{g/g dw}$) of exposed samples, effect detection limit (EDL), Exposed-to-Control (EC).

	Control	Exposed	EDL	EC median
Al	496 \pm 20	619 \pm 209	698	0.84
As	0.21 \pm 0.01	0.32 \pm 0.06	0.35	0.88
Cd	0.06 \pm 0.01	0.07 \pm 0.02	0.11	0.64
Cr	1.67 \pm 0.09	3.63 \pm 0.90	2.53	1.34
Cu	3.79 \pm 0.03	16.6 \pm 8.6	4.06	3.76
Fe	442 \pm 10	837 \pm 214	543	1.49
Pb	1.96 \pm 0.08	5.84 \pm 2.39	2.78	1.75
Sb	0.11 \pm 0.01	0.76 \pm 0.32	0.17	4.00
Zn	27.1 \pm 1.5	38.2 \pm 13.1	42.6	0.90

In Fig.2A is reported the map of CI elaborated by the IDW algorithm and sorted into quintiles. The CI ranges from 1.36 and 4.49, indicating that the whole area is interested by contamination and severe contamination by trace elements. In Fig. 2B, the boxplot shows the contamination data for concentric areas. The Centre shows higher values of contamination, even though a significant difference did not emerge between groups. Disaggregating for Pedestrian areas (Fig. 3), a new result emerges: being protected by urban forests and the limitation to vehicular traffic, these sites show the lowest values of contamination.

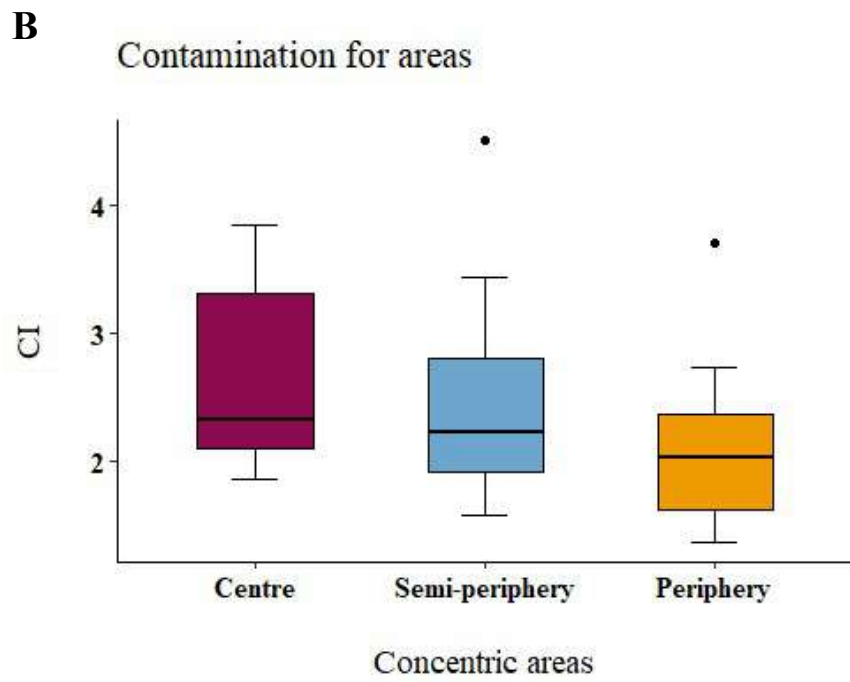
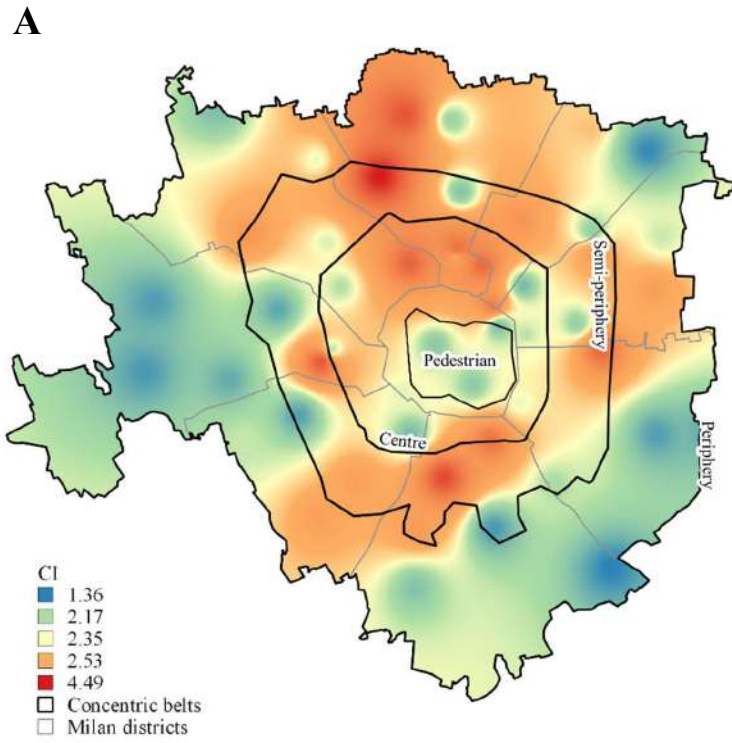


Figure 2: A) Spatial distribution of Contamination Index (CI) and B) CI elaborated for concentric areas

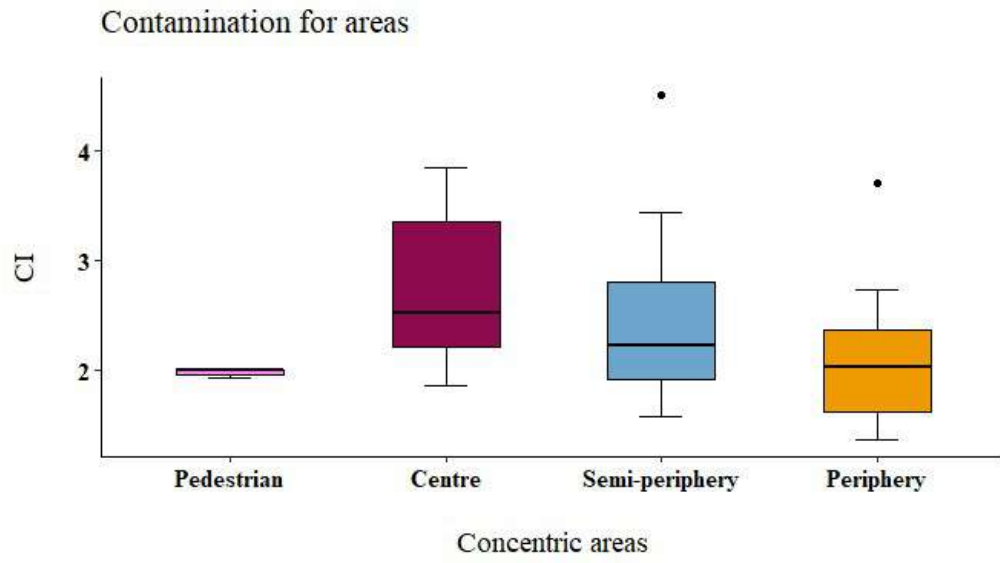


Figure 3: Values of CI elaborated after the disaggregation of Pedestrian Area

3.2 Source apportionment

A three factor solution was selected as optimal for the PMF model, with no factor resulting uncorrelated. The correlation between predicted and observed data was high ($R^2 > 0.6$) for all the elements, except for Al and Cd ($R^2 = 0.16$ and 0.21 , respectively). The standardized residuals were in the range of ± 2 , indicating all the elements were well modelled.

The factor profiles are separately reported.

The profile of the first Factor (F1) and its distribution all over the area is reported in fig 4A and Fig. 4B. This factor accounted for 45.2% of the total variability and it is dominated by Cu (50.4 %) and Sb (30.1%).

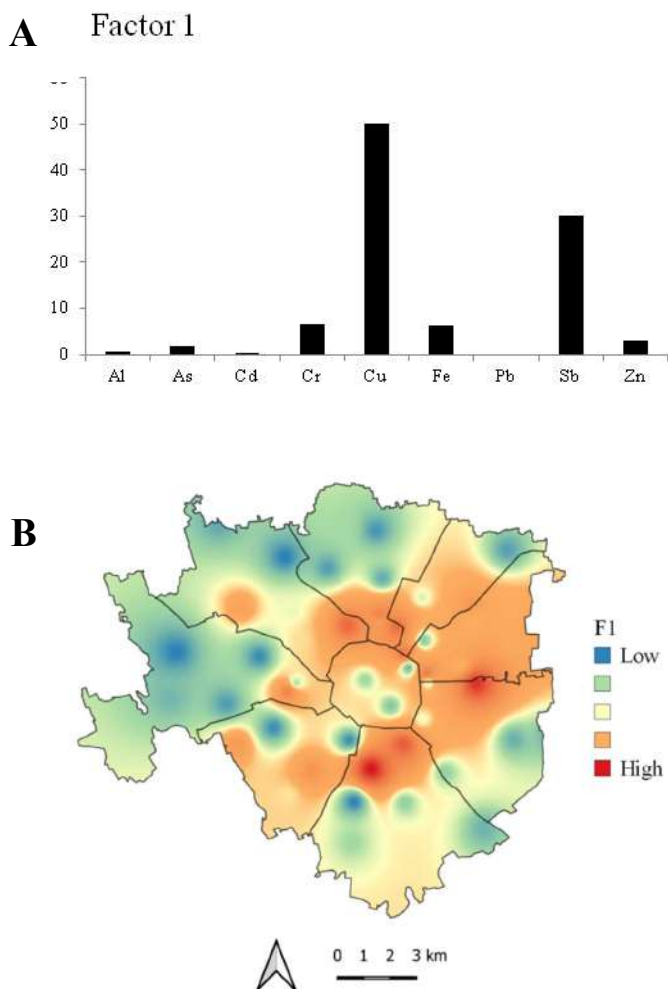


Figure 4: **A)** Elemental profile of the F1. Cu contributes for 50.4% and Sb for 30.1% **B)** Spatial distribution of F1. The pattern suggests the presence of diffuse emission sources

These elements are common tracer of brake abrasion of vehicles, and, combined with the homogeneous distribution of the factor, suggested the vehicular and train traffic the most reliable emission sources. Thus, the major roads (>25 m) (Fig. 5A) and railway lines (Fig. 5B) were mapped over the area in order to visualize a possible common distribution.

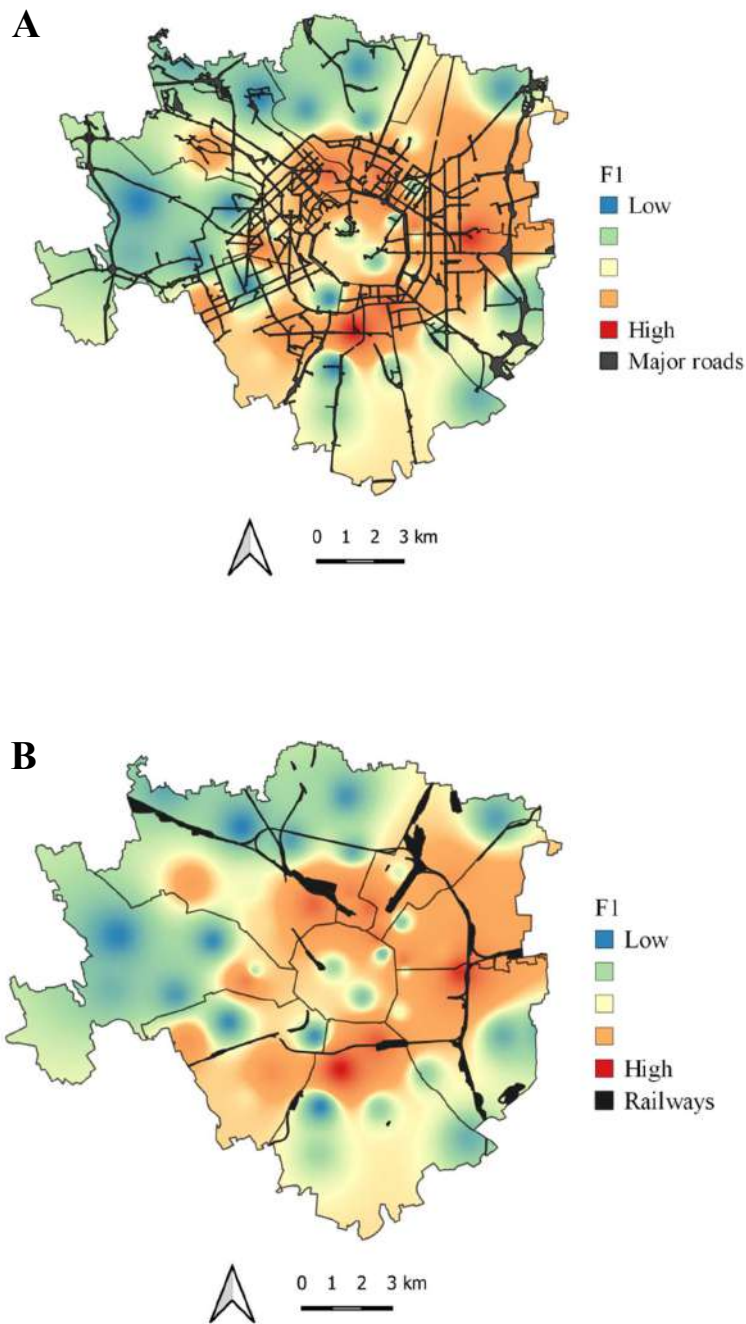


Figure 5: A) Spatial distribution of F1 with the network of major roads and B) Location of railway lines is overlaid to the distribution map of the F1

The correlation between the factor values and the distance from the major roads and the railway lines as potential emission sources is reported in Fig. 6A and B.

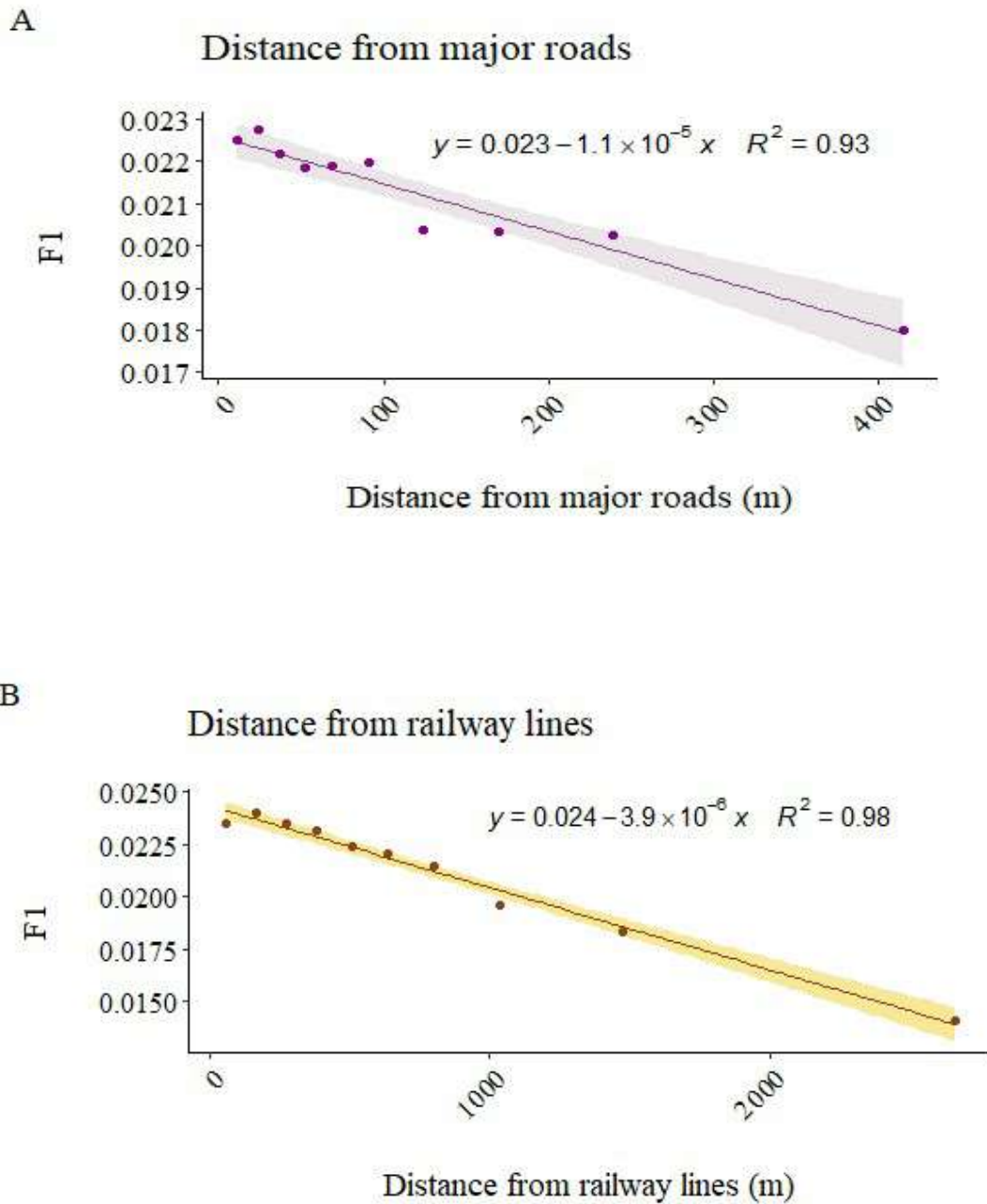


Figure 6: **A)** Linear correlation between the factor values and the distance from major roads and **B)** linear correlation between the factor values and the distance from railway lines

The second factor (F2) profile accounted for 25.4 % of the total variability and it is dominated by Pb (53.9%) and Sb (34.3%) (Fig.7 A). The distribution of this factor is highly localized in the northern part of the city, suggesting a punctual source more than diffused sources (Fig. 7B). Given the co-presence of Pb and Sb, a research on industrial activities was carried, finding in the interested area the existence of a large glasswork and crystal factory, active from 1964 to 2004. This industry was pinpointed as potential emission source given the fundamental use of Pb and Sb in glass production (Fig.8)

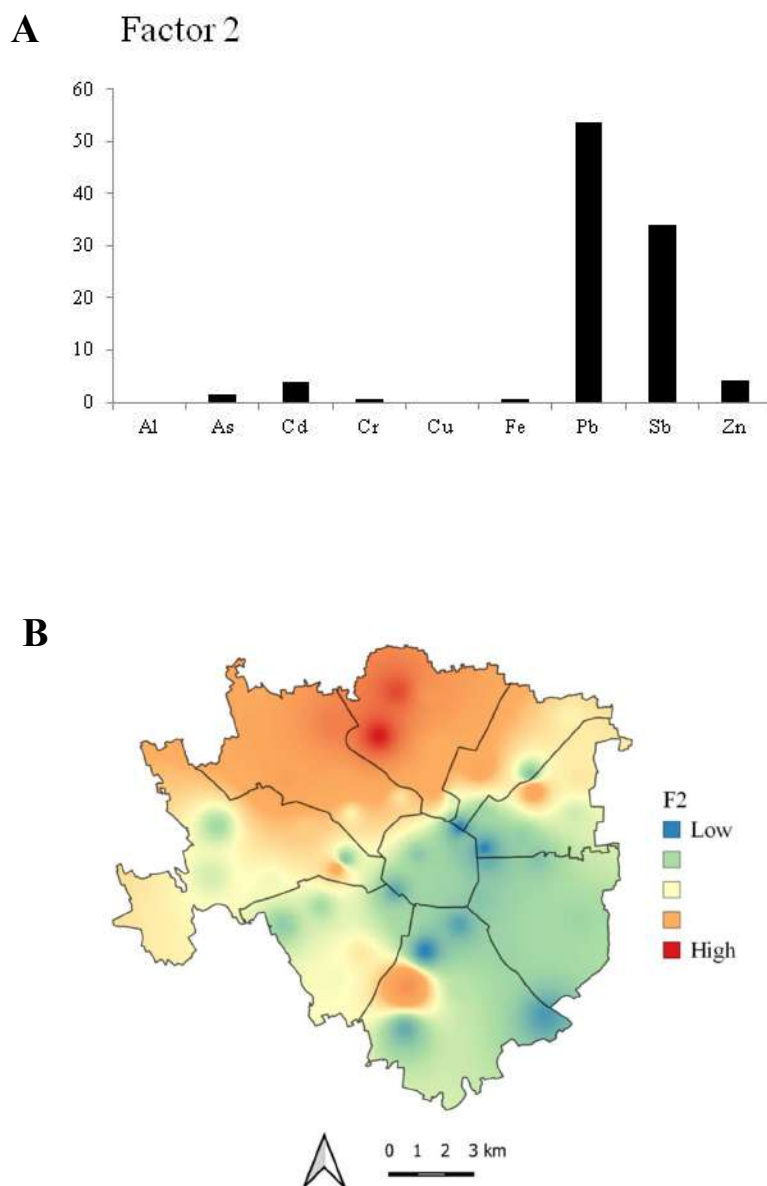


Figure 7: A) Elemental profile of F2. Pb contributes for 53.9% while Sb for 34.3%. **B)** Spatial distribution of F2 over the area.

The correlation between the factor value and the distance with the potential source is reported in Fig. 8. The correlation follows an exponential shape, typical of a decay from a punctual source.

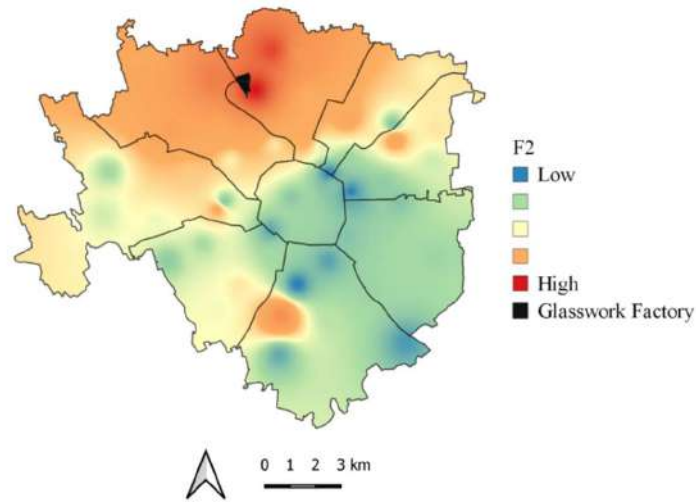


Figure 8: Spatial distribution of F2 and the position of the glasswork factory

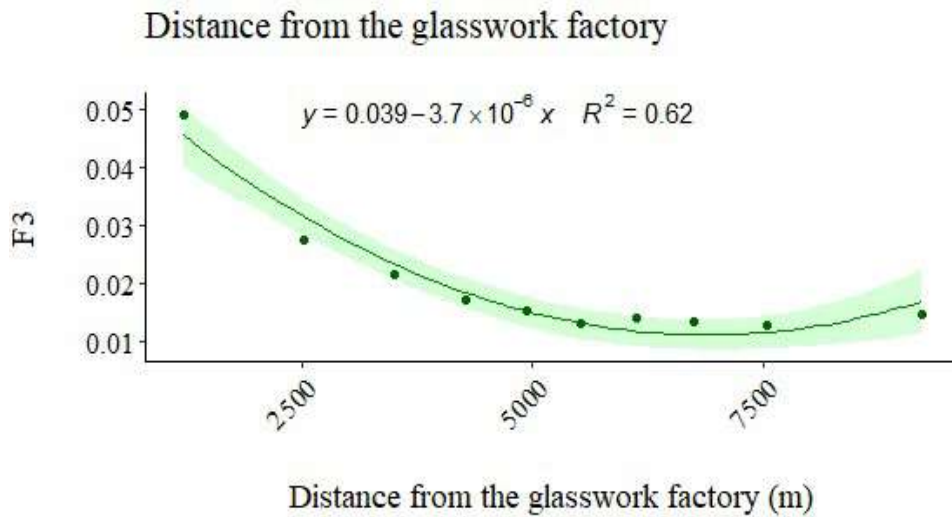


Figure 9: Exponential correlation between the distance from the glasswork factory and factor values

The third factor (F3) accounted for 29.3 % of the total variability. No elements seem to dominate the profile, however the main contribution is from Fe (20.2%), Cr (17%) and Al (16.1%), typical of soil re-suspension (Fig 10A). No urban elements seemed to fit with this distribution, however the prevalent presence in the southern part, where more agriculture is present, can confirm the lithogenic origin (Fig 10B).

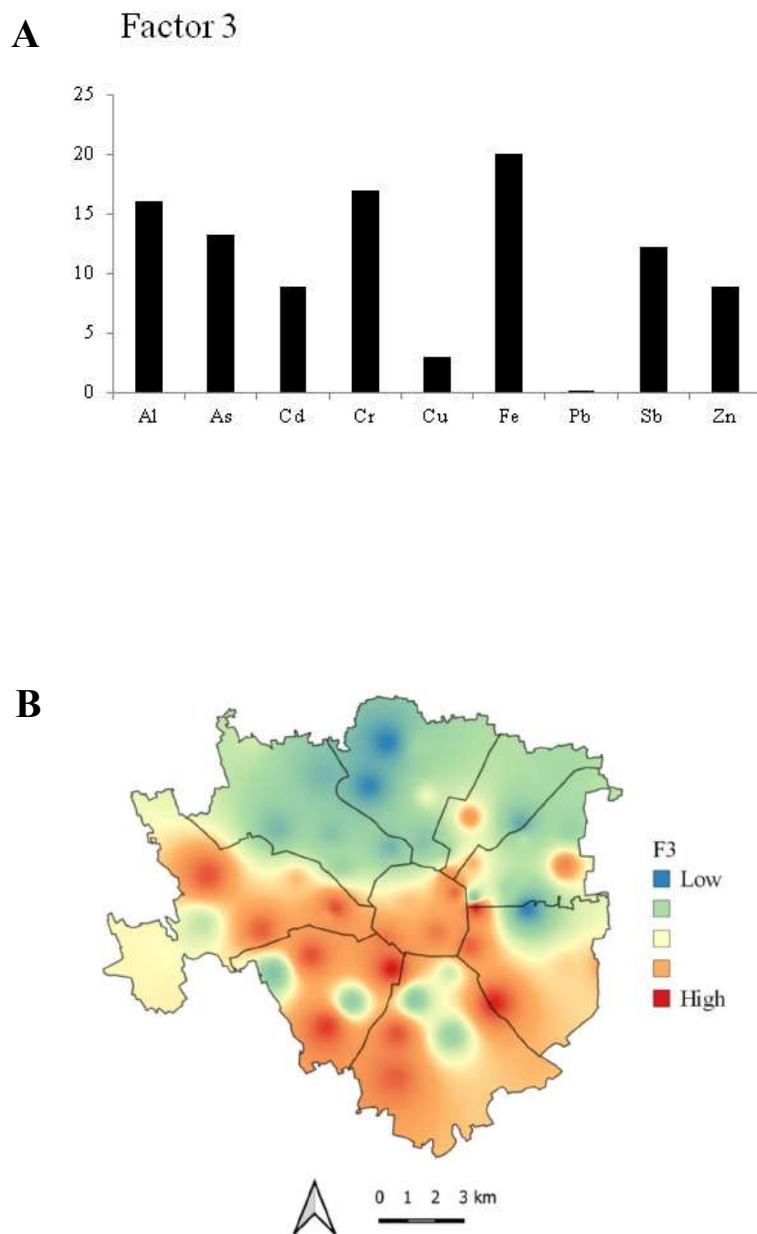


Figure 10: **A)** Elemental profile of F3. Fe contribute for 20.2%, Cr for 17% and Al for 16%. **B)** Spatial distribution of F3. No evident pattern was highlights, nor urban element seem to fit with the distribution

4. Discussion

The results of lichen biomonitoring showed a general high pollution from trace elements in the study area, especially for Cu and Sb. The effectiveness of bioaccumulation of these elements is witnessed by several other lichen biomonitoring studies carried in urban areas (Paoli et al., 2013; Loppi and Paoli, 2015; Loppi et al., 2019; Vannini et al., 2019), as well as inside the car cabin for the assessment of PM exposure inside the car (Paoli et al., 2019).

The high level of Cu and Sb is not surprisingly in urban areas, since, as showed by Zereini et al. (2005), urban PM is particularly enriched of these elements, mainly distributed in the finest fractions. Both Cu and Sb find a common origin in the brake system components, of which abrasion is the main source of their release: Cu from brake ware can account up to 75% of the total Cu emissions in European roads (Van der Gon et al., 2007), while Sb is widely used as a tracer of vehicular traffic (Wåhlin et al., 2006; Jeong et al., 2019). Non-exhaust emissions are gaining more and more relevance in air contamination, due to the fact that main abatement campaign were addressed especially to the exhaust emissions (EEA, 2018).

The results of Contamination Index show a generally high level of pollution registered in almost each sampling site. The elaboration of CI for concentric areas highlighted no significant differences even a clear decreasing trend of pollution is detectable from the central areas to the peripheries. Interestingly, disaggregating for Pedestrian area elucidates a new scenario for which the pollution level in this new area is dramatically lower than the others. Green areas, thus, are confirmed to provide reliable protection against air pollution (Baraldi et al., 2019). Trees, in facts, can help in reducing PM by adsorption on leaf surface as a consequence of dry deposition process (Manes et al., 2016).

The source apportionment analysis through PMF, highlighted 3 factors characterized by specific elemental profiles. The first factor, dominated by Cu and Sb was associated to non-exhaust emissions, especially brake abrasion (Sanders et al., 2003; Sternbeck et al., 2002). This result is in line with the global trend of the trace element pollution (Massimi et al., 2019): while exhaust emissions were strongly fought through technological improvements (the removal of Pb from gasoline, the introduction of catalytic converter etc...), less was done for non-exhausts that are, nowadays, in prevalent proportion over the formers (Thorpe and Harrison, 2008). The strong linear correlation ($R^2= 0.93$) between the factor score and the distance from the major roads reinforces the hypothesis that vehicular traffic is a key factor for Sb and Cu emissions, detectable up to 100 m. The railway lines

represent another potential source for these elements, since they are contributors of non-exhaust emissions (Timmers and Achten, 2016). The impact of the railways on air and water quality is well documented (Wierzbicka et al., 2015) and other authors pinpointed the train traffic as the most contributors of these trace elements in lichens (Massimi et al., 2019). Moreover, the strong linear relation between the factor score and the distance from the railway lines ($R^2=0.98$), confirms the not-secondary role of train traffic in Cu and Sb emission, detectable up to 1000 m, according to Wiłkomirski et al. (2011).

The second factor was dominated by Pb and Sb, with a really concentrated spatial distribution in the northern part of the city. Lead was traditionally used as tracer of vehicle traffic, however, after the ban of leaded gasoline in the '90 in Italy, a remarkable decline was registered by lichen biomonitoring in many Italian cities (see Loppi and Pirintsos, 2003; Loppi et al., 2004). Nowadays, it is estimated to derive mainly from road dust re-suspension as showed in Monaci et al. (2000) and industrial activity (Dong et al., 2017). Thus, an industrial origin of this factor was hypothesized. A brief research on the main industries active in that area led to pinpoint a glasswork factory active from 1964 to 2004. The glass and crystal production uses Pb and Sb as fundamental elements in production processes (Apostoli et al., 1998) and high level of Pb are often recorded nearby the plants due to the scarce mobility of Pb (Helmfrid et al., 2019; Young et al., 2002). The factory position, in facts, coincided with the highest score of the factor. The clear exponential decreasing trend ($R^2=0.63$) between factor score and the increasing distance confirms the industrial hypothesis. This result suggests the key role non only of the actual emission sources but also the past residuals.

The third factor did not show dominant elements, but the main contributor (Fe, Cr and Al) suggest the lithogenic origin of this factor. Also the spatial distribution did not show any correspondence with urban elements, however the agricultural origin can be hypothesized since this factor distributes complementary to the previous one, in the southern part of the municipality where more agricultural areas are present.

5. Conclusions

The city of Milan shows different degree of contamination based on the distance from the city centre, in particular are the Peripheries that show a better air quality compared to the Centre. However, high level of bioaccumulation were recorded in the whole area suggesting that TE contamination is severe in the whole area, in particular for those elements linked to non-exhaust emissions (Cr, Cu, Fe, and Sb). The source apportionment analysis managed to highlight roads and railways as the most important contributor of non-exhaust related elements, while the industrial activity as the main contributor for Pb in the area.

Chapter 2

Physiological response of the lichen *Evernia prunastri* L. (Ach) exposed in Milan (N Italy)

1. Introduction

Due to the high sensitivity to air pollution, lichens have been widely used as bioindicators of environmental changes and air pollution episodes. The first, not eye visible, reactions occurring in the physiological status when some stressors happen in the environment, are called “early warning signals” of a potential damage to the organism. These physiological markers express both ongoing damages (as the production of oxidative species) and attempts of protection (as the production of anti-oxidant molecules) and represents a precious tool for forecasting possible scenarios (Lačkovická et al., 2013). In lichens, many biomarkers are used for this purpose as the damage to DNA structure (Cansaran-Duman et al., 2011), membrane lipid peroxidation and permeability (Pisani et al., 2009; Munzi et al., 2009), the production of specific secondary metabolites (Basile et al., 2015), as well as the evaluation of photosynthetic activity (Paoli et al., 2010).

The alteration of the physiological status may turn in different bioaccumulation capacities: even if is demonstrated that devitalized thalli may accumulate higher amounts than living thalli (Ceconi et al., 2020), the former status fails to provide the biological interaction between biota and the surrounding environment, that is the key point of the biological monitoring.

It is noteworthy that the biological response is the product of the synergy of contaminants acting in the real environment, thus, it provides the actual health status of the biota.

In this chapter it is investigated the biological effect of the exposure of *Evernia prunastri* in the study area through the evaluation of selected physiological parameters: damage to photosynthetic apparatus, anti-radical activity and changes in cell membrane permeability.

2. Materials and methods

Before all the analysis, the samples were revitalized through a regular hydration with water and kept overnight in a climatic chamber at 16 C°, RH = 65%, 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photons PAR in order to recover the vital status (Paoli et al., 2015).

2.1 Photobiont

2.1.1 Photosynthetic activity

Environmental stress can cause several impairments to the photosynthetic activity of the photobiont (Strasser et al., 2004) with consequences on the global vitality of the organism. A rapid, non-invasive test to assess the photosynthetic activity consists in evaluating the chlorophyll *a* fluorescence emission (Tretiach, 2008).

Ten distal fragments were randomly selected for each sample and dark adapted for 10 minutes using a clip, then lightened with a saturating red light for 1 s (650 nm, 3000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) with a light emitting diode (LED). Measurements of chlorophyll *a* fluorescence were carried out with a Plant Efficiency Analyzer fluorimeter (Handy PEA, Hansatech, Norfolk, UK). Three parameters were investigated: F_v/F_M ; PI_{ABS} and the OJIP transient.

- The ratio F_v/F_M (where $F_v = F_M - F_0$) is an indicator of the maximum quantum yield of PS II (Butler and Kitajima, 1975). At the T_0 , all the reaction centres of PSII are opened and the fluorescence is minimum (F_0), along with time the centres close until the fluorescence is maximum (F_M) when all the centres are closed (Maxwell and Johnson, 2000). The optimal value of the ratio F_v/F_M for hydrated lichens is around 0.6-0.7 (Manrique et al., 1993), and it reduces when some stresses occur. This parameter is the most used and found in literature, thus it is nowadays the most comparable. However it considers exclusively the PSII performance.
- The Performance Index (PI_{ABS}) summarizes the contribution of all parameters involved in the functionality of the electron flow through PSII, thus it is used as a global indicator of the photosynthetic performance (Strasser et al., 2004, Paoli et al.,

2010). This parameter is less used than the former, even though it is more accurate and it considers the entire photosynthetic apparatus. The comparability with literature values is, thus, less possible than the former value.

- The OJIP transient, also called “the Kaustky effect”, is the characteristic transient of chlorophyll fluorescence emitted in a period of 1 s, after a period of dark adaptation (10 or more minutes). This transient represents the fast rise of chlorophyll intensity before a slow phase of return to the fundamental state, that occurs within few minutes. The fluorescence intensity starts with “O” (origin) phase and reaches the maximum in “P” (peak) phase in 1 s. Two intermedia flexions are in J and I phases. Reported in a logarithmic scale, it is possible to clearly distinguish the fluorescence emission curve and phases. The fluorescence yield is suggested to be regulated by the plastoquinone A (QA) of Photosystem II, in its oxidized state, thus, as the concentration of reduction of QA (Q_A^-) rises, the emission of fluorescence rises as well. That leads to consider the OJIP transient as a good indicator of the photochemical quantum yield of Photosystem II, widely used for the evaluation of environmental stresses (Stirbet and Govindjee, 2011; Malaspina et al., 2015).

2.2 *Mycobiont*

2.2.1 *Antiradical activity*

The production of free radicals and reactive oxygen species (ROS) is related to metabolic processes as well as environmental stressors. High level of these species turns into damages to biomolecules that, losing their functionality, may lead also to cell death.

Antioxidant molecules are naturally present in living system with the aim to minimize the free radical actions.

A colorimetric test to quantify the free radical scavenging potential activity is the DPPH test (Mishra et al., 2012). Samples of ca. 50 mg (previously air-dried) were homogenized in 1 mL of a solution of ethanol/water (80:20; v/v). 100 μ L of the homogenate were added to 1 mL of a 100 μ M DPPH solution prepared by dissolving 3.9 mg of this compound in 100 mL of methanol/water (80:20; v/v) solution. The test was performed at room temperature and the reaction occurred in 1 hour, then the samples were read at 517 nm and the results were expressed as % Antiradical Activity (ARA%) according to the formula:

$ARA\% = 100 * [1 - (\text{control absorbance}/\text{sample absorbance})]$

where control absorbance = the absorbance of the reagents only.

Three measures were performed for each experimental unit.

2.2.2 Integrity of the cell membrane

The integrity of the cell membrane proved to be sensitive to environmental stress such as phytotoxic gases and potentially toxic elements (Branquinho et al., 1997). The alteration of cell membrane permeability due to environmental stress leads to the leakage of electrolytes from the cell, that is proportional to the damage. The leakage of electrolytes is easily measurable by soaking the sample in deionized water and measuring the change in electrical conductivity of the medium (Munzi et al., 2009). At first, the conductivity of deionized water was measured with the conductivity meter (Crison Basic 30) as T_0 . From each sample, 10 aliquots of about 50 mg were randomly selected and washed for 3 s with deionized water to remove the superficial dust. Then, the sub-samples were soaked in 50 mL of deionized water for 1 hour and the conductivity was newly measured to assess the increase from the T_0 .

To obtain the maximum damage to membranes, after the first measurement, thalli were frozen at -20°C overnight, then, newly soaked in 50 mL of deionized water for 1 hour. The electrical conductivity associated with the maximum damage was used to compare the electrical conductivity of the former analysis according to the formula:

$(\text{Electrical conductivity of retrieved thalli}/ \text{maximum induced damage}) * 100$

2.3 Statistical analysis

The degree of vitality compared to control samples was assessed by a direct comparison between the measures and expressed as “Exposed/Control”.

The differences in vitality performances by concentric areas were tested by using ANOVA test and Tukey test for post-hoc comparison for parametric analysis. The normality of data was previously tested through the Shapiro Wilk test. Data in this way analyzed were those aggregated for 3 concentric areas (Central, Semi-periphery, and Periphery).

Disaggregating also for Pedestrian area, a non-parametric analysis was necessary, thus it was used the Kruskal-Wallis test and Pairwise Mann Whitney U-test for post-hoc comparison.

Correlation between bioaccumulated elements and vitality performances was tested through Pearson correlation method.

Elaborations were performed with R software (R Core Team, 2020).

3. Results

The vitality performances of control and exposed samples are reported in Tab. 1 for each sampling site. Control values showed optimal vitality performances while exposed samples showed, in general, a reduction of vitality.

Table 1: Mean values of eco-physiological parameters measured in control and exposed samples

Site	FvF _M	PI _{ABS}	Mb %	ARA %
CTRL	0.710	0.221	26.3	56.2
1a	0.605	0.129	29.3	67.9
1b	0.539	0.055	36.6	67.8
1c	0.578	0.071	31.5	62.0
21a	0.546	0.083	35.4	62.2
21b	0.594	0.107	42.7	61.9
22a	0.631	0.104	31.8	68.5
22b	0.606	0.111	35.9	57.7
23b	0.571	0.092	33.3	68.8
31a	0.604	0.101	34.4	53.3
31b	0.527	0.051	28.5	66.9
32a	0.548	0.056	37.1	61.4
32b	0.601	0.144	40.1	75.0
33a	0.697	0.202	35.4	65.9
33b	0.632	0.140	37.1	62.5
41a	0.705	0.193	25.7	67.7
41b	0.485	0.038	32.6	57.9
42a	0.613	0.091	39.9	75.3
42b	0.605	0.104	35.5	69.5
43a	0.669	0.153	44.6	80.3
43b	0.713	0.227	36.1	61.2
51a	0.516	0.071	32.0	69.1
51b	0.566	0.098	49.0	61.8

52a	0.653	0.167	35.4	66.5
52b	0.573	0.084	36.2	65.1
53a	0.597	0.102	27.2	64.9
53b	0.641	0.130	47.6	59.3
61a	0.485	0.038	29.6	63.3
61b	0.515	0.074	27.7	57.9
62a	0.692	0.177	42.6	60.9
62b	0.534	0.093	36.2	61.4
63a	0.660	0.170	42.6	72.1
63b	0.575	0.120	34.5	53.0
71a	0.488	0.050	33.0	54.1
71b	0.568	0.110	37.1	56.1
72a	0.577	0.083	33.0	66.6
72b	0.678	0.156	33.7	54.2
73a	0.544	0.105	41.1	58.8
73b	0.592	0.094	35.1	52.4
81a	0.687	0.141	37.0	49.3
81b	0.594	0.083	41.1	54.2
82a	0.549	0.071	26.6	59.6
82b	0.694	0.193	25.1	67.5
83a	0.442	0.036	54.7	64.4
83b	0.594	0.081	35.2	65.1
91a	0.569	0.072	42.3	67.1
91b	0.570	0.055	38.3	62.2
92a	0.646	0.138	23.1	44.3
92b	0.616	0.100	24.2	55.7
93a	0.616	0.102	27.4	66.2
93b	0.691	0.189	36.9	63.3

Tab. 2 shows the mean \pm standard deviation of the investigated parameters calculated for control samples and the mean for all the area, and the ratio between the exposed samples and the control ones. The control samples showed a general optimal vital status, thus it can be excluded the effects of the transplant process on lichen physiology.

The photosynthetic performance expressed as F_v/F_m and PI_{ABS} of the exposed samples, showed a general decrease respect to the control samples (16% and 50%, respectively), while the antioxidant activity (ARA) and the damage to cell membrane (Mb) increased by 11% and 38 % respectively.

Table 2: Mean value \pm standard deviation of investigated physiological parameters in control and exposed samples and the rate Exposed on Control

	Ctrl	Exposed	Exposed/Control
F _V /F _M	0.710 \pm 0.05	0.60 \pm 0.06	0.84
PI _{ABS}	0.221 \pm 0.09	0.11 \pm 0.05	0.50
Mb%	26.3 \pm 4.2	36.2 \pm 8.7	1.38
ARA%	56.2 \pm 5.8	62.6 \pm 6.8	1.11

Figures 2-5(A-B) show the boxplots of the investigated parameters sorted by centrality level, with and without the disaggregation of Pedestrian area. Comparison between groups showed significant differences only between the Centre and the peripheral area ($p < 0.05$, marked with *) in photosynthetic activity, with the former being significantly lower than the latter. By disaggregating the Pedestrian area, no differences were outlined. However, a clear trend is detectable with the central area that shows a general lower vitality performance for all parameters, that increases with increasing distance from the city centre.

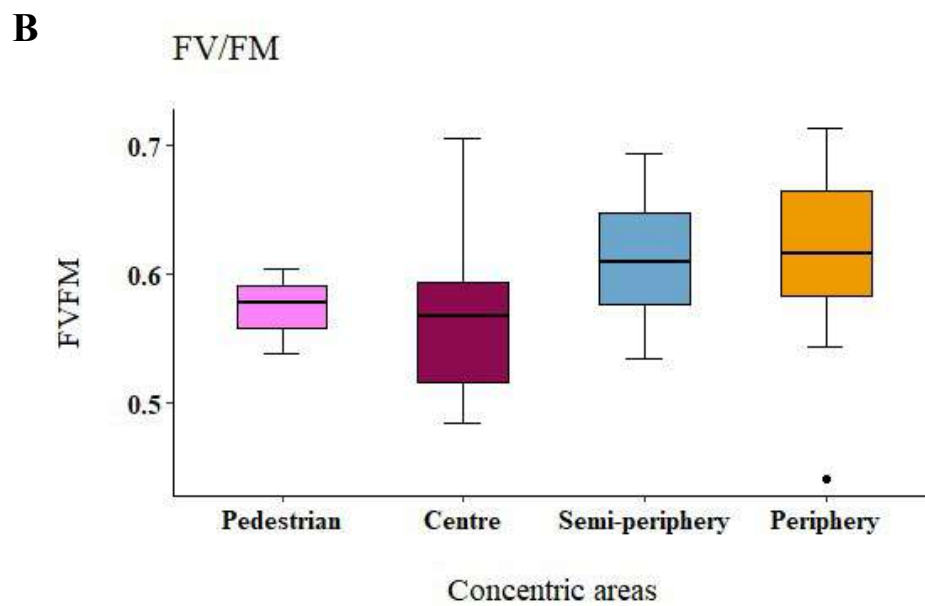
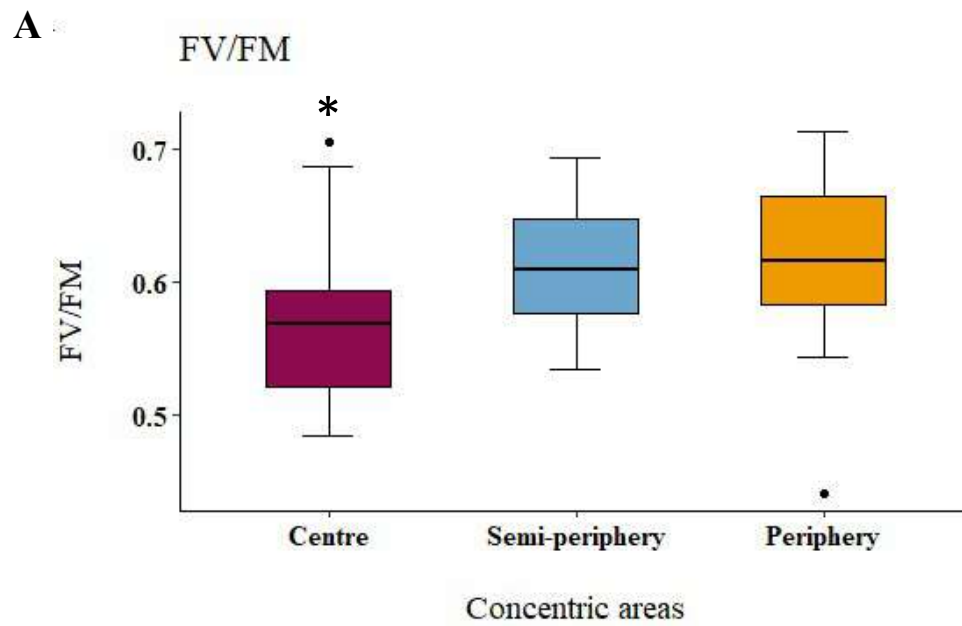


Figure 2: A) F_V/F_M elaborated for the 3 main areas and **B)** Disaggregating also for Pedestrian area. The * indicates significant difference

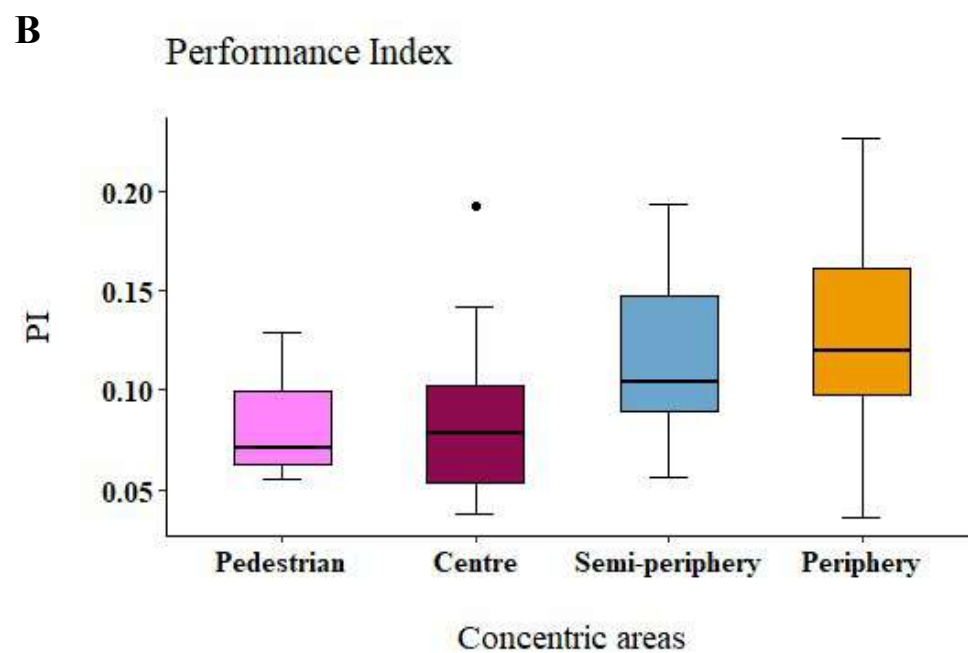
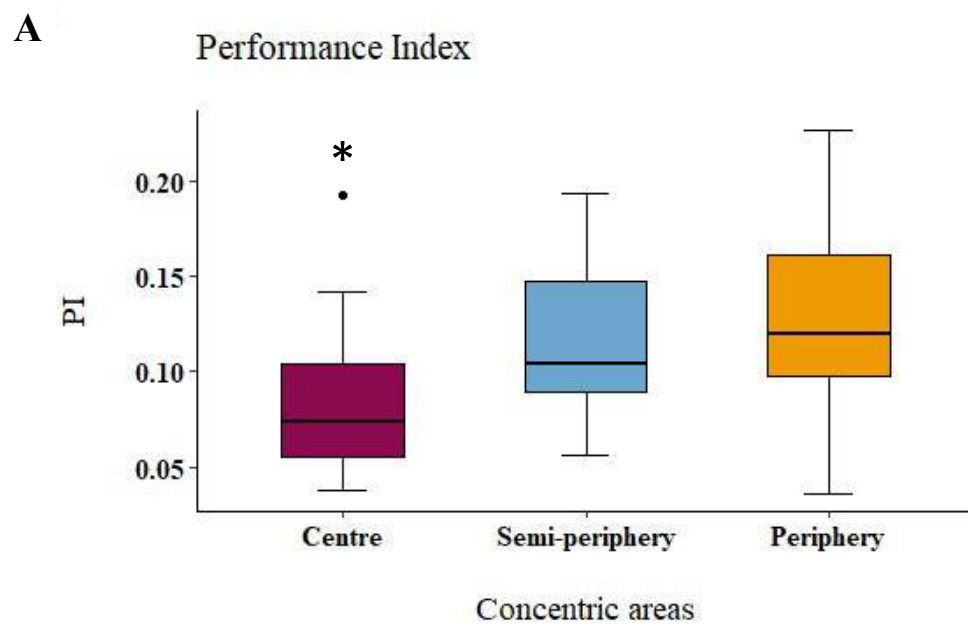


Figure 3: A) PI_{ABS} elaborated for the 3 main areas and B) Disaggregating also for Pedestrian area. The * indicates significant difference

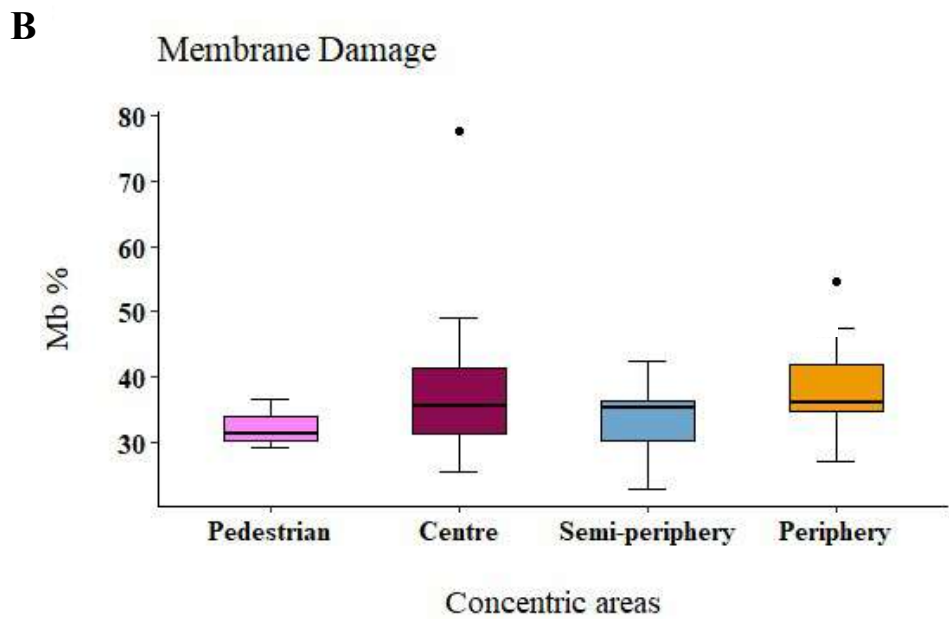
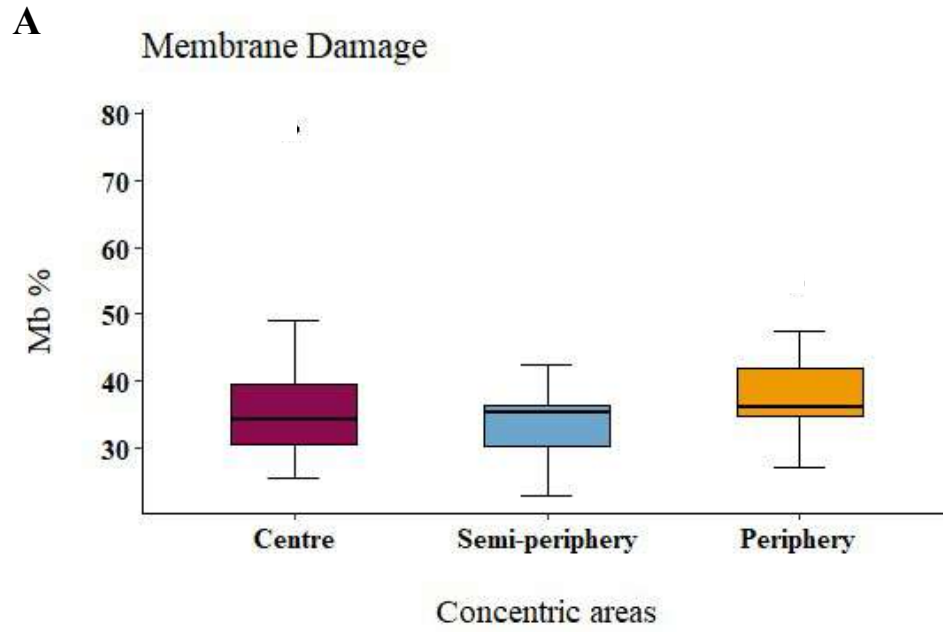


Figure 4: Boxplot of Membrane damage (%) sorted for concentric areas. **A)** Pedestrian area is included in the central one and **B)** Pedestrian area is considered separately

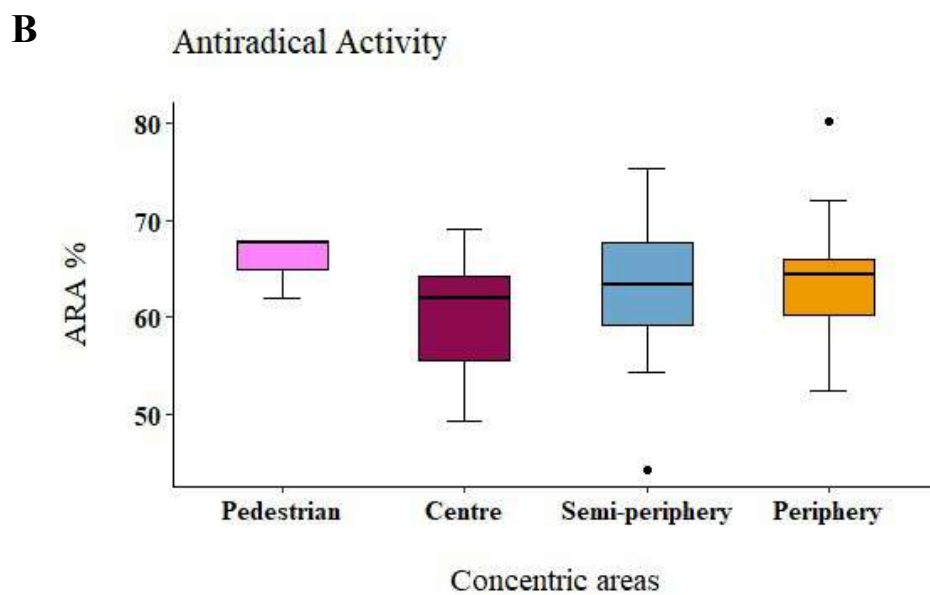
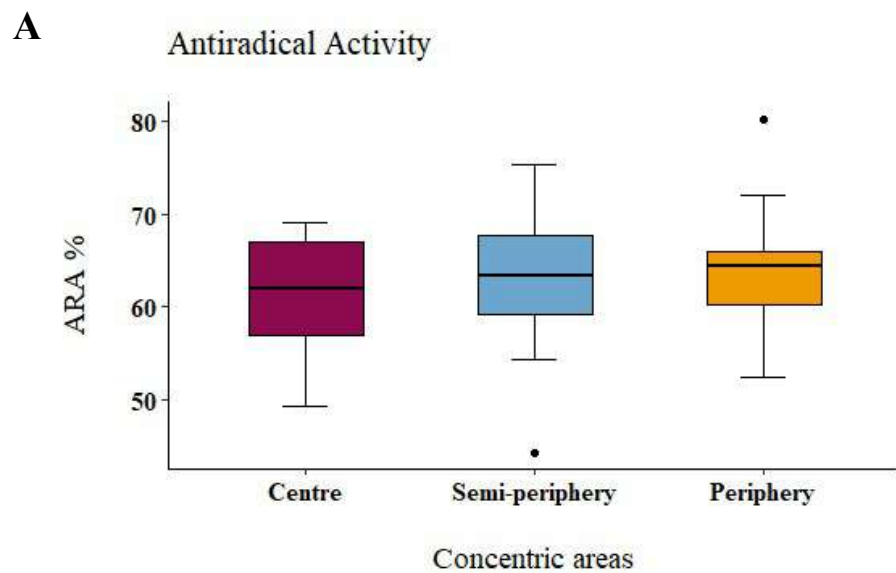


Figure 5: Boxplot of Antiradical activity (%) sorted for concentric areas. **A)** Pedestrian area is included in the central one and **B)** Pedestrian areas is considered separately.

JIP Test

The O-J-I-P transient of control and exposed sample is reported in Fig 6. In 6A the average of the exposed samples is reported with the transient of controls. An evident but not severe flattening is observable for exposed samples, however the typical shape of the curve is maintained, while controls show the classical shape and intensity of unstressed plants (Strasser et al., 2004). In 6B, values are reported sorted for concentric areas, disaggregated for the Pedestrian area.

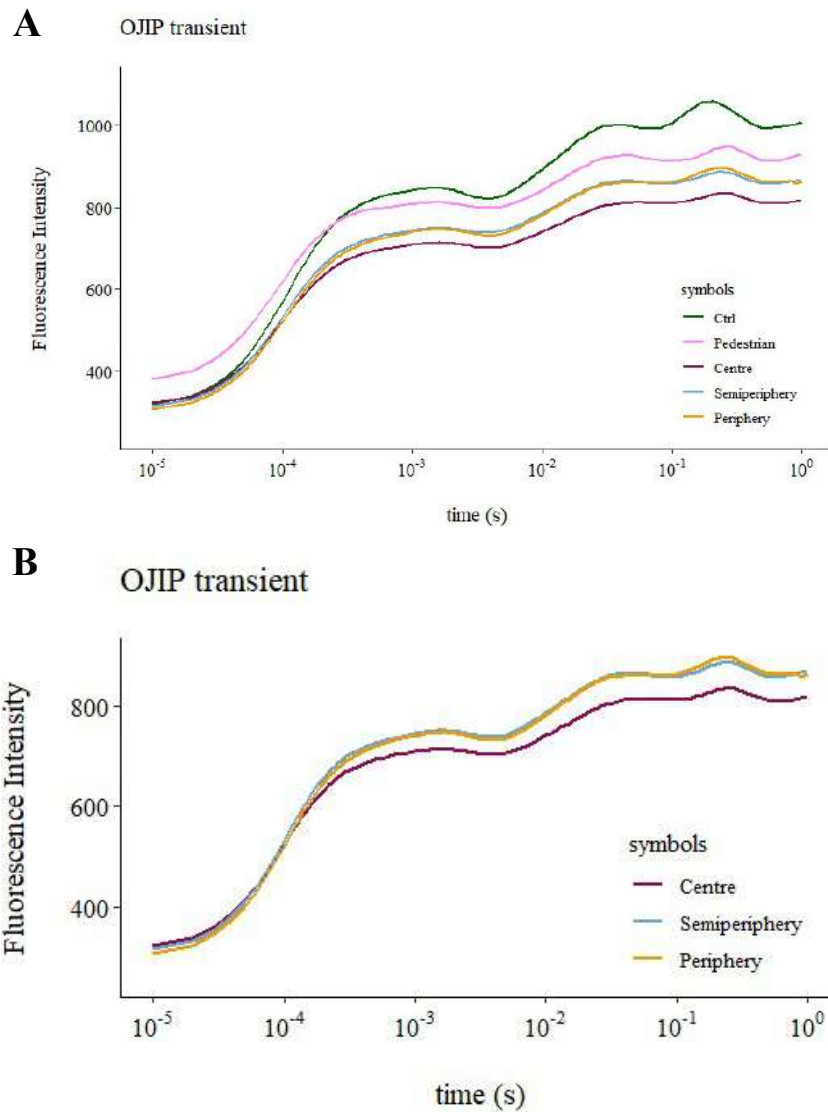


Figure 6: OJIP transient. **A)** Pedestrian area is included in the central one and **B)** Pedestrian area is considered separately

Correlation between trace elements and physiological parameters was performed with the Pearson coefficient and is reported in Tab. 3. No significant correlations emerged, except for a weak correlation between the content of Cu and cell membrane damage (0.28, $p < 0.05$).

Table.3: Pearson matrix correlation between trace element content and physiological parameter values.
*= $p < 0.05$

	Cr	Cu	Fe	Pb	Sb	Cl
F_V/F_M	-0.17	-0.12	-0.23	0.02	-0.08	-0.11
PI_{ABS}	-0.20	-0.18	-0.26	-0.04	-0.10	-0.18
MD	0.22	0.28*	0.22	-0.11	0.14	0.17
ARA	-0.05	0.07	-0.10	-0.22	-0.17	-0.13

4. Discussion

The results show an overall good vital status of the lichen samples after the exposure in Milan, indicating that the atmospheric contamination did not affect significantly their physiology. This result is also confirmed by the correlation matrix between the bioaccumulation of trace elements and the physiological parameters, since no significant correlation emerged with the exception of weak one between Cu and cell membrane integrity (0.28, $p < 0.05$). Copper is a fundamental component of many enzymes, and its uptake into the cell is mainly active (Hauck et al., 2009), but when supplied in excess, the uptake turns into a passive dynamic, even reaching toxic levels for the organism. Laboratory experiments demonstrated that Cu induces a strong plasmolysis measurable as the loss of K^+ ions proportionally to the Cu supply in the lichens *Ramalina fastigiata*, *Usnea* spp (Branquinho et al., 1997) and *Umbilicaria muhlenbergii* (Puckett, 1976). The impairment caused to cell membrane was reported also by Guschina and Harwood (2006) who showed that lipid metabolism of lichen photobionts may be affected by this element when supplied in excess

to the organism. Our results, thus, are in line with these observations and are in agreement with other field studies e.g. Carreras and Pignata (2002), that reported a correlation between Cu and the content of hydroperoxide conjugate dienes in lichen samples, indicating a strong oxidative stress caused by this element.

The lack of physiological effects caused by metal accumulation could be surprisingly since the damage induced by trace elements on lichen vital mechanisms is well known (see Backor and Loppi, 2009), but on the other hand, it could be explained by the fact that in urban areas trace elements are mainly deposited on the thallus surface in form of dust and only a little portion is absorbed to the thallus (Vannini et al., 2017; Vannini et al., 2019), where they can effectively cause damage to vital mechanisms (Branquinho et al., 1997). Our results are in line with other field studies. For example, Paoli et al. (2011) transplanted *Evernia prunastri* in the surrounding of an industrial area and detected no or few relations between trace element accumulation in lichen thalli and the physiological performance. Similarly, Sujetovienė et al. (2019) noticed that physiological impairment occurring in lichen samples transplanted nearby a landfill was not related to the metal accumulation into the thalli.

A plausible explanation for the physiological impairments can be found in the synergic activity of typical urban gaseous pollutants as NO_x, SO₂ and NH₃. SO₂ was considered the most affecting compound for lichen physiology, as reported by many authors (Nimis et al., 1991; Nash III, 1973) but after its strong decrease in the last decades, NO_x represent nowadays the most relevant pollutant in urban areas (EEA, 2015). It is formed by combustion reactions of vehicles, house heating and industrial plants.

At physiological level, it is widely reported the role of oxidized forms of N as NO and NO_x compounds: decreased of ergosterol content and increased permeability of the cell membrane were registered as consequence of acute and chronic exposure to N compounds (Gaio Oliveira 2005, Munzi et al., 2009) and the peroxidation of membranes was further observed as a consequence of pollution exposure (Godinho et al., 2004). NO_x act also as acidifier, increasing metal solubility in wet depositions and, thus, their availability to be absorbed by the organism.

However, the role of NO₂ in lichen physiology damage is still debated, since some authors reported a decrease in lichen vitality nearby NO₂ hotspots (Malaspina et al., 2018; Freitas et al., 2011; Piccotto et al., 2001), while other studies report an enhanced net photosynthesis where NO₂ concentrations were higher (von Arb et al., 1990; Sujetoviene and Sliumpaitė, 2013). On the other hand, the solely action of NO₂ fumigated on lichens showed to be dangerous for physiological mechanisms only at high and very high

concentrations (4 and 8 ppm), far above the values found in very polluted areas (Nahs, 1976). Piccotto et al. (2011) suggested a synergic effect of gaseous pollutants and the mesoclimate of urban areas, that is characterized by dryness due to dust deposition on substrata, and revamping the so called “draught hypothesis” advanced by Rydzak (1969). This is a reliable explanation for the urban area of Milan, also because of the scarcity of precipitations during the exposure period (data not shown).

Ammonia is the most present alkaline gas in the atmosphere and in urban areas (Decina et al., 2019). It is produced mainly by agricultural activities but urban sources are catalytic converter of diesel-engine vehicles (Sutton et al., 2000). The wind transportation of this gas, makes it very diffused and evenly distributed in many environments. Many studies in urban and rural areas report that this compound is responsible of the eutrophication of tree barks, favoring the colonisation nitrophilous species. The main releases of this gas in Lombardy region come from agricultural activities, however, the Milan urban area is surrounded by an agricultural belt that is an effective source of NH_3 . Thus, it can be suggested a synergic action of metals and gaseous pollutants.

In this scenario, the impairment in the physiology of *E. prunastri* is in line with the theory. This species in fact, is an acidophytic one that lives in N-poor environment, and has developed efficient mechanisms to uptake N for nourishment, while its shrubby morphology and high surface/volume ratio allow to accumulate more N than a tolerant species as *Xanthoria parietina* (Munzi et al., 2013). This results in accumulation of N to toxic quantities for the organism.

In the last 20 years the concentration of NO_x in Milan dropped significantly from an average of $260 \mu\text{g}/\text{m}^3$ in early 2000 to an average of $160 \mu\text{g}/\text{m}^3$ in the most recent years (ARPA Lombardia, 2020). However, these values, even though encouraging, are still far from being hospitable for *E. prunastri*: Davies et al., (2007) reported for the London city the occurrence of *E. prunastri* in areas where values of this compound are not higher than 50-70 $\mu\text{g}/\text{m}^3$. The fact that samples were, however, alive and vital at the end of the campaign suggests that Milan urban area could be not suitable for an acidophytic new colonisation, but the global condition are not so severe to induce a rapid death of the organisms.

In European urban areas where a new colonisation is occurring, the vegetation is characterized by nitrophilous species, typical of the *Xanthorion* alliance such as *Physcia adscendens* and *Xanthoria parietina* (Davies et al., 2007). Moreover, according to the “drought hypothesis”, the deposition of dust enhances the dryness of the substrata, favouring more tolerant and nitrophilous species rather than less tolerant as *Evernia prunastri* which prefers humid environments (Loppi and De Dominicis, 1996).

5. Conclusions

Samples of *Evernia prunastri* exposed in Milan showed physiological impairments in all the investigated parameters even though not so severe to induce the devitalization of samples. Rather than TE contamination, is the NO_x elevated presence that prevents the normal vital status of less tolerant species as *Evernia prunastri*, thus, even the air quality is greatly ameliorated during the past decades, the environmental conditions are not suitable for a new colonisation of less tolerant lichen species.

Section 2

Environmental Justice and Perception of air pollution

This section explores the interaction between air quality and human life. Firstly, the environmental disparities will be investigated considering the socio-economic status of population and the degree of air pollution experienced. In the second chapter, the perception of air pollution and the associated risk will be investigate through the administration of a questionnaire to the citizens.

Chapter 1

Air quality disparities at urban scale: the contribution of lichen biomonitoring

1. Introduction

The uneven distribution of environmental hazards over the population is the core of a new and growing discipline called “Environmental Justice”. Born as “Environmental Racism” in the USA (Brulle and Pillow, 2006), this discipline aimed to investigate the ethnic composition of the territories most affected by environmental degradation and pollution, but soon it turned in “Environmental Justice” when it was clear that not only ethnic reasons, but also socio-economic and demographic characteristics could lead to a disparity in the burden of environmental degradation (Heiman, 1996). Even though the USA in 1994 and Europe in 1998 officialized the right to Environmental Justice (Presidential Executive order 12898, 1994; UNECE, 1998), i.e., the right of everyone to live in a clean environment regardless of ethnic belonging or economic status, the disproportion of environmental hazards is still evident worldwide. Since a scanty environmental quality is also the cause of numerous health injuries, as mentioned in the previous chapters, it can be concluded that the most disadvantaged groups experience the so called “triple jeopardy” (Jerrett, 2001): poor environment, poor economic status and poor health. Each of these “poorness” enhances and attracts the others (Martins et al., 2004). For example, the low socio economic status leads to live in an economic neighborhood, often characterized by crowding, scarcity of green areas and more industrial activities (Wustemann et al., 2017), that influences greatly both life quality and health status (Grineski, 2007). The poor health status, in turn, is often an obstacle for the improving of life quality. A consequent polarization of the society, with healthier population that chose for better environment, better life quality and thus, achieve a better health status and disadvantaged groups that live in worse hazards, is thus expected and recognized in many realities (Tonne et al., 2018; Lanier et al., 2019). Due to its multidisciplinary nature, this topic receive contributes by several scientific fields as environmental sciences, geography, sociology, urban studies and economy, among many others, while due to its recent creation a methodological consensus for its study is not achieved yet. The environmental disparities, in facts, are differently declined on the basis of the situation in exam: culture, history, urban organization as well as geography and the

spatial scale to which the phenomenon is studied greatly influence the parameters to consider (Germani et al., 2014; Banzhaf and Walsh, 2006). For example, while in the USA the ethnic composition is a determinant driving force for a disproportionate burden of environmental hazards as pollutant plants (Bullard, 2001), in Europe the racial question provides a very little contribute, while it is the socio-economic status that mainly leads to different environmental quality. In particular, Laurent (2011Bae) in his review showed that in European countries, the Environmental Justice can be declined as “the poorest population experiences the worse air quality”.

To assess Environmental Justice, therefore, it is necessary to match the socio economic characteristics with the air quality data. At regional or national scale, both the air quality and socio-economic data represent macro areas and thus a fine grain of detail is not needed. At the opposite, at urban scale, a high spatial resolution distribution of air contamination as well as a specific characterization of socio economic status of neighborhoods or districts is required.

However, at so small spatial scale, data as income are not available for privacy reasons, and also air pollution distribution is not finely determined. The local monitoring devices in facts, are generally few in territories due to their high costs of installation and maintenance, thus the air pollution model is based on few monitoring points, carrying evident smoothing of the pollution hotspots (Martins et al., 2004; Li et al., 2018). Many authors use the proximity to a given emission source as surrogate for human exposure to air pollution (Jerrett et al., 2005), however this method assumes the equally propagation of air pollutants from the emission source, ignoring the numerous meteorological and physical factors that affect air dispersion (Maantay et al., 2009). Other authors model themselves air pollution data from emission source or use geo-statistics methods as the land use regression (LUR) or geographically weighted regression (Briggs et al., 2000; Gilbert and Chakraborty, 2011). However, as declared by most authors, to improve air monitoring data is a crucial point for a better evaluation of air pollution dispersion.

Lichen biomonitoring, as proved in the previous chapter, may greatly contribute to provide a high spatial resolution map of air contamination and, through the source apportionment analysis, a further study can be carried considering the effective main emission sources. Ocelli et al. (2016) were the first to show the effectiveness of this method for a large conurbation highlighting that pollution hotspots and economic disparities matched with air quality measures. Contardo et al. (2018) proved the effectiveness of the approach also for monitoring environmental justice in the area around a municipal waste incinerator.

In this chapter we explore the use of lichen biomonitoring for environmental justice purposes at urban scale: the data collected in the previous chapter about trace element bioaccumulation, the source apportionment and the physiological response of lichens will be here matched with the socio-economic status of Milan population.

2. Materials and Methods

2.1 Socio-economic status and spatial units

To assess the socio economic status of Milan population and to assess if the more economic disadvantaged groups experience a lower air quality, an index of socio-economic deprivation (SDI) was elaborated on the basis of the last census campaign (ISTAT, 2011). Following Caranci et al. (2016), 5 items were explored as proxy of socio-economic deprivation: schooling, employment, crowding, family composition and house rent. Through 14 parameters, the rate of low schooling, unemployment, family with 5 or more components, house rent, and the crowding were calculated. The sum of these indicators resulted in the socio economic deprivation index. The crowding was firstly normalized in 0-1 scale. In Tab 1 is reported the list of selected parameters and the elaboration of the index.

The index was elaborated for the smallest spatial area for which data are available: the census unit. These units are small patches not homogeneous in size, shape nor demographic characteristics but cover the entire territory with no overlap and are the local administration units for census and territory politics activities. In Milan there are 6,085 census units from which were subtracted those with no inhabitants or that missing values for selected parameters.

Table 1: Economic and demographic parameters selected for the elaboration of the socio economic index

Area	Indicator	parameters and elaboration
Education	low schooling	rate of illiterate+literate+ primary and secondary school on the population with 6 and more years
Exclusion from employment	unemployment	unemployed on total work force
Income housing	owning houses	rent of families that stay in rent on the total families
Family composition	Numerosity of families	rate of families with 5 members+ families with 6 or more members on the total of families
overpopulation	Density	total population on the area of census unit

2.2 Environmental Justice assessment

The environmental justice was assessed by comparing the air pollution data provided by the outcome of lichen biomonitoring and the socio-economic status elaborated for census units.

For the overall situation, the Contamination Index and the Socio economic deprivation index were compared. To each census units was assigned a value of CI calculated as the mean of the pixel values inside each boundary, as also described in the previous chapter. Then, the CI values were sorted into deciles and the mean of each decile was calculated. The mean SDI of corresponding census units was elaborated for the relative deciles and a linear correlation was searched for.

In order to explore if the proximity to the city centre may be a reliable parameter to predict the socio-economic status, a comparison between CI and SDI elaborated for concentric areas was performed, using the mean value of both indices. The Pedestrian area is included in the central area because of the scarcity of inhabited census units

2.3 The contribute of the Source Apportionment Analysis

The lichen biomonitoring data elaborated by PMF highlighted the role of 3 principal emission sources in the study area: roads, railways and industrial activity. These elements are, thus, considered relevant for the search of environmental inequalities. In particular, a proximity-based analysis was conducted, hypothesizing that closer the emission source, higher the socio economic deprivation. The minimum distances between the census units and each emission source was calculated by the QGIS software considering the central point of each census unit. For the first factor (F1), that relies on major roads and railways, the distance from the census units to the emission sources were sorted into deciles and the mean for each decile was calculated also for the corresponding socio-economic deprivation. The correlation between distance and SDI was investigated using the Pearson coefficient. Since the distance from roads and railways can assume different meaning in different situations, the elaboration of the proximity analysis was carried also disaggregating for the concentric areas.

For the second factor (F2), a different approach was used. Since a clustered distribution in the northern part of the city, one of the most industrialized area, was evident, only the census units most interested by this factor were included in elaboration of environmental justice assessment. The designed source, the glasswork factory, was taken as the central point from which 4 different distance classes were calculated: 0-250 m, 250-500 m, 500-

1000 m, 1000-2000 m. For each class, the average SDI was calculated. The overall SDI of the “Industrial area”, the area interested by Factor 2 was also calculated.

2.4 Vulnerability index

Beyond the correlation between environmental and economic parameters, an interesting approach is to combine in a more comprehensive index those factors that can determine a vulnerability condition for a given area, in order to highlight those areas that experience the overall worse condition, that are also those to which specific socio-environmental politics should be addressed. The elaboration of a “Vulnerability Index” was firstly proposed by Lanier et al. (2019), who considered three parameters to analyze separately: environmental quality, socio economic deprivation and the degree of population susceptibility, i.e., the rate of old and young people and the crowding, considered of epidemiological relevance.

In this work we follow the approach of Lanier et al. (2019) adding a fourth parameter that relies on neighborhood quality. To each census units was assigned a cumulative score as the sum of single scores of the considered parameters, the following section describes the elaboration.

2.4.1 Score of the environmental quality (S_{ENV})

The data provided by lichen biomonitoring were combined in the score of environmental quality (S_{ENV}). The F_vF_M , PI_{ABS} , ARA and M_b were calculated for each census units starting from the interpolated map (as made for CI). Then, all data were ranked by increasing order for the worse performance, for example, higher the CI, higher the rank, higher the PI, lower the rank, for each census unit. Then, the sum of each rank provided the S_{ENV} for the given census unit. The values of the final scores were then sorted into sextiles in order to obtain more classes of environmental conditions than the other parameters (that will be sorted into quartiles).

2.4.2 Score of the population susceptibility (S_{POP})

The rate of young (<15 years) and old (>65 years) people, combined with the population density are considered for epidemiological relevance since the susceptibility of some age categories and the effect of crowded living (Annavarapu and Kathi, 2016) Thus, a score of population susceptibility was successfully used to pinpoint more vulnerable categories of

population (Lanier et al., 2019). These 3 parameters were ranked by increasing order, assigning, thus, the highest values to census units with more old and young people and more crowded. The sum of the ranks provided the population score (S_{POP}). The values of the scores were then sorted into quartiles.

2.4.3 Score of the socio-economic deprivation (S_{DEP})

The socio economic deprivation previously elaborated for each census unit was ranked assigning the higher values to the most deprived areas, to provide the score of socio economic deprivation (S_{DEP}). The values were then sorted into quartiles.

2.4.4 Score of the neighborhood quality (S_{URB})

The physical quality and organization of the neighborhood in which one can live can be a reliable indicator of the life quality (Mears et al., 2020). The parameters considered for the elaboration of the score rely on organization of neighborhood and are: the average age of buildings, the average conservation status of buildings, the percentage of industrial land use, the distance from the green areas, and the distance from the first public transport for each census units. The values were ranked and sum in order to assign to the worse situation the highest value. The score values were then sorted into quartiles.

2.4.5 Vulnerability index

The vulnerability index was calculated as the multiplication of the quantiles of each parameters in each census units. Thus, the minimum possible value was 1 (1 x1x1x1) and the maximum was 384 (6x4x4x4). The vulnerability index was then sorted into 10 classes for a better visualization and interpretation.

Finally, since both the susceptible population and the neighborhood quality could affect the contamination, correlations was searched for between CI and these two scores.

3. Results

3.1 Overall Environmental Justice

A first result of the overall situation of Environmental Justice is reported in Fig 1 as the correlation between deciles of contamination and deciles of socio economic deprivation. The deciles of CI ranged from 1.91 (first deciles) and 3.15 (tenth deciles) while SDI values ranged from 0.80 to 0.95. No significant relation ($p>0.05$) links the deprivation to the contamination level, however a weak negative trend is detectable ($R=-0.38$), for which with the increasing of CI a decreasing of SDI is registered.

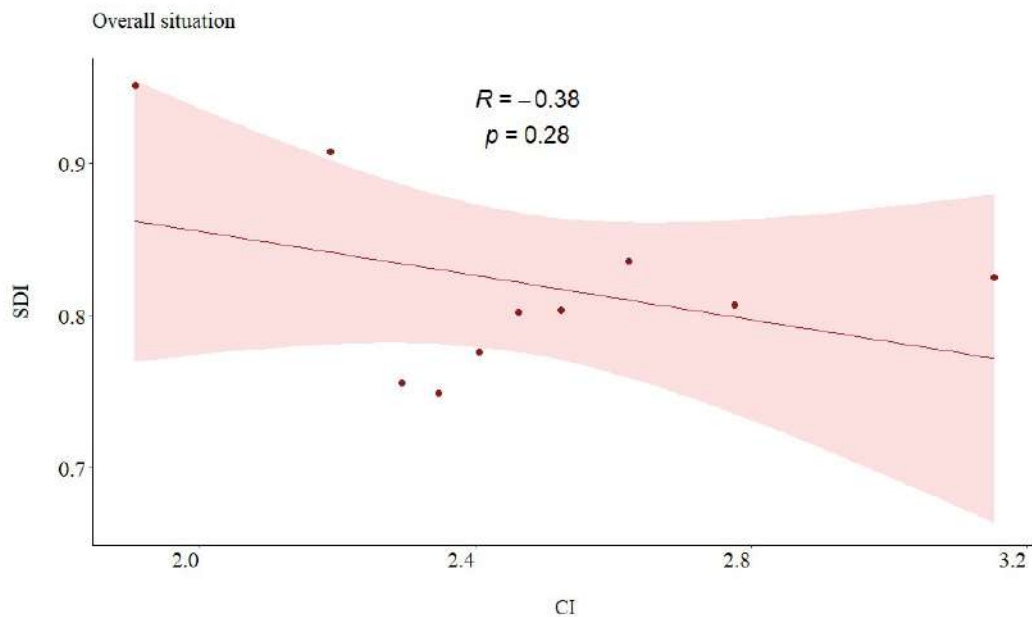


Figure 3: Relation between deciles of CI and SDI

The hypothesis that the socio economic deprivation depends on the proximity to the city center has been tested evaluating the SDI for each concentric areas (Tab. 2) and the associated CI is also reported. The SDI follows a decreasing trend from the peripheries to the central areas, at the opposite does the contamination index, for the exception of Pedestrian area. Even though the lack of significant differences between the concentric areas, the intrinsic significance of the trend suggests that Peripheries experience an overall better air quality than the Centre, that, in turn, is characterized by a better life quality (lower deprivation). However, the Pedestrian area shows both lower contamination (CI=1.97) than

the mean (CI=2.41) and lower deprivation (SDI=0.68) than the mean (SDI=0.82). The central area shows a SDI value (0.68) that is lower than the mean, but the highest CI values (CI=2.81), while the peripheral areas show the highest value of deprivation (SDI=0.96) with lower CI (CI=2.18) than the average. The Semi-peripheral area shows in general values similar to the mean both for SDI (0.85) and for CI (2.52).

Table 2: Mean of SDI and CI elaborated for the entire area and for concentric areas

AREA	Mean SDI	Mean CI
Milan	0.82	2.41
Pedestrian	0.68	1.97
Centre	0.68	2.81
Semi-periphery	0.85	2.52
Periphery	0.96	2.18

3.2 *The contribute of the Source Apportionment Analysis*

3.2.1 *Factor 1: Roads and railways*

The proximity to the roads and railways was tested to be a determinant factor for the socio economic deprivation status in the study area. In fig 3 is reported the relation between deciles of distances from the major roads and deciles of the SDI. A significant positive correlation ($R=0.89$, $p<0.005$) shows that increasing the distance, the socio economic deprivation increases linearly as well. The census units closest to major roads (the first deciles of distance) averagely are 11 m far and show the lowest SDI (0.77), while the farther census units are averagely 570 m far from roads and show the highest SDI (0.90). In general, the minimum distance between census units and the major roads is 0 m (census units that include major roads or that are tangent to them), while the maximum distance is ca 965 m. The mean distance is about 87 m, while the median is 0 m, indicating that half of census units includes or is tangent to major roads.

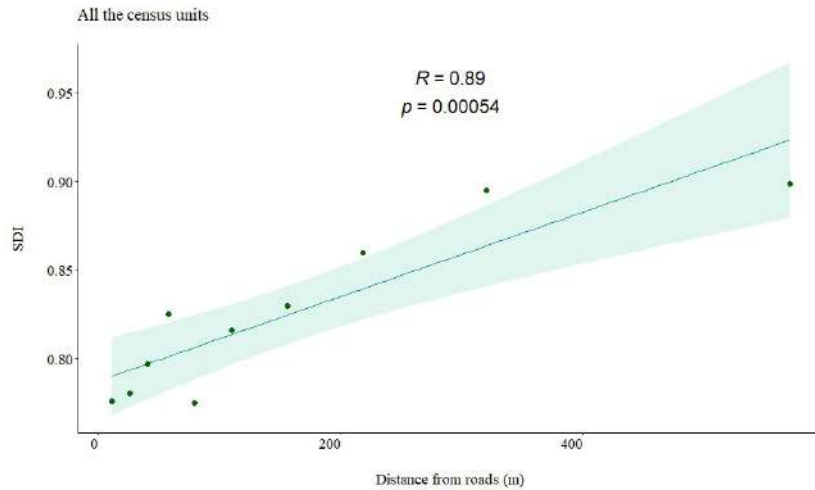


Figure 2: Linear relation between the CI and the distance from roads elaborated for all the area

An opposite situation is showed considering the distances from the railways (Fig 3): increasing the distance, the SDI linearly decrease ($R=-0.87$, $p<0.005$). The closest census units to the railways (the first deciles of distance) are 140 m far, while the farther are ca 2800 m far. The last deciles was not included in the elaboration because the associated SDI was considered not representative of the distance from the railways, thus, the last deciles was the 9th, with average distance from the railways of ca 1600 m. The mean distance is ca 860 m, the closest census unit is only 0.37 m far while in general, the farthest is ca 5500 m far. The median of the distance is ca 664 m, indicating that half of census units are more or less close to the railways. This result indicates that wealthier classes choose a to live quite far from the railways.

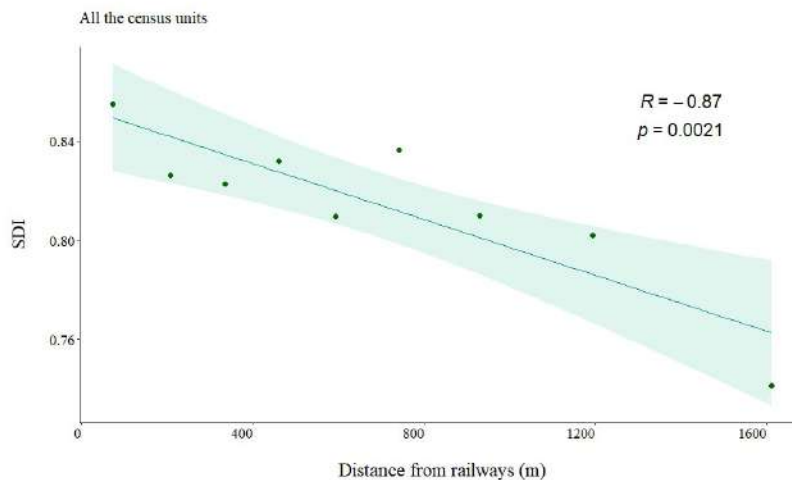


Figure 3: Linear relation between the CI and the distance from railways elaborated for the all area

3.2.1.1 Proximity analysis elaborated for concentric areas

The proximity analysis disaggregated for the concentric areas is here reported.

In the Pedestrian area SDI is not significantly related to the distance from the major roads ($R=-0.39$, $p>0.05$) (fig 4), even though the weak negative trend is in contrast with the overall situation. By sorting into deciles, the first 3 deciles were 0, in fact 109 census units on 354 total are tangent to the roads or include them, thus, only 7 classes were considered. The fourth decile is averagely 9 m far from the roads, while the tenth is ca 440 m. In general, the closest census unit is 0 m far, while the farthest is 607 m with a mean of 160 m and a median of 133 m.

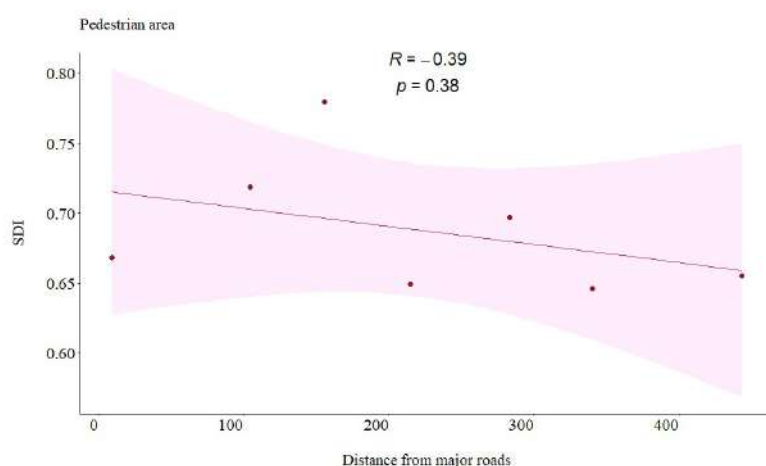


Figure 4: Linear relation between the CI and the distance from major roads in Pedestrian area

A similar situation is reported considering the railways (Fig 5): a weak and not significant negative trend links the distance from the railways and the socio economic deprivation status.

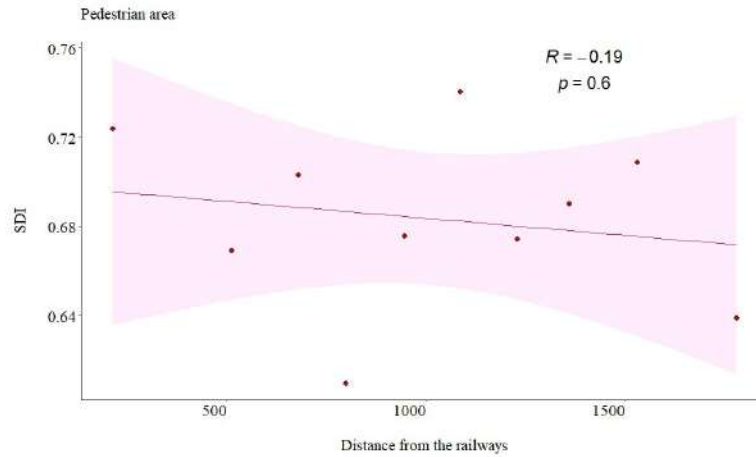


Figure 5: Linear relation between the CI and the distance from railways in Pedestrian area

The trend is, however, in line with the overall situation. The first decile is averagely 212 m far while the farthest deciles is ca 1700 m far, with a mean of ca 1000 m. The closest census unit is 0 m far while the farthest is ca 2000 m far, with a median of 1010 m.

In Fig. 6 it is reported the relation between the distance from major roads and SDI elaborated for the Central area. Only 3 distance classes were calculated, since 1235 census units over 1719 (total) include or are tangent to the major roads (distance = 0 m).

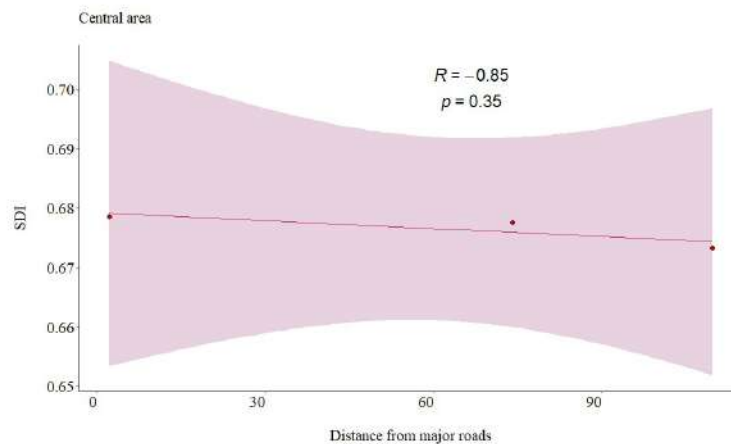


Figure 6: Linear relation between CI and distance from roads in Central area. Only 3 deciles were > 0

Sorting the non 0 distances into deciles (Fig 7), no relevant changes appear, confirming the non-relation between distances from roads and SDI. In general, the census units in this area are averagely 29 m far from roads, with a median of 0 m and a maximum distance of ca 523 m.

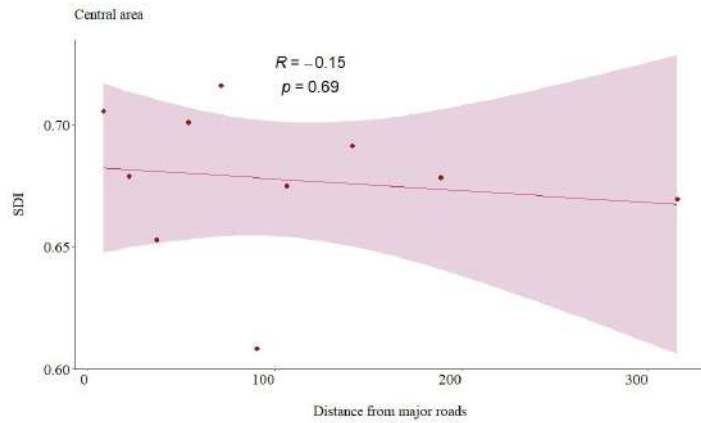


Figure 7: Linear relation between SDI and the deciles of distances from major roads in Central area.

The distance from the railways (Fig 8), instead, shows a negative and almost significant relation ($R=-0.58$, $p=0.0077$), indicating that higher is the distance and lower is the SDI. This trend is in line with the overall situation showed above. The first deciles is averagely 24 m far from railways while the farthest is ca 590 m far. Averagely, the census units in this area are 656 m far from railways, with a minimum of 0 m and a maximum of 2021 m and a median of 576 m.

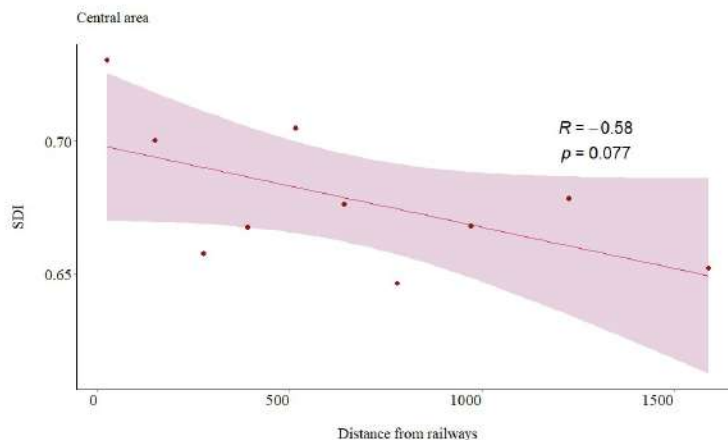


Figure 8: linear relation between SDI and the distance from railways in Central area

For the Semi-peripheral area, the relation between distances from the sources and the socio economic deprivation status is reported in Fig. 9. No significant trend is detectable, with a high dispersion of data. The closest census units are averagely 10 m far while the farthest ca 470 m far from roads. In general, the minimum distance is 0 m, the maximum distance is 1125 m with a mean of 128 m and a median of 82 m.

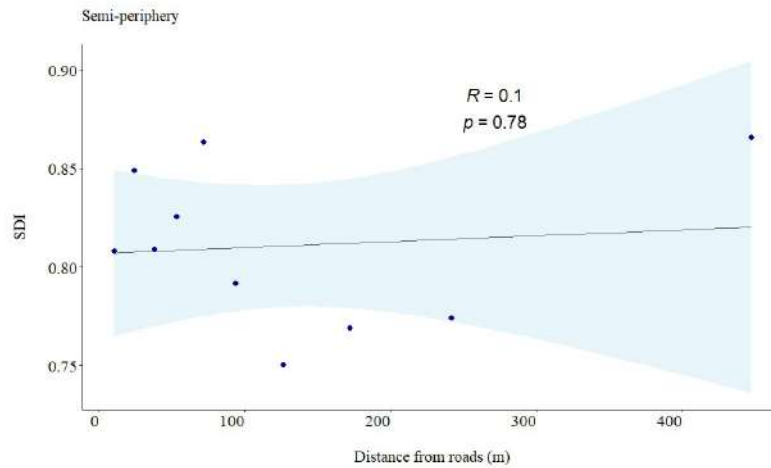


Figure 9: Linear relation between SDI and distance from major roads in Semi-Peripheral area

A nonlinear relation was used to assess the relation between the distance from the railways and the SDI (Fig. 10). Even though an inverted U shape trend is detectable, no significance emerged. The trend may suggest that between 500 m and ca 1200 m far the SDI is higher while it decreases for distances less than 500 m and higher than 1200 m. Averagely, the census units closest to the railways are 52 m far while the farthest 2386 m far. The minimum distance is, in general, 0 m while the maximum is ca 3500 m, the mean distance is ca 790 m and the median is ca 590 m.

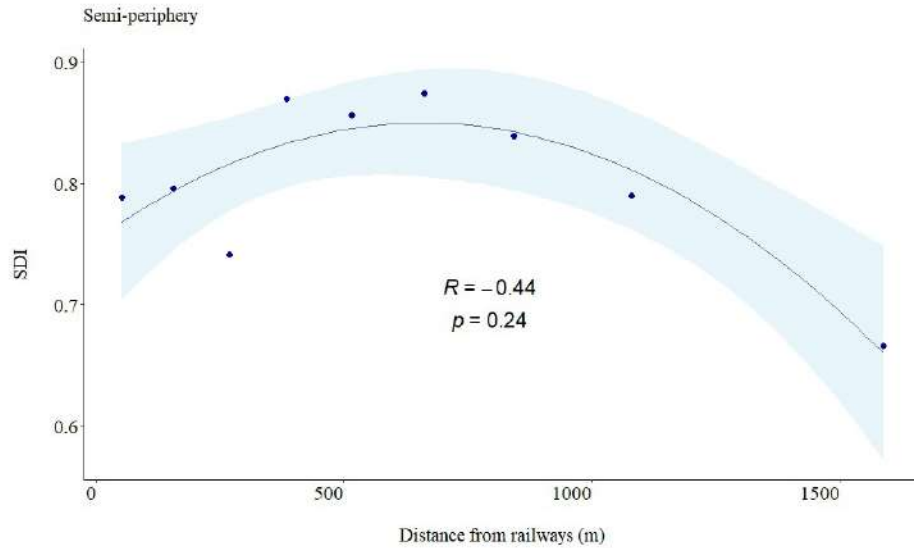


Figure 10: Nonlinear relation between SDI and the distance from the railways in Semi-peripheral area

Also Peripheral areas did not show any significance in the elaboration between distance from sources and SDI (Fig. 11, 12). In particular, the distance from the major roads show a weak negative trend ($R=-0.31$, $p>0.05$), in contrast with the overall situation but in line with the previous data aggregation. The closest census units are ca 20 m far while the farthest ca 720 m far. In general, the closest unit is 0 m far, while the farthest is 1247 m far with a mean value of 249 m and a median value of 185 m.

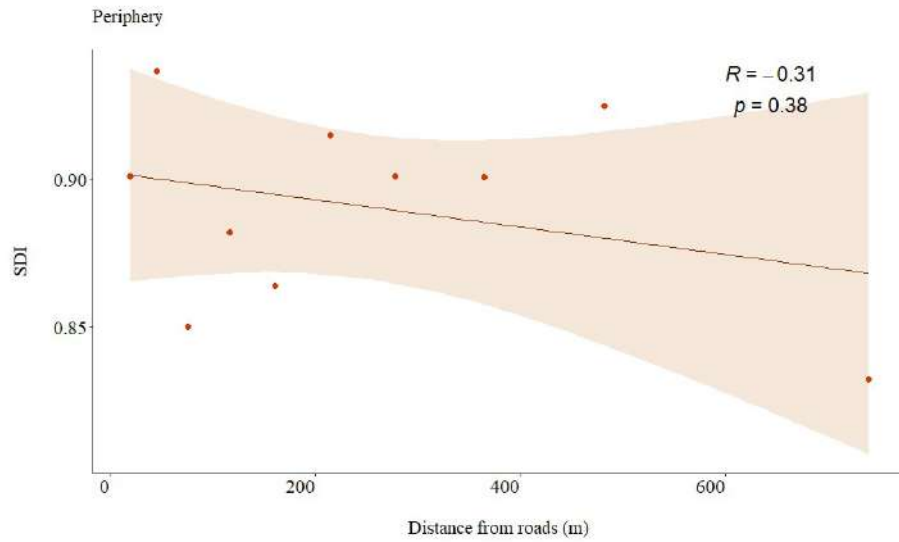


Figure 11: Linear relation between SDI and the distance from major roads in Peripheral area

The distance from railways appear with no trend at all ($R=0.1$, $p>0.05$), suggesting the total lack of relation between the source proximity and the socio economic deprivation in Peripheral areas (Fig. 12). In this area, the closest census units are averagely 68 m far while the farthest deciles has a mean of 3695 m. In general, the closest census unit is 0 m far while the farthest is 5550 m far with a mean of ca 1100 m and a median of ca 670 m.

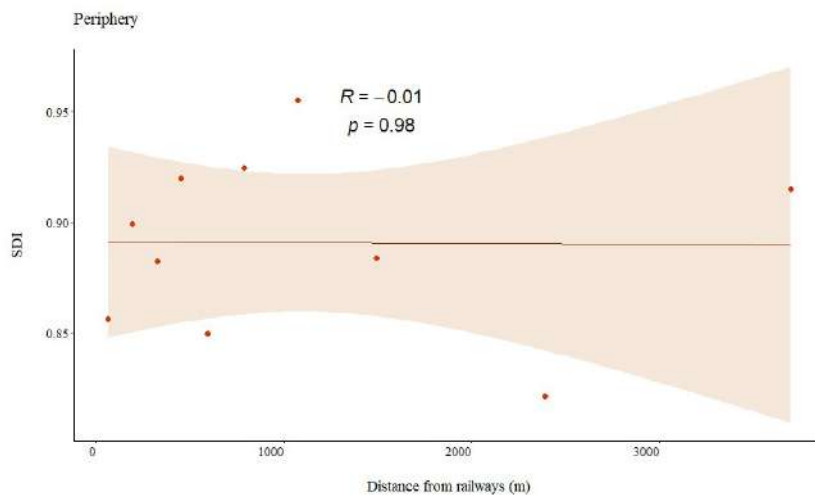


Figure 12: Linear relation between SDI and the distance from railways in Peripheral

In Tab. 3 are summarized the distances from major roads and railways for concentric areas. From the Central area (C) to the Periphery (P) the distances increases both from railways and major roads. The Pedestrian area, not surprisingly, show a general higher distance from emission sources than the Central area, being more similar to Periphery than to Central for mean values of distances.

Table 3: Summary of the distances between major roads and railways elaborated for the concentric areas. Value reported rely on minimum, maximum, mean and median distance

		PD (n=355)	C (n=1719)	SP (n=2291)	P (n=1962)
Roads	min	0	0	0	0
	max	607.5	523.4	1125.0	1247.6
	mean	158.0	29.1	82.3	249.9
	median	133.9	0	128.0	185.1
Railways	min	0	0	0	1.6
	max	2017.4	2021.4	3520.6	5558.7
	mean	1010.8	656.4	793.5	1108.7
	median	1010.1	576.5	592.1	676.8

3.2.2 Factor 2: Industrial activity

Through the spatial interpolation of Factor 2 (F2) of the source apportionment analysis, a clustered area emerged as the most interested by pollution of Pb and Sb. The highest value of the F2 was registered nearby a dismissed glasswork factory but all the area can be classified as one of the most industrialized of Milan. Since the clustering and the industrial-based nature of the pollution, the proximity analysis was carried considering just this area, as showed in Fig.13 (A-C). Extrapolating only the census units of this area, the average of SDI was calculated and, considering the glasswork factory as the most relevant point for contamination, different distance rays were selected, inside each one the SDI was elaborated.

The census units in the “industrial area” are 1569, thus about the 28% of the total census units. In Tab. 4 is reported the result of the data elaboration. The average SDI for the city is

0.82, while excluding the industrial area the SDI decreases to 0.81. The only industrial area has a SDI slightly higher than the mean (0.87), while some differences are detectable by sorting for different distance classes. The census units at maximum 250 m far from the glasswork shows a SDI of 0.84, that increases to 1.02 and 1.06 for census units between 250-500 m and 500-1000 m respectively. Over than 1 km the SDI starts to decrease (0.912) to reaches a mean value over 2 km of distance.

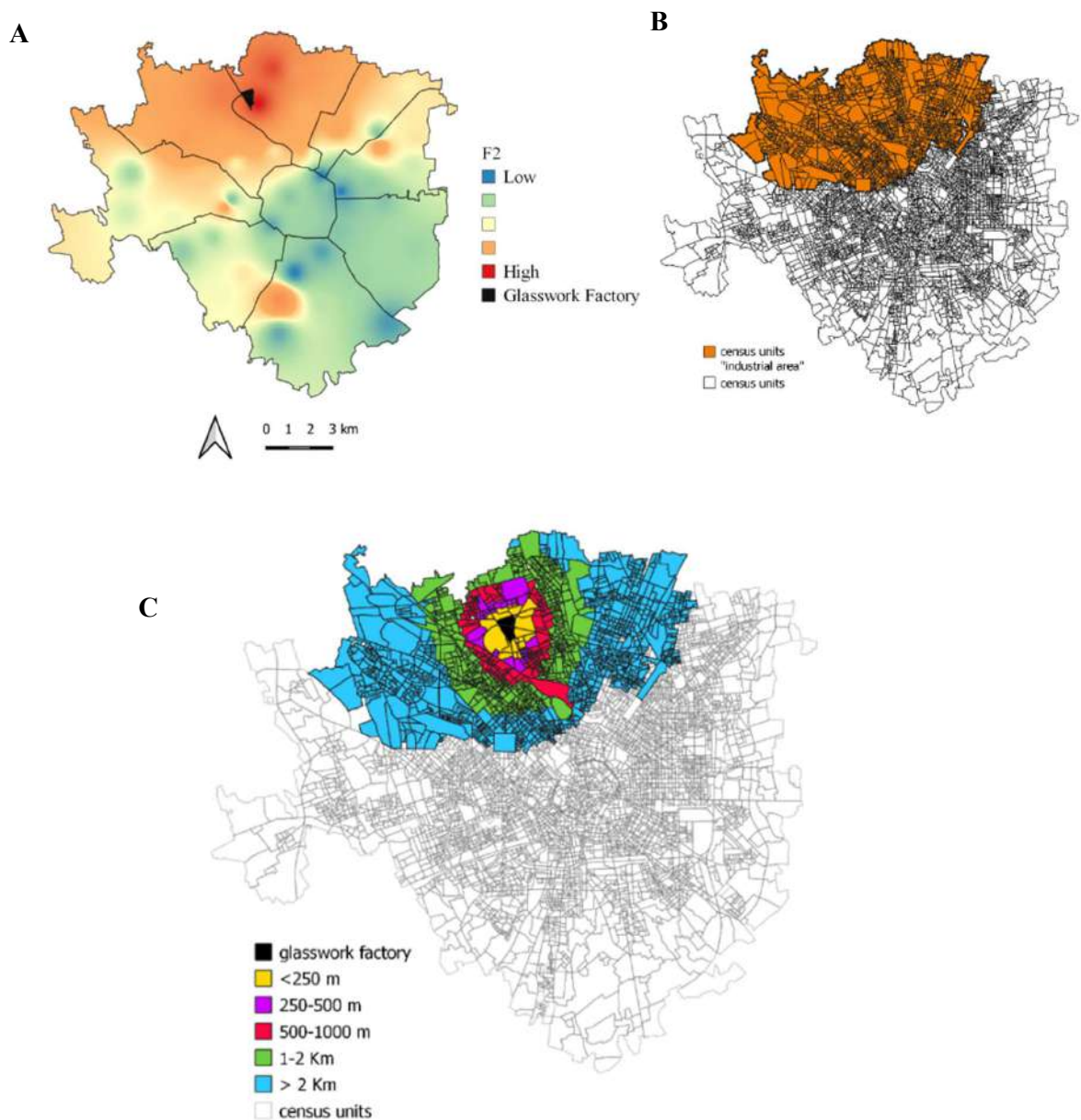


Figure 13: A) Spatial distribution of Factor 2, the position of the glasswork factory and in B) in orange is highlighted the “industrial area” and the census units considered for the proximity analysis. In C) the different distance classes are highlighted in different colors

Table 4: Values of SDI elaborated for all the study area, the industrial area, the study area subtracted of the industrial area and for each distance class

Area	SDI
Milan	0.82
Milan (w/o industrial area n=3981)	0.81
industrial area (total, n=1569)	0.87
<250 mt (n=27)	0.84
250-500 mt (n=36)	1.02
500m-1000m (n=140)	1.06
1-2 km (n=478)	0.91
> 2 km (n=888)	0.82

3.3 The Vulnerability Index

The results of the vulnerability index are reported for each elaborated score.

3.3.1 S_{ENV}

In Tab. 5 is reported the contribute of each sextile on the total for each concentric area calculated as number of census units for each sextile/total census units for the area * 100. All the census units were considered, also those with missing values for some parameters, thus the total of percentages is less than 100%. The area with the best environmental score is the Semi-periphery, where the 1st sextiles, indicating a best performance, is represented by the 19% of the census units, while the Pedestrian area is the one with the lowest rate of environmental score (4.3%). The weight of the worse environmental performance is highest in the Central area (29%) while the lowest rate is registered for Peripheral area (7%). However, it is noteworthy that the 63% of the census units in Peripheral area shows an environmental score between the 1st and the 3rd sextiles, that is the highest rate compared to the other areas: 29.3%, 31%, 38% for Pedestrian, Central and Semi-peripheral area respectively.

Table 4: Percentage of census units for each sextiles of SDI elaborated for concentric areas

sextiles S ENV	PED	C	SP	P
1 st	4.3 %	11 %	19 %	15 %
2 nd	13 %	8 %	9 %	28 %
3 rd	13 %	12 %	10 %	20 %
4 th	15 %	15 %	19 %	11 %
5 th	26 %	19 %	18 %	6 %
6 th	18 %	29 %	20 %	7 %

The Fig. 14. Shows the map of S_{ENV} elaborated for each census unit. The difference between areas is easy detectable at a glance, with the Central and Semi-peripheral areas that show a more intense presence of highest sextiles. Excepting some Peripheral census units, the biological monitoring highlight these areas as the most polluted.

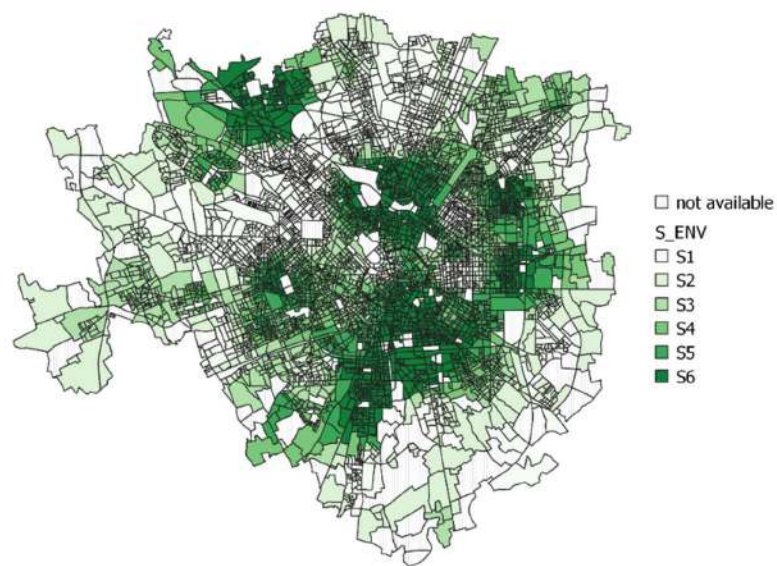


Figure 14: Distribution map of the sextiles of S_{ENV} in the study area

3.3.2 S_{DEP}

In Tab. 6 is reported the contribution of each quartiles of deprivation in each concentric area. The 1st quartiles, i.e., the lowest deprivation, is highest in Pedestrian area, where 36% of census units are really low deprived. The lowest percentage is reported in Peripheral areas, where just the 19% of the census units is low deprived. At the opposite, the Pedestrian area has only 6% of census units considered as deprived, while in the Peripheral area the percentage rises until 32. The sum of the 1st and the 2nd quartiles shows that 65% of the census units in the Pedestrian area are wealthy, while the lowest percentage is 38%, registered in the Peripheral area. While for the Centre, Semi-periphery and Periphery the percentages remain quite balanced in all the quartiles, a evident disproportion is present in the Pedestrian area.

Table 6: Percentage of census units for each quartiles of S_{DEP} in each concentric area

S_{DEP}	PD	C	SP	P
1 st	36 %	29 %	21 %	19 %
2 nd	27 %	28 %	22 %	19 %
3 rd	19 %	23 %	26 %	22 %
4 th	6 %	14 %	26 %	32 %

The Fig. 15 reports the distribution of S_{DEP} elaborated for each census unit. The pattern is slightly visible, however, the peripheries clearly show a higher proportion of deprived census units.

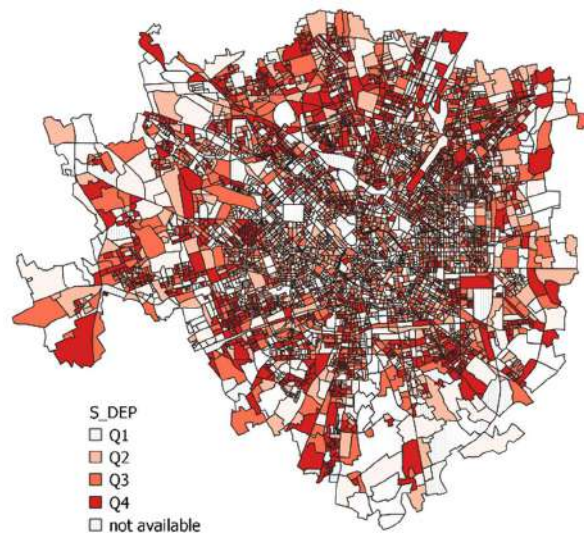


Figure 15: Distribution map of the quartiles of S_{DEP} in the study area

3.3.3 S_{POP}

In Tab. 7 is reported the percentage of the census units for each quartiles of S_{POP} . The Central area shows the highest percentage of census units with very susceptible population (26%), and also the lowest percentage of census units “not susceptible” (19%), while the Pedestrian area shows the highest rate of “not susceptible” population (33%) and the lowest of susceptible population (14%). The rates of this score are evenly distributed in the quartiles of each area, with no evident disproportion between quartiles: about half of census units are distributed in the first 2 quartiles for each area.

Table 7: Percentage of census units for each quartiles of S_{POP} in each concentric area

S_{POP}	PD	C	SP	P
1 st	33 %	19 %	22 %	29 %
2 nd	21 %	24 %	24 %	24 %
3 rd	21 %	27 %	23 %	20 %
4 th	14 %	26 %	25 %	18 %

In Fig. 16 the map of distribution of S_{POP} is reported, with a visible trend for which Periphery appears less interested by susceptible population, that is concentrated more in Central and Semi-peripheral area.

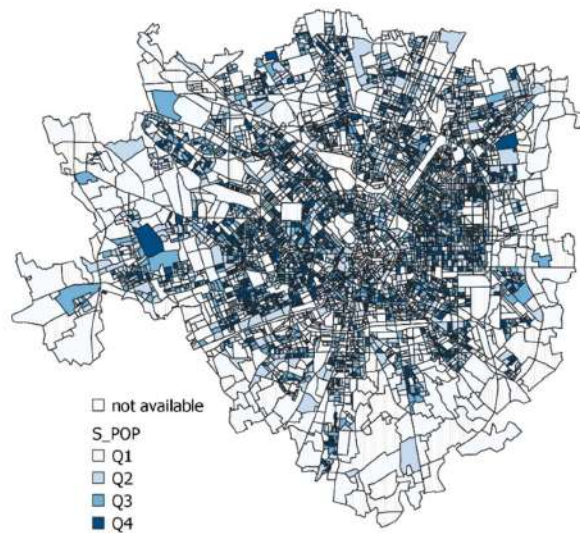


Figure 16: Distribution map of quartiles of S_{POP} in the study area

3.3.4 S_{URB}

The neighborhood quality summarized by S_{URB} is reported in Tab 8. The Pedestrian area shows the highest rate of a good neighborhood quality with 39% of the census units in the 1st quartiles while only 4.3 % in the 4th quartiles, that are also the extreme values compared to the other areas. The area with the lowest percentage of good neighborhood quality is the Semi-peripheral area (21%) while the area with the highest percentage of census units in the 4th quartiles is the Peripheral area (27%). In general, the sum of the first 2 quartiles demonstrates that Pedestrian area has 67% of census units in good neighborhood quality, percentage that decreases away from the center: 49% for central area, 45% for Semi-peripheral area and 42% for Peripheral area.

Table 8: Percentage of census units for each quartiles of S_{URB} in each concentric area

S_{URB}	PD	C	SP	P
1 st	39 %	25 %	21%	23%
2 nd	28 %	24 %	24%	19 %
3 rd	17 %	24 %	24%	23 %
4 th	4.7 %	21 %	26%	27 %

The quartiles of the score are mapped in Fig. 17, with an evident uneven distribution in the city. The Peripheral area is clearly more interested by low quality neighborhood compared to the central ones.

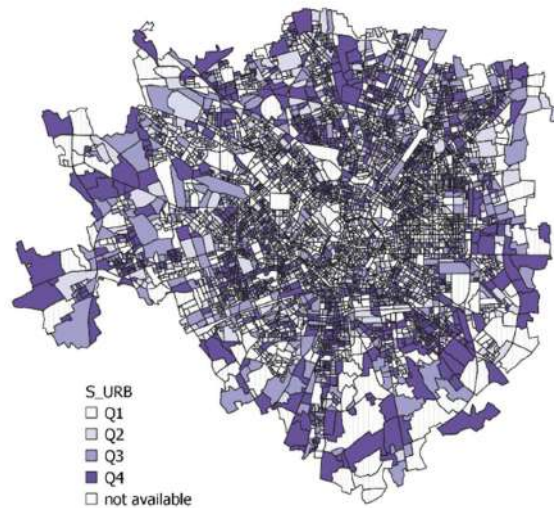


Figure 17: Distribution map of the quartiles of S_{URB} in the study area

3.3.5 Vulnerability Index

The summary of the vulnerability index elaborated for the census units and then sorted for concentric areas is reported in Tab. 9. The Pedestrian area is the one with the highest rate of census units in the 1st deciles (18%), while the central area shows the lowest rate (9%). In the 10th deciles, generally all the concentric areas show among the minimum values, however the Pedestrian area has the lowest rate (0.9 %) while the highest rate is reported for the Semi-peripheral area (10%). The sum of the first 5 deciles shows Pedestrian area concentrates in this range 65% of the census units, the central area 58 % ; the Semi-periphery 46% and the Periphery 57% . Only the Semi-periphery is slightly unbalanced to the most vulnerable part, while the Pedestrian area shows an evident unbalancing to the less vulnerability.

Table 9: Deciles of Vulnerability index elaborated for concentric areas and the percentage of census units in each deciles

VULN	PD	C	SP	P
1 st	18 %	9 %	11 %	14 %
2 nd	14 %	9 %	7 %	9 %
3 rd	11 %	9 %	8 %	11 %
4 th	12 %	9 %	10 %	10 %
5 th	10 %	12 %	10 %	13 %
6 th	7 %	9 %	9 %	9 %
7 th	6 %	7 %	8 %	5 %
8 th	8 %	14 %	14 %	12 %
9 th	4 %	8 %	9 %	6 %
10 th	0.9 %	8 %	10 %	3 %

In Fig. 18 is reported the vulnerability index elaborated for census units and sorted into deciles in the study area. The map shows clearly that the most vulnerable census units are clustered in a specific belt around the city center.

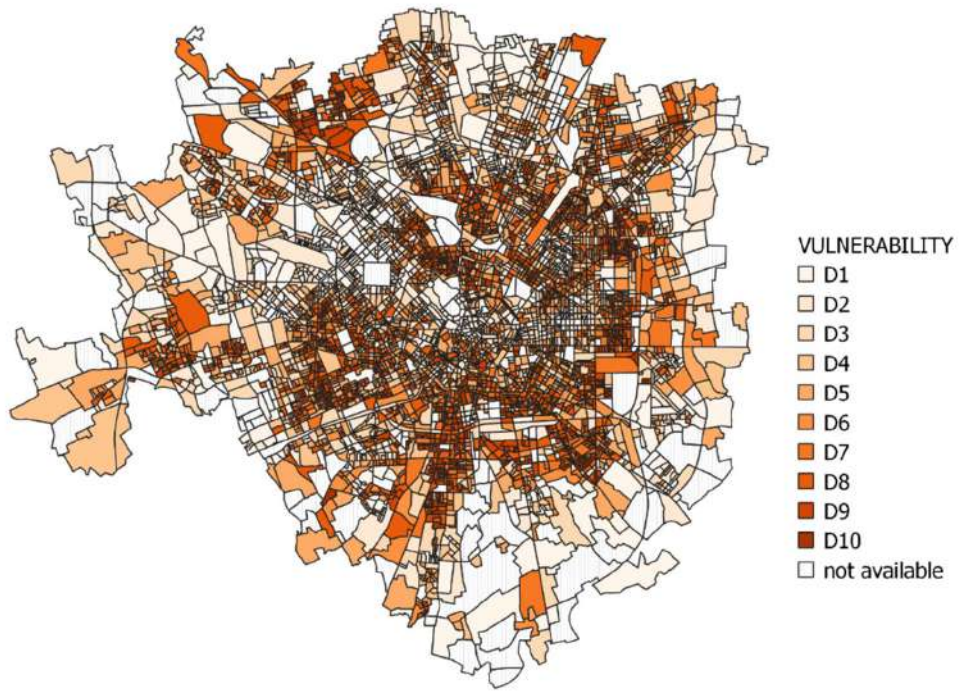


Figure 18: Census units are represented by the color of the Vulnerability class they belong to.

The relation between S_{POP} and the CI is showed in Fig. 19. A significant positive relation ($R=0.63$, $p<0.05$) suggests that more susceptible population basically experiences a worse air quality, while in Fig. 20 is showed the relation between the contamination and the neighborhood quality.

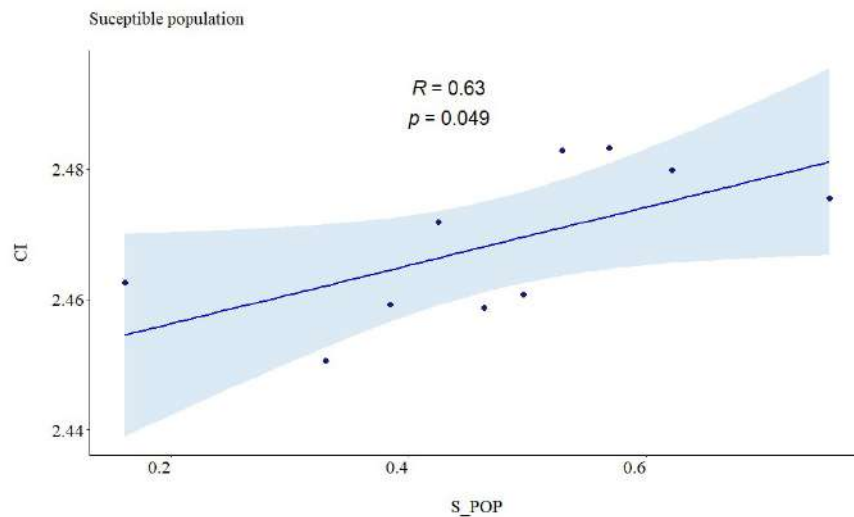


Figure 19: Linear relation between CI and S_{POP} .

Even though not significant, the U shape of the relation highlight that both the worse neighborhood quality and the best ones are characterized by higher level of air pollution than the classes in the middle.

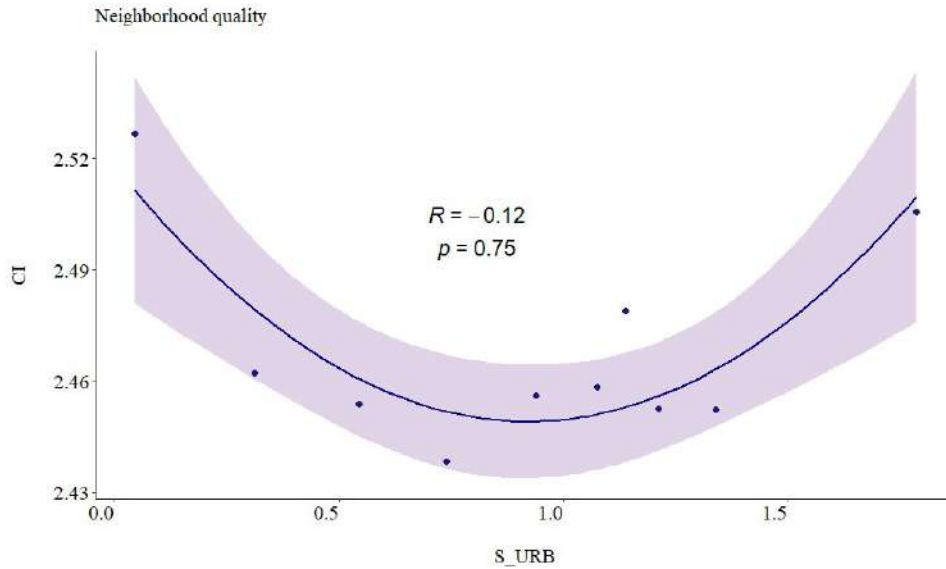


Figure 20: Nonlinear relation between CI and S_{URB}

4. Discussion

In this work the use of lichen biomonitoring techniques for the assessment of EJ at urban scale was tested. Firstly, the overall contamination by trace elements was compared to the socio-economic deprivation elaborated at census unit scale, then, the emission sources emerged from the source apportionment analysis addressed the evaluation of EJ to a proximity-based approach to the given sources. Finally, the socio economic, demographic and environmental data were used to create an overall vulnerability index to highlight those areas more interested by different type of fragilities.

Our results showed the suitability of lichen biomonitoring for EJ assessment, since it managed to overcome the main limitation of such studies at small scale, i.e., the scarcity of high resolution data of air pollution. To the best of my knowledge, in facts, the most used approach to collect air pollution data is to refer to available data as local registers, data from air monitoring devices or modeled data over the area. This leads authors to create coarse grain size resolution maps of air pollution, that hampers the detailed assessment of environmental disparities, especially at small scale.

Through lichen biomonitoring, instead, a high resolution was achieved: comparing to studies that used air monitoring devices, in this work we got a density of 0.28 monitoring

site per km² (50 sampling sites/181 km²), while Li et al. (2018) had 0.014 monitoring site per km² in Hong Kong (15 monitoring sites/ 1106 km²), Branis and Linhartova (2012) had the maximum value in Praha with a density of 0.030 monitoring sites per km² (15 monitoring sites in 496 km²) and Martins et al. (2004), had 0.006 monitoring devices per km² in São Paulo (9 monitoring sites/1521 km²). Thus, our air quality biomonitoring network was ca. 20 times denser than Hong Kong, 45 times than São Paulo and 9 times than Praha.

Considering also the epidemiological implications, in this study there are ca 3.57 monitoring sites every 100.000 inhabitants while in Hong Kong only 0.012, in São Paulo 0.012 and in Praha 0.11. Thus, the exposure of citizen to air pollution can be better estimate than with monitoring devices.

The high degree of detail provided by lichen biomonitoring resulted in a high resolution map of contamination, with evident hotspots and fluctuations within the city, thus, it was possible to assign to each census units a value of contamination highly representative. The mean contamination elaborated for the whole city is 2.41 and the mean value of socio economic deprivation is 0.82. The less deprived areas are the Pedestrian and the Central one with a SDI value of 0.68, while the Semi-periphery and Periphery have 0.85 and 0.96 values respectively. The contamination index decreases with distance from the center, with the exception of the Pedestrian area. Even though no significant differences emerged between the groups, a clear trend is detectable indicating that central areas are those less deprived but experience a higher air pollution level compared to the other areas, in particular to Periphery that experiences a worse socio-economic status but also a lower air pollution level. This trend could be explained by the fact that Milan is a city that is changing rapidly its organization: in the last decades the industrial vocation left space to a new conception of urban life, based on services to the citizens, requalification of peripheries, promotion of green and pedestrian areas and so on (Armondi and Bruzzese, 2017), and the process is not yet concluded. This leads the city center to represent the main core of the activities, offices and services while peripheries are slowly changing from the previous industrial or agricultural based economy to the new one. Thus, transports, roads and people also from other cities pour into the central areas more than peripheries, and the wealth is here concentrated.

Searching for the overall situation of EJ, thus correlating the CI and SDI sorted into deciles regardless of the belonging area, the trend reduces drastically its behavior until it becomes non-significant.

The lack of any type of significant correlation could indicate that in Milan the air quality is not a decisive factor for the overall life quality, thus the wealthier citizens don't

choose a less polluted area to live, but rather they chose the location on the basis of economic availability. However, it is noteworthy that Milan is a city that is changing rapidly but these fast changes are not always met by the people fluxes within the city, in fact, many households continue to live in the same place despite the rise or fall in the neighborhood quality and regardless of their economic status. For an exhaustive comprehension of the phenomenon, maybe more peculiarities of the city have to be taken into account as the house market, the urban planning for the future years and the demographic changes due to the new economic characteristics of the city.

4.1 The contribute of the Source Apportionment Analysis

Through the source apportionment analysis three emission sources were highlighted: major roads, railways and the industrial activity. These sources were used to conduct a proximity analysis.

The proximity analysis is a useful tool for evaluate the personal exposure to air pollution (Jerrett, 2005), however it does not take into consideration factors as precipitations, humidity and winds that are crucial for air pollution dispersion (Maantay et al., 2009). The proximity analysis here conducted, instead, started from the opposite point of view, or better said, the emission sources were extrapolated from the measured concentration of effective air pollution, thus, the air pollution data is intrinsic in the proximity analysis. In this way, the uncertainty linked to the natural factors is restricted. Moreover, through this method the contribution of industrial activity (that would not have been considered since the industrial era of Milan ended more than a decade ago) was highlighted and taken into consideration for EJ assessment.

4.1.1 F1: major roads and the socio-economic deprivation

Correlating the deciles of distance from major roads and the socio economic deprivation status, a significant positive relation emerged, indicating that increasing the distances, increases also the socio economic deprivation. These results are apparently in contrast to what commonly showed in literature for which the exposure to traffic related pollution is higher in disadvantaged groups (for example: Kingham et al., 2007, Jerrett, 2009). However, are many the cases for which the wealthier population is exposed to traffic related air pollution, both at urban and national scale. Cesaroni et al. (2010) studied in Rome the relation between health status, socio economic deprivation and proximity to roads finding that elderly people and those living in medium and high socio economic position were more

exposed to traffic related air pollution. The same results were also found by Havard et al., (2011) in Paris, Hajat et al. (2013) in New York city and Buzzelli and Jerrett (2007) in Toronto. At wider scale, Richardson et al. (2013) in a cross national study conducted in Europe reported that are the wealthier countries to suffer more of air pollution than the poorest, while Perlin et al. (1995) evaluated the association between ethnic composition and the industrial air emissions in the United States finding that the regions with the higher rate of industrial activity were also those with higher rate of ethnic minorities and the higher income for household.

4.1.2 F1: Railways and the socio-economic deprivation

The opposite situation emerged from the relation between railways and socio economic deprivation status: in this case a significant positive relation links the two parameters indicating that the closest census units to the railways are also the most deprived. A reason can be found in the fact that the noise from the railways reduces the house prices (Day et al., 2007) and generally the railway stations are perceived as more hazardous in terms of public security than actually are (Cozens et al., 2003). Many study, in facts, demonstrate that the more disadvantaged groups are more likely to be exposed to railway noise (Tonne et al., 2018; Verbeek, 2019; Lakes et al., 2013). Emissions from railways are, however, hardly considered in environmental justice studies (Mitchell and Norman, 2012). Our results thus, pinpoint that the railways emissions are as important as the noise for air pollution contribution, suggesting that they have to be more considered for Environmental Justice and air pollution politics.

4.1.3 F1: Major roads, railways and the socio economic deprivation for concentric areas

The distance from major roads and railways can have different meaning in different areas of the city (Power, 2012), and for this reason a detailed evaluation was performed by disaggregating the distances and socio economic data for the concentric areas. The only significant relation emerged was between distance from railways and SDI elaborated in the Central area ($R=-0.58$, $p<0.05$) while the other elaboration did not show significative trend. Even though the lack of significance, it is noteworthy that in general, in each concentric area, the relation of SDI with the distance from the major roads followed an opposite trend if compared to the general one. The importance of distances to the major roads in different part pf the city was previously tested also by Cesaroni et al (2010) who divided the city of Rome on the basis of the highest trafficked road finding that in the central areas were the less

affluent and less educated people to live closer to roads, at the opposite to what registered in Peripheral area. The distance from the railways, instead, followed in each concentric area the same trend of general situation, in particular the only significant relation emerged in Central area. Since the high socio economic status of this area, it is presumable that the proximity to the railways is easily avoidable by households.

Considering also the contamination data, it is not surprisingly that the Central area shows the highest value of CI: comparing the concentric areas, the Central is the one with the average lowest distance from roads, with a mean of 29 m and a median equal to 0 m, and also the lowest average distance from railways, that is ca 650 m.

4.1.4 Factor 2: Industrial activity

The area most interested by F2 was indicated as the “industrial area” also because of the high presence of industrial activities in the past as in the present. The clustered distribution led to consider only this area rather than the whole city. The SDI elaborated only for this area (0.84) is higher than the average of the whole city (0.82), indicating that a socio-economic disparities are here present. Moreover, considering the distance classes from the industry, in the ray of 500-1000 m the deprivation is even higher (1.06). The presence of industry is traditionally considered when searching for EJ from its initial movement (Bullard, 2001) but is yet an important factor for predicting environmental inequalities also in Europe (Viel et al., 2011). Our results confirm the previous literature, indicating that industrial areas and the presence of factories can be yet drivers of environmental disparities in a city.

4.2 The vulnerability Index

The elaboration of separated scores related to environment, socio-economic, demographic and quality of neighborhood allowed to both identify those areas more affected by each fragility and those areas that showed a general bad or good situation. This approach is considered complementary to the correlation research since it provides a frame of the overall situation rather than a casual-effect scenario (Lanier et al., 2019). Our results, in facts, successfully provide the four elaborated scenarios and, by disaggregating for concentric areas, clearer interpretation of the study area is possible.

The environmental score (S_{ENV}) reflected partially the contamination index distribution because of the aggregation in the scores of physiological parameters. The cumulation of both bioaccumulation and eco-physiology data provided thus a changed

situation, for which Pedestrian area is no more the “cleanest” but it is the Peripheral area that shows an overall better environmental situation, while the Central area confirmed the worse environmental data.

The deprivation score (S_{DEP}), since it derives from the SDI, shows no differences, but highlights that Pedestrian area is effectively the less interested by socio-economic deprivation.

The susceptibility score (S_{POP}) shows where more fragile people are located into the area. Surprisingly, is the Central area that host more susceptible population, however the percentages are quite similar in all areas. This uniform distribution suggests that in the city the more fragile groups are not clustered in scanty situation as proved, instead, in other works.

The score of neighborhood quality (S_{URB}) indicates those neighborhood more or less cozy for a good life quality as the distance from green areas or the presence of industrial activities. It is not surprising that the Pedestrian area is the one with best neighborhood quality, while the Peripheral shows the highest score (thus, the worse quality). This score highlighted that the Peripheral area don't experience an overall better air quality due to some requalification, but rather it is the combination of the socio economic deprivation and the scarcity of services that lead these areas to be less attractive and, thus, lived by population.

The cumulative combination of these scores led to the elaboration of the vulnerability matrix that pinpoints the most fragile areas. As expected, Pedestrian area shows the highest rate of a good quality of life because of a combination of a good air quality and socio-economic status. The worse overall situation is showed in Semi-peripheral area where nor the socio economic nor the environmental parameters were particularly good. This results is in line to that of Havard et al. (2009) who showed that midlevel deprivation categories were also the more exposed to air pollution compared to the highest income or the lowest income categories. Through this kind of approach, a specific socio-environmental politics can be addressed (Bae, 1997).

4.3 The relation between CI and scores

The relation between air contamination and S_{POP} and S_{URB} was finally investigated. A significant positive correlation was found between the CI and the susceptible population, thus increasing the susceptibility increases also the contamination. This results suggests that elderly and younger people are generally more exposed to air pollution, a situation already proved in Italy (Germani et al., 2014) but also in USA (Ewans et al., 2004). The causal-effect

dynamic, however, is not obvious because the personal behavior of elderly and youngest could lead to a higher releases in the atmosphere, but on the other side, as showed in Germani et al. (2014) some social categories as such are more often subjected to worse air quality.

The nonlinear relation between air contamination and neighborhood quality, instead, did not provide significant results, even though it suggests that both best neighborhoods and the worse one experience a high level of contamination. This result confirms partially the previous elaboration for which the central and wealthy areas experience best life quality but worse air quality, while the contribution of industrial activity in Peripheral area can enhance the deprived situation by elevating the contamination.

5. Conclusions

Lichen biomonitoring allowed to obtain a detailed information of the air contamination in the city that in turns allowed a reliable assessment of environmental disparities in Milan. The overall situation suggests that the traditional trend of economic-related disparities in the exposure to air pollution is not present, at the opposite, the areas where the deprivation was higher were also those with better air pollution. This indicates that in Milan a strong connection is still present between life quality and the presence of vehicles and roads. The use of source apportionment analysis allowed a precious insight in Milan dynamics, highlighting that near industrial activities, the socio-economic deprivation was generally higher

Chapter 2

The perception of air pollution and of its risk for human and environmental health

1. Preface

The final part of this thesis was carried to obtain a subjective point of view about air pollution in the city of Milan directly from Milan citizens. People are, in facts, not only the cause of air pollution with their activities but also the victim of the effects of the prolonged exposure, and their perception and habits can greatly improve the interpretation of environmental data. The study of perception can also improve the addressing of environmental politics, since that it was demonstrated that more than the effective risk, is the perceived risk to drive changes in human behavior. However, behind the perception study a large number of variables that interact each other are present: socio-economic status, personal beliefs, cognitive structures as well as ethnic culture, are just few of the factors to consider for perception study. Since the interaction between these variables roots in discipline as psychology and sociology, a comprehensive study is not proposed in this work. Thus, this chapter primary aim is to present the phases of the elaboration of the questionnaire and the preliminary and explorative results. Further analysis and discussions will be carried to improve the data interpretation in a wider lecture key.

The themes explored in this work are:

- The physic perception of air pollution, given its importance in mediating between air pollution and health symptoms (Kasperson et al., 1988; Cutter, 1995)
- The change in habits during an intense smog day, to investigate how the quality of life can effectively be affected by air pollution (Bresnhan et al., 1997)
- The health symptoms usually associated to air pollution, in order to highlight the role of perception in signaling health symptoms (Orru et al., 2018)
- The risk perception of air pollution to evaluate the average degree of concern of population
- The “halo effect”, i.e., the effect for which people tend to consider their own neighborhood or, in general, their place, less polluted than others (Hofflinger et al., 2019)

- The interest for the current study with lichens as bioindicators of air pollution, in order to test if the risk perception can lead to be more interested in scientific studies
- The frequency of information in different media to investigate the role of information channels in risk perception (Huang and Yang, 2020)
- The frequency of use of different transports within the city, to observe if the normal behavior is matched with the degree of concern about air pollution

People were categorized by Age, gender, education level since it was demonstrated to be reliable characteristics for differentiating responses (Kim et al., 2018; Laws et al., 2015).

2. Creation of the questionnaire

We selected to elaborate a self-completion paper questionnaire, directly administrated by the interviewer to randomly selected people in Milan. The questionnaire was anonymous.

2.1 Elaboration of the questionnaire

2.1.1 Phase 1: Focus Groups

The first step of the questionnaire elaboration was the organization of the “Focus Groups”. A focus group is, briefly, a small group of target people (in general no more than 12 participants) that, under the guidance of the moderator, discuss freely about the given topic.

The focus group is a widely used method as qualitative research itself or as preliminary phase for questionnaire elaboration, because it allows people of different backgrounds and experiences to discuss and compare opinions and perceptions, bringing out memories, new ideas and enhancing their point of view on a given item. People selected, in facts, even with different backgrounds and personal stories, share cognitive structures, culture, and adhere to similar normative beliefs (Folch-Lyon and Trost, 1981) and the discussion enhances and brings out shared and different thoughts. In this way, the phenomenon object of the discussion is all-round considered. The researcher gains the different aspects of the phenomenon as seen by laypeople, thus, themes to consider for the questionnaire elaboration.

In this study, 2 focus groups were organized with 2 and 7 participants, and the key words used were “Environment”, “Air pollution perception”, “Air pollution risk”, “Life quality and air pollution”.

The interviews lasted ca 2 and 3 hours respectively, they were recorded and the main items highlighted by participants were used as basis for the first elaboration.

2.1.2 Phase 2: Administration of drafts

A first draft of questionnaire was proposed both to laypeople and expert researchers in order to check the clarity of questions, understandability of response typology, if the rightness of scientific items was maintained and the time requested to completely fill the questionnaire. Different drafts were proposed, and the final version was finally elaborated.

2.1.3 Phase 3: Elaboration of the final version

The final questionnaire was composed by 30 questions with 3 different types of response methodology:

- Dichotomous: Yes/No,
- Likert scale of agreement: 1-5 where 1 means “totally disagree”; 2 “disagree”; 3 “don’t know”; 4 “agree”; 5 “totally agree”
- Choose of frequency: Never, rarely, regularly, often, always. In the case of media we included also “don’t use this media”.

Personal data collected referred to Gender, Education, Age and the reasons to stay in Milan: work/study and/or live in the city. To respondents was also asked to select which district they attend more for living or working. The questions covered 9 main themes (other than personal data) and organized in sections, as listed below:

Table 5: Themes subject of survey and sections of questionnaire

Section	Theme
Section 1A	The physical perception of air pollution
Section 1B	The change in habits during a very smoggy day
Section 1C	The health symptoms the respondents have, that they refer to air pollution
Section 2A	The risk perception of air pollution for the self and other people
Section 2B	The importance of green areas for improving air quality and protection from air pollution
Section 2C	The “Halo effect”
Section 3	The interest in the current study about the use of lichens as biomonitors of air pollution
Section 4	The frequency of reading of hearing news about air pollution on different media
Section 5	The frequency of use different type of transport to move within the city
Section 6	Personal data

The questionnaire elaborated was averagely filled in no more than 3 minutes. The questionnaire was written in Italian language, the English version of the final draft is reported in Fig.1 (lateral indication of sections are here reported for a better understanding of results).

Air pollution in Milan

What the citizens and the workers think?

Thank you for participating to this survey about air quality in Milan. The questionnaire is anonymous. Could you indicate on the map where...

Where do you **live** in Milan?

I don't live in Milan



Where do you **work** or **study** in Milan?

I don't work or study in Milan



1A "Physical perception"

When I am in Milan I can perceive the air pollution (please answer to all the questions)

...From bad smells or sensation of heavy atmosphere Yes No

...By seeing heavy smog Yes No

...By seeing dust covering outdoor objects Yes No

...By associating with the car noise Yes No

...On my body (es. skin, face, hair) Yes No

1B "Change habits"

During a intense smoggy day

...I avoid to open the windows Yes No

...I avoid to do outdoor sports Yes No

...I go to work by car Yes No

...I go to work with public transports Yes No

1C "Health symptoms"

In Milan I often have

...Sore throat Yes No

...Itchy eyes Yes No

...Breathing problems Yes No

2A "Risk perception"

Thank you. Now we would like to know how much you are agree with the following statements, referred to those who live in Milan

	Totally not agree	Not agree	I don't know	Quite agree	Totally agree
There is more probability to develop health problems related to air pollution than to be involved in a car accident	1	2	3	4	5
Air pollution is a serious problem for those who live here	1	2	3	4	5

Figure 1: Front page of the questionnaire

		Totally not agree	Not agree	I don't know	Quite agree	Totally agree		
2A	"Risk perception"							
	children are more damaged than adults by air pollution	1	2	3	4	5		
2B	"Halo effect"							
	The area where I live in Milan has a better air quality than others	1	2	3	4	5		
	The area where I work in Milan has a better air quality than others	1	2	3	4	5		
2C	"Importance of green areas"							
	Green areas as urban parks and tree rows improve the air quality of the city	1	2	3	4	5		
	By studying plants is possible to evaluate the air pollution in the city	1	2	3	4	5		
3	"Interest in the current study"							
	Currently, we are carrying a study on air quality in Milan by using plants							
	How important is it for Milan citizens in your point of view?	Not important				Very important		
		1	2	3	4	5		
	Would you like to know more about this kind of study?	Not at all				Very interested		
		1	2	3	4	5		
4	"Frequent information"							
	How frequently do you hear about air pollution in these media							
	Television and radio	<input type="checkbox"/> don't use it	<input type="checkbox"/> Never	<input type="checkbox"/> Rarely	<input type="checkbox"/> Regularly	<input type="checkbox"/> Often		
	Newspapers and magazines	<input type="checkbox"/> don't use it	<input type="checkbox"/> Never	<input type="checkbox"/> Rarely	<input type="checkbox"/> Regularly	<input type="checkbox"/> Often		
	Websites (local portals)	<input type="checkbox"/> don't use it	<input type="checkbox"/> Never	<input type="checkbox"/> Rarely	<input type="checkbox"/> Regularly	<input type="checkbox"/> Often		
	Social networks (es. Facebook)	<input type="checkbox"/> don't use it	<input type="checkbox"/> Never	<input type="checkbox"/> Rarely	<input type="checkbox"/> Regularly	<input type="checkbox"/> Often		
5	"Sustainable mobility"							
	How often do you use these means of transport within the city?							
	Car or scooter	<input type="checkbox"/> Never	<input type="checkbox"/> Rarely	<input type="checkbox"/> Regularly	<input type="checkbox"/> Often	<input type="checkbox"/> Always		
	Bicycle	<input type="checkbox"/> Never	<input type="checkbox"/> Rarely	<input type="checkbox"/> Regularly	<input type="checkbox"/> Often	<input type="checkbox"/> Always		
	Public transports	<input type="checkbox"/> Never	<input type="checkbox"/> Rarely	<input type="checkbox"/> Regularly	<input type="checkbox"/> Often	<input type="checkbox"/> Always		
	By foot	<input type="checkbox"/> Never	<input type="checkbox"/> Rarely	<input type="checkbox"/> Regularly	<input type="checkbox"/> Often	<input type="checkbox"/> Always		
6	"Personal data"							
	Sex	<input type="checkbox"/> Male	<input type="checkbox"/> Female	Education level	<input type="checkbox"/> Elementary	<input type="checkbox"/> Secondary	<input type="checkbox"/> high school	<input type="checkbox"/> university
	Age	<input type="checkbox"/> 18-25	<input type="checkbox"/> 26-40	<input type="checkbox"/> 41-55	<input type="checkbox"/> 56-65	<input type="checkbox"/> more than 65		

Figure 2: Back page of the questionnaire

2.2 Administration of the questionnaire

The administration lasted 4 weeks during February-March 2019. Respondents were randomly selected, avoiding people < 17 years old, inside urban parks of the city

2.3 Data elaboration

The data were elaborated by section rather than by single question, in order to obtain a first overall picture of the distribution of perception along the category of respondents (gender, age and education).

In particular, for the sections with Yes/No answers (1A, 1B, and 1C), if the answer “Yes” was chosen for at least half of the choices, the final answer to the section was considered “Yes”.

For sections with response typology the Likert scale, if a high agreement (score 4 and 5) was chosen in at least half of the questions, the final score was considered as “agree” versus “not agree” if more than half of the responses showed a low agreement level (1-2). The score 3 “I don’t know” was considered as a not agreement with the proposition.

The data of frequency choice was elaborated as follow:

For information channels, if the respondent frequently read or searched air pollution information in 2 or more channels, he was considered as “interested” or “informed”.

For type of mobility within the city, if the respondent chosen a high frequency of use of eco-sustainable type of transport, the mobility was considered “sustainable”.

A summary score was finally elaborated by each category as the average of score achieved in each section.

3. Descriptive results

A total of 302 questionnaires were filled, excluding the personal data, most of them were fully completed (82.8%), 14.6% completed for more than half (75-99%), 2.6% were completed in a range of 50-75%, and no questionnaire was filled for less than half, as summarized in Tab.2.

Table 6: Summary of questionnaire rate of completement

tot	complete 100%	complete 75%-100%	complete 50%-75%	complete <50%
302	250	44	8	0
%	82.8	14.6	2.6	0

The composition of participants characterized by sex, age, and education is reported in Tab. 3. A balance between Female and Male was achieved, with females being slightly more abundant than males (54.3% vs 45.7% respectively). Most part of the respondents live and/or study in Milan (90.8%) while less than 10% of participants stood in the city for other reasons (as visiting relatives, sporadic days of work). The age of respondents distributes unevenly through age classes, with the class 18-25 being the most abundant (36.4% of respondents), followed by the class 26-40 (25.8%), 41-55 (17.5%), 56-65 (9.3%), while the extreme classes were the less represented, in particular the class >65 represents the 9.6% while the class <18 represents only the 1.32% of the total. No respondent had an education level under the secondary school (elementary school= 0 respondent), while the most represented education level was the university and the high school (50 and 44.9% respectively). The remaining part was represented by the secondary school (5.1%). In general, people were available to leave these kind of data, as showed by the high rate of response achieved in each category (100% of respondent indicated their age, while the lowest rate was achieved in the question about living in Milan, 86.1%).

Table 7: Demographic composition of respondents

Personal data	Options	N answers	% of the total	Answer rate %
Sex	F	163	54.3	99.4%
	M	137	45.7	
live and/or study in Milan	Yes	237	90.8	86.1%
	No	24	9.2	
Age	<18	4	1.32	100%
	18-25	110	36.4	
	26-40	78	25.8	
	41-55	53	17.5	
	56-65	28	9.3	
	>65	65	9.60	
Education	Elementary s.	0	0	98.1%
	Secondary s.	15	5.1	
	high s.	133	44.9	
	University	148	50	

Sorting the total complement of the questionnaire by personal parameters, in general the females, the people with high school diploma and the people between 18 and 25 years old were the category who gave back the highest number of completely filled questionnaires, as reported in Tab.4. The categories who gave back the lowest number of completed questionnaires, instead, were people with a middle school diploma and eldest people: only ca the 13% of these participants fully completed the questionnaire.

Table 8: Percentage of completion of questionnaires sorted by personal data: Sex, Education level and Age

Parameter	options	Filled 100%	%
Sex	F	69	42.3%
	M	55	40.1%
Education	middle s.	2	13.4%
	high s.	58	43.6%
	university	64	43.2%
Age	<18	2	50%
	18-25	56	51%
	26-40	33	42.3%
	41-55	21	39.6%
	56-65	8	28.6%
	>65	4	13.8%

While looking by single section, the rate of answer is reported in Tab.5. In general, most part of the questions were answered, with the lowest rate for the section 1C, that regarding the health symptoms. No section about 2C (regarding the “halo effect”) reported no answer at all.

Table 9: Rate of answers by section

	Fully answered	Partially answered	No answer
Section 1A	95.7%	4.3%	0
Section 1B	93.7%	6.3%	0
Section 1C	92.4%	7.6%	0
Section 2A	99.7%	0.33%	0
Section 2B	97.8%	2.2%	0
Section 2C	96.0%	2.3%	1.6%
Section 3	99.3%	0.66%	0
Section 4	97.0%	3%	0
Section 5	96.0%	4%	0

3.1 The overall situation

In Fig. 2 are summarized the answers for each issue of the questionnaire. 71% of the total respondents declared they physically perceive the air pollution as by seeing dust on objects, or by bad smells for example, while a higher percentage of people (91.4%) automatically changes its behavior in an intensely polluted day, as, for example, they avoid to open the windows. Health symptoms linked to air pollution as the sore throat or itchy eyes are perceived by 66.6% of respondents, but a higher percentage of people (88.4%) perceive air pollution as risky for the health. The importance of green areas for a better air quality and protection from air pollution is perceived by 70.9% of respondents. Testing for the “halo effect”, i.e., the effect for which the own area is thought to be less polluted than others, only 31.1% of people showed to be agree with the proposition. Most part of respondents showed interest also for the current study (76.2%) through lichens as bioindicators of air quality. Asking for the daily habits of information and mobility, about 60% of respondents declared to regularly find information about climate and environment on media as TV or websites for example, and 83.4% of respondents use from regularly to always eco-friendly way as bike, public transports and walking to move within the city.

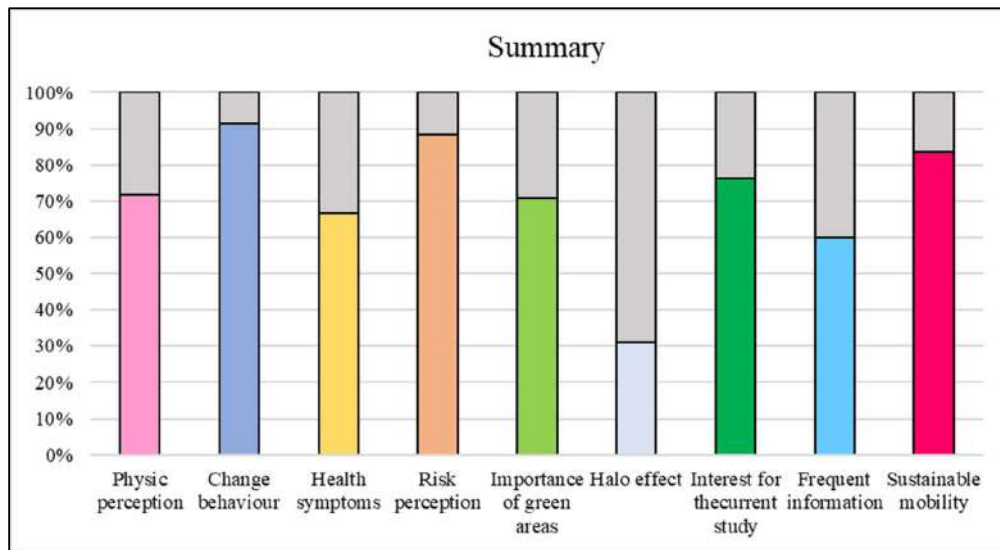


Figure 2: Rate of agreement by section

3.2 Disaggregated data for personal characteristics

3.2.1 Gender

The answers were then separately explored by personal characteristics. In Fig. 3, the preferences were sorted for gender. Female in general perceive more than males the air pollution physically (75.5% vs 67.9% respectively) but are the males who modify some behavior in a dense smog day (94.9% vs the 83.3% of female). The experience of health symptoms, the risk perception and the importance of green areas, instead show balanced results between the two categories, around respectively 66-68%; 86-90% and 66%. The “halo effect” is slightly more pronounced in males, but however low values for both are registered (23.4% vs 15.3% of female). The interest for the current study is higher in females than males (74.8% vs 23.4%) but are males who more frequently search or read about air pollution on media (24,1% vs 17.2% of females). The eco-friendly mobility is a frequent habitude for both, even if females are those who use it more (88.3% vs 77.4%)

Participants of both sexes showed a great agreement in the changing behavior, risk perception and sustainable mobility items. A slightly more marked differences in responses were recorded in the other items, however in all cases more than 50% of participants agreed each other.

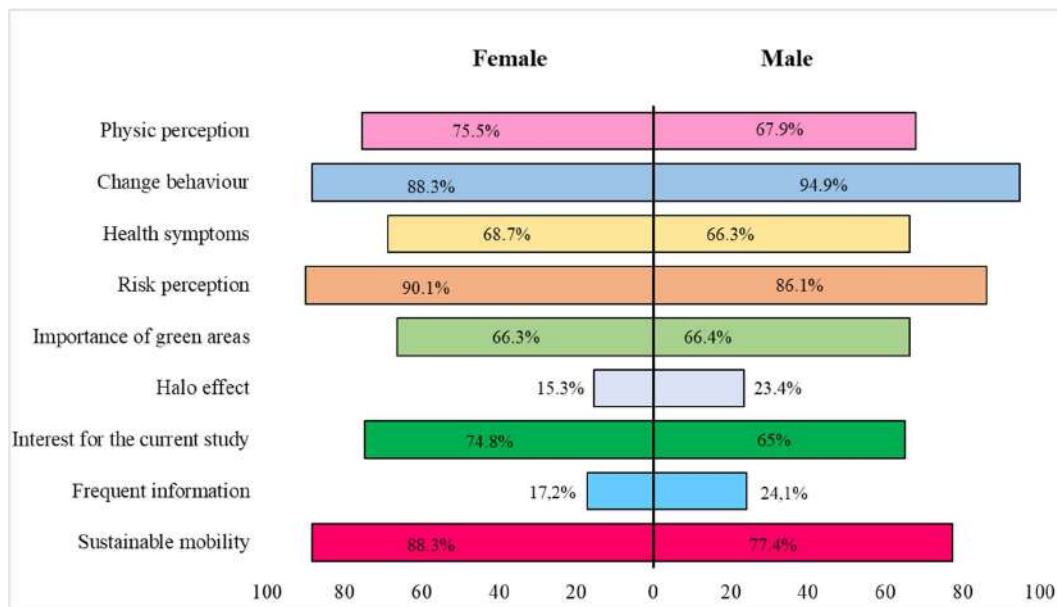


Figure 3: Rate of agreement for section by gender

3.2.2 Education

Results sorted by education level are showed in the Fig.4. Since no questionnaire was filled by the lowest available education level (elementary school), this category was deleted. The physical perception appears to be quite balanced between categories and generally high, with the lowest value recorded for the middle school (80%) and the highest for University (86.5%). The change in habits follows the education trend, with only 40% of people with middle school who changes the behavior during a smoggy day, while is 66.9% of graduated people that modify its habits. Also the health symptoms follows this trend, with 53% of people with middle school who declared to feel some health symptoms while it is 74.3% of graduated people who perceive more health problems. At the opposite, the risk perception is higher for people with the lowest education level (26.7%) while people with high school showed the lower perception of air pollution related risk. The importance of green areas is perceived quite equally by the three categories, with a rate of ca 66% of respondents agreeing with their importance for a better air quality. The interest for the current study was highest for people with the lowest education level (86.7%) and lowest for graduated people (62.8%). The sustainable mobility is preferred by 86.7% of people with middle school while the category with the lowest rate is that of people with the high school (67.7%).

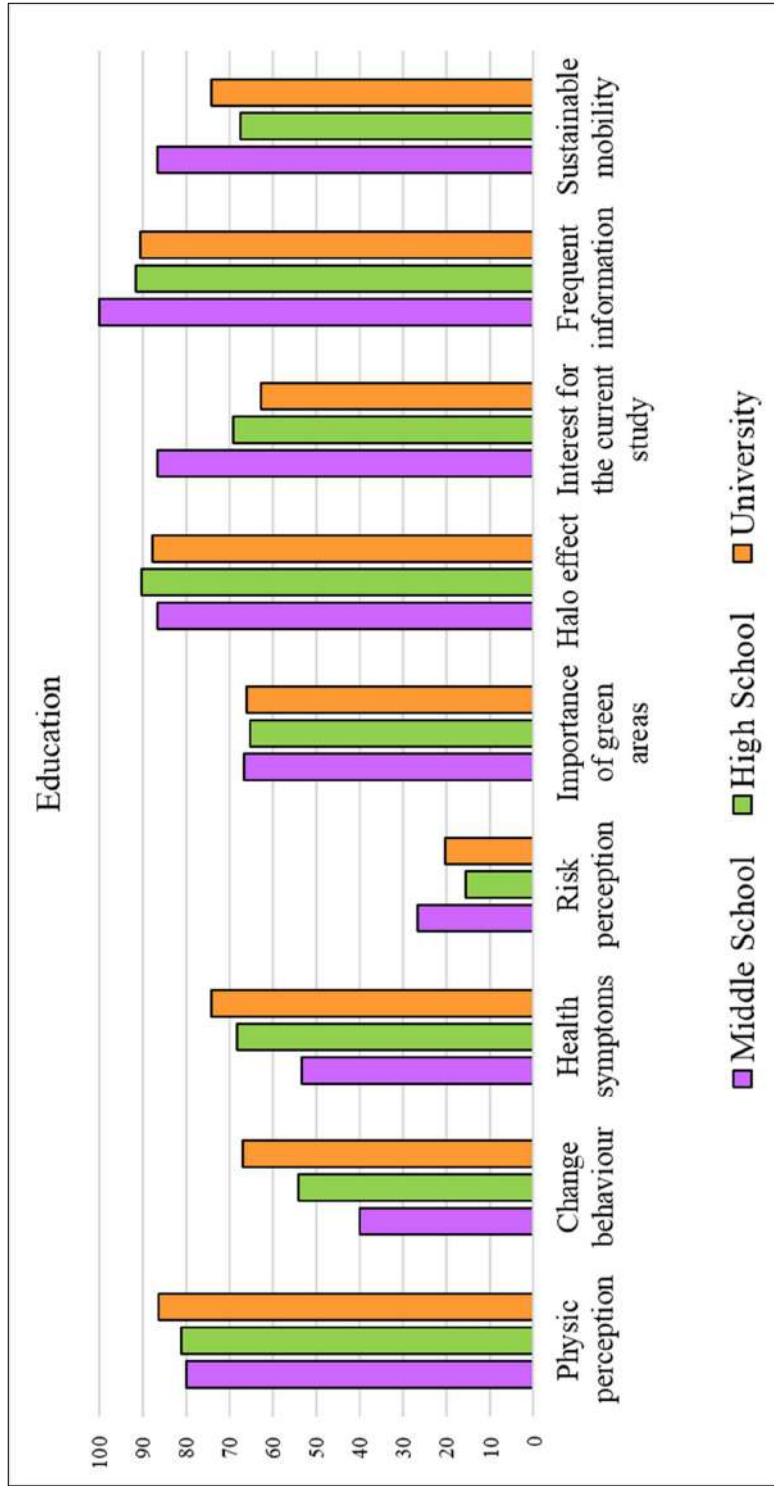


Figure 4: Rate of agreement for each section by Education level

3.2.3 Age

In Fig. 5 the preferences are reported by age classes. The physical perception appears to increase with age, in fact only 35% of <18 declared to perceive it while 86.2% of <65 years old declared to perceive it. A different trend is shown for the change in the habits during a smog day, for which 100% of the youngest prefer to change some habits while the lowest percentage is shown for the class 26-40 (85.9%). The other classes are in the range 89-96% of respondent who changes some behavior. The youngest also declared to feel also the health effects of air pollution (100%), while only 57% of the class 18-25 experiences symptoms. The other classes are balanced around a percentage 67-73%. The risk is generally highly perceived, with percentages in the range 83-100%, with the lowest percentage showed for the class 18-25 years and the highest for the youngest class (<18 years old). The importance of green areas appear to be particularly thought by the youngest and the eldest age class (75% and 75.9%) while the class 18-25 showed the lowest rate of respondent in agreement with the importance of green areas (60%). The “halo effect” shows a great variability through classes, with the youngest that show no halo effect, the class 18-25 showing 18.2%, the class 26-40 10.3%, the class 56-65 showing 21.4% and the eldest class showing 20.7%. In general all classes showed interest for the current study in Milan with lichens, however 100% of the youngest was interested while only 58.6% of the eldest people declared to be interested and are willing to know something more about the study. The most informed category about air pollution was the youngest (100%), followed by the class 56-65 years old, while the lowest informed is the class 18-25 with 64.5% of respondent who said to read or search about environment and air pollution on media. The eco-friendly mobility is used by 100% of the youngest, obviously because they have not the legal age for driving license, but among the other classes, the class 26-40 has 50% of respondents who use frequently a eco-friendly mobility within the city. The percentages are lower for the other classes, which are distributed in the range 37.7 % (class 41-55) and 42.9% (56-65).

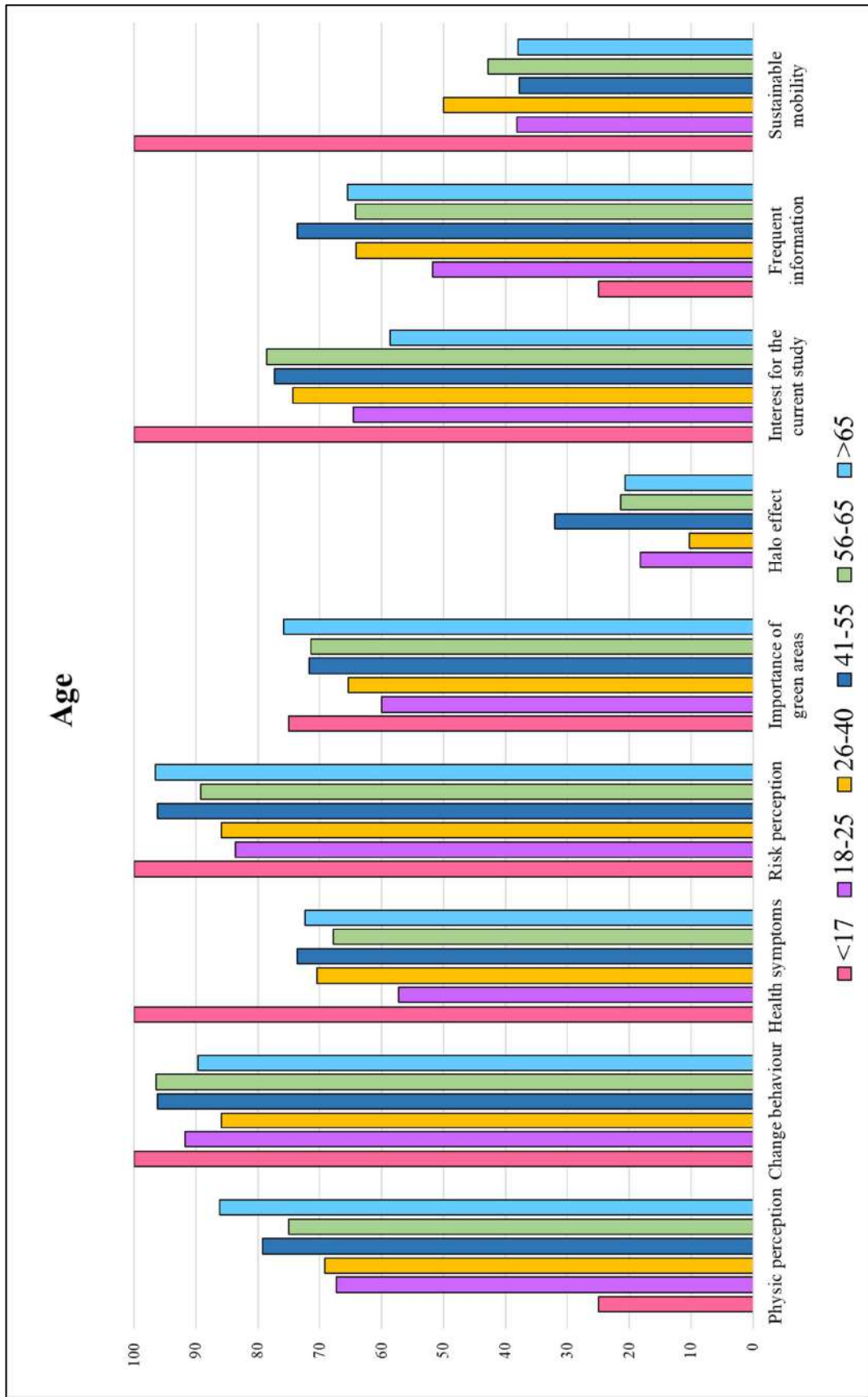


Figure 9: Rate of agreement for each section by Age

The summary for each category is reported in Tab. 6. This “score” indicates the average meeting of each category with the proposed thematic. In general, 65% of respondents perceive pollution, perceive its risk and do practically something to protect themselves, improve environment or know more about the problem. Females and Males achieved a quite equal score with a mean of 69.1 and 68.3%. Also for the education level no important differences between scores were registered. The three categories in facts, are in the range of 67.1-70.0%. Greater gaps were instead recorded between age classes. The category with the lowest score is the 18-25 class age with only the 59.2% of respondent aware or willing to do something for the environment, while the highest class is the 41-55 years old, with 70% of people who perceive the air pollution, its risks and do something to protect them and the environment. In general, the category with the highest score was the age class 41-55, that achieved 70.9%, while the lowest score is recorded for the age class of 18-25 years, with a score of 59.2%.

Table 10: Average score achieved by each personal parameter.

Parameter	Options	%
General		65.7%
Gender	F	64.9%
	M	63.5%
Education	Middle School	69.6%
	High School	67.1%
	University	70.0%
Age	<17	69.4%
	18-25	59.2%
	26-40	64.0%
	41-55	70.9%
	56-65	67.5%
	>65	67.0%

4. Conclusions

The high rate of fully and quasi-fully completed questionnaire (97.4%) indicates that questions and typology of response were clear to participants, as well as the time required for the compilation did not discourage the interviewed people. Participants were also available to indicate their personal data, with the lower rate of response for the question about living or studying in Milan (86%). Selecting randomly the pool of participants, many of them could, in facts, be in the city for other reasons rather than those proposed, as visiting parents frequently for example. The high rate of answers of each single question confirmed the understandability of questions and the capability to easily give an answer for participants. The only section that registered also no answers was the 2C, the one referred to the “halo effect”. Probably, since the 2 question that composed the section referred one to the living place and the other to the working place, people in Milan for other reasons did not answered. Additionally, the questions required time to think the comparison between the areas, that could have discouraged some participant.

It can be concluded that among participants a high level of perception of air pollution and its risk is felt even with some differences between some categories. The fact that Milan is among the most polluted areas not only in Italy but also in Europe could have led people to increase their perception of air pollution and risk. However, it is noteworthy that elderly people were attracted by the questionnaire, adding personal verbal comments on the air quality of Milan. Most of them reported the great improvement of air quality since some decades, remembering the severe condition experienced in the period 1950-1970. At the opposite, youngest people seemed somehow used to the air pollution situation, that can also explain their low perception of the air pollution. Moreover, they reported a higher disappointment with actual environmental policies. These data were not recorded because they were freely added in words by participants, that at the end of questionnaire were in the most cases glad to have declared their opinion about the air quality.

5. Limitations and further analysis

This work presents several limitations. The first one is the choice of administrating questionnaires inside green parks of the city center, thus creating a bias for the creation of a reliable sample of Milan citizen, excluding people who do not go usually to parks for many reasons. The limited number of interviewed people, selected in the same place, thus, with similar perception at least of green areas, could have smoothed the potential differences by categories.

Final conclusions and considerations

In this work is provided the effectiveness of the use of lichen biomonitoring techniques for environmental justice assessment at urban scale: the high spatial resolution of air pollution data increased notably the accuracy in the elaboration of pattern of environmental disparities, providing also new and unexpected scenarios of air contamination. The combination of the source apportionment analysis and the research of environmental inequalities highlighted, in facts, 2 different scenarios: the first one is linked to the actual situation, characterized by intense traffic and the determinant role of transport inside the city. The closeness to roads and traffic-related air pollution does not discourage wealthier people, because it carries also a higher life quality standard. Areas with better air quality, instead, are characterized by lower life quality. The second scenario was unexpected since it highlighted the role of the past industrial activity as determinant of air pollution in the northern part of the city but also determinant of environmental inequalities. This scenario would have not been considered without the dense network of sampling sites and the source apportionment analysis.

The strength of this approach is that it starts from a high quality data of air pollution that lead to the main emission sources, and from this point a specific research of environmental disparities can be carried. This is the opposite of what traditionally done in EJ research, where emission sources are hypothesized and their impact on the area is not considered, thus, a high level of uncertainty is carried in the elaboration.

Further improvements should be apported for what concerns more specific dynamic of the city in exam, as the traffic fluxes and considering other factors as the house market for example.

Asking people their point of view revealed many interesting aspects of air quality in Milan that could surely improve also the Environmental Justice interpretation, further analysis should be carried in this sense.

For its nature, this multidisciplinary work did not studied deeply all the aspects presented but provided a first walkable pathway for an all-round evaluation of air pollution at urban area.

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APPENDIX A

Raw data of bioaccumulated trace elements expressed in ppm dry weight.

ID	Concentric Belt	District	Al	Cr	Fe	Cu	Zn	As	Cd	Sb	Pb
IAEA336 C	/	/	680±110	1.06±0.017	430±50	3.6±0.5	30±3.4	0.63±0.08	0.117±0.017	0.073±0.01	4.9±0.6
IAEA336 F	/	/	611±17	1.11±0.015	393±10	4.1±0.25	31±1.0	0.61±0.01	0.111±0.017	0.081±0.007	5.0±0.1
IAEA336 F	/	/	613±18	1.11±0.06	389±15	3.8±0.21	30±1.1	0.58±0.03	0.103±0.004	0.077±0.007	4.5±0.2
CTRL1	/	/	510±9	1.59±0.08	441±10	3.81±0.27	27.1±0.7	0.215±0.05	0.062±0.004	0.116±0.008	2.0±0.04
CTRL2	/	/	296±7	1.68±0.12	433±14	3.77±0.32	28.7±0.9	0.188±0.022	0.066±0.007	0.134±0.007	1.86±0.05
CTRL3	/	/	482±10	1.76±0.09	453±11	2.32±0.13	25.6±0.6	0.212±0.027	0.056±0.004	0.108±0.009	2.01±0.05
10A	Pedestrian	1	442±15	3.84±0.26	809±25	12.23±0.28	39.6±1.0	0.215±0.009	0.063±0.009	0.48±0.026	4.42±0.11
10B	Pedestrian	1	305±13	3.68±0.14	842±26	11.56±0.66	49.4±1.3	0.336±0.033	0.089±0.005	0.497±0.017	4.67±0.10
10C	Pedestrian	1	520±13	3.54±0.25	871±11	10.49±0.38	39.3±2.0	0.301±0.01	0.064±0.006	0.506±0.019	4.19±0.07
21A	Centre	2	681±18	4.96±0.30	1112±27	28.96±0.47	40.8±1.5	0.389±0.051	0.072±0.008	1.136±0.032	6.74±0.22
21B	Centre	2	765±17	5.84±0.38	1426±34	28.26±1.31	32.8±1.1	0.318±0.06	0.057±0.006	0.754±0.055	4.47±0.10
22A	Semi-periphery	2	698±15	4.64±0.21	955±14	15.87±0.59	35.7±1.3	0.402±0.046	0.073±0.01	0.89±0.039	8.19±0.25
22B	Semi-periphery	2	309±10	2.96±0.36	599±16	20.58±0.88	29.7±1.5	0.285±0.044	0.057±0.007	0.569±0.03	4.50±0.28
23B	Periphery	2	279±6	2.33±0.30	471±17	6.51±0.40	33.8±1.3	0.245±0.03	0.059±0.003	0.546±0.012	4.13±0.16
31A	Centre	3	654±24	3.09±0.25	697±16	11.41±0.60	27.2±1.1	0.254±0.026	0.074±0.007	0.545±0.022	4.49±0.18
31B	Centre	3	544±14	3.78±0.21	696±10	25.31±0.53	36.4±1.2	0.267±0.044	0.053±0.006	0.645±0.022	3.15±0.10
32A	Semi-periphery	3	371±4	10.90±0.50	1138±25	93.70±1.28	42.5±0.3	0.333±0.042	0.08±0.007	1.105±0.037	6.87±0.11
32B	Semi-periphery	3	486±15	2.95±0.36	726±25	17.04±0.47	31.1±1.1	0.289±0.043	0.067±0.006	0.563±0.021	4.66±0.26
33A	Periphery	3	362±8	3.25±0.18	645±21	15.49±0.48	51.0±1.4	0.278±0.063	0.093±0.01	0.928±0.033	4.91±0.17
33B	Periphery	3	681±26	4.16±0.23	1055±51	18.47±1.12	53.0±1.6	0.346±0.042	0.072±0.008	0.843±0.028	5.81±0.12
41A	Centre	4	894±12	4.39±0.17	1021±24	13.85±0.50	30.8±0.8	0.394±0.032	0.064±0.002	0.754±0.012	4.00±0.10
41B	Centre	4	870±10	3.97±0.39	896±14	15.39±0.48	34.4±1.1	0.336±0.017	0.068±0.006	0.68±0.037	4.73±0.26
42A	Semi-periphery	4	465±10	4.61±0.34	926±6	43.03±0.44	46.6±2.2	0.369±0.032	0.072±0.009	1.081±0.04	6.37±0.25
42B	Semi-periphery	4	847±28	3.29±0.16	920±25	15.09±0.98	74.3±1.4	0.377±0.04	0.073±0.002	0.492±0.027	5.60±0.19
43A	Periphery	4	589±12	2.74±0.27	708±18	8.99±0.29	25.7±1.2	0.26±0.02	0.07±0.003	0.543±0.042	4.16±0.10
43B	Periphery	4	429±9	3.09±0.19	553±8	8.75±0.19	28.6±0.8	0.305±0.046	0.069±0.003	0.481±0.012	4.03±0.08
51A	Centre	5	865±15	4.85±0.15	1158±27	32.03±0.84	40.0±0.8	0.377±0.037	0.071±0.003	1.115±0.028	5.02±0.05
51B	Centre	5	989±30	5.72±0.25	1349±23	42.44±1.47	53.4±1.0	0.399±0.022	0.067±0.007	1.639±0.067	4.10±0.10
52A	Semi-periphery	5	464±5	2.20±0.10	492±14	12.24±0.40	29.0±0.3	0.24±0.037	0.072±0.009	0.385±0.021	4.90±0.17
52B	Semi-periphery	5	935±14	4.07±0.20	981±19	11.75±0.41	35.7±0.5	0.422±0.019	0.089±0.009	0.748±0.037	11.90±0.30
53A	Periphery	5	581±11	2.57±0.17	627±8	9.21±0.10	16.8±0.8	0.279±0.04	0.055±0.005	0.29±0.014	2.87±0.05
53B	Periphery	5	733±10	3.38±0.22	813±11	13.14±0.53	17.4±0.5	0.347±0.066	0.104±0.012	0.534±0.028	3.83±0.16
61A	Centre	6	1060±21	3.25±0.10	827±15	8.28±0.17	27.2±1.0	0.332±0.037	0.062±0.008	0.546±0.015	4.76±0.14
61B	Centre	6	822±14	4.51±0.30	962±30	15.38±0.71	29.0±0.5	0.246±0.013	0.048±0.001	0.618±0.024	4.05±0.14
62A	Semi-periphery	6	495±17	3.72±0.19	870±34	20.46±0.63	39.5±1.2	0.33±0.043	0.081±0.009	1.015±0.028	5.91±0.15
62B	Semi-periphery	6	784±12	2.79±0.33	710±15	7.07±0.21	28.5±1.4	0.308±0.019	0.072±0.007	0.521±0.023	4.24±0.21
63A	Periphery	6	733±7	4.81±0.19	902±15	16.09±0.34	41.1±1.2	0.525±0.077	0.085±0.007	0.949±0.031	5.76±0.19
63B	Periphery	6	341±12	3.23±0.22	727±11	17.98±0.44	39.5±1.3	0.28±0.02	0.069±0.003	0.822±0.018	4.12±0.09
71A	Centre	7	1042±18	4.99±0.25	1214±27	25.24±0.75	50.4±1.8	0.365±0.059	0.082±0.006	1.335±0.02	8.03±0.23
71B	Centre	7	1090±28	3.33±0.16	909±16	15.26±0.24	38.4±1.2	0.322±0.046	0.057±0.006	0.576±0.034	5.14±0.11
72A	Semi-periphery	7	525±15	2.73±0.05	656±30	8.80±0.43	58.7±1.5	0.271±0.017	0.121±0.012	0.551±0.03	4.73±0.04

72B	Semi-periphery	7	509±8	2.62±0.17	699±11	7.27±0.24	28.6±0.8	0.278±0.036	0.071±0.007	0.473±0.032	5.89±0.18
73A	Periphery	7	368±7	2.45±0.12	549±18	7.78±0.19	30.4±0.9	0.267±0.02	0.057±0.003	0.607±0.021	3.81±0.08
73B	Periphery	7	529±16	3.21±0.20	784±27	5.45±0.43	26.3±0.6	0.332±0.044	0.059±0.007	0.508±0.017	4.02±0.06
81A	Centre	8	442±14	3.20±0.52	709±9	14.39±0.21	38.1±2.0	0.259±0.034	0.063±0.005	0.754±0.025	5.32±0.24
81B	Centre	8	606±15	4.99±0.27	1115±27	31.12±1.07	40.9±1.5	0.318±0.035	0.067±0.001	1.06±0.037	7.67±0.18
82A	Semi-periphery	8	438±18	3.53±0.39	797±38	20.16±0.86	60.6±1.6	0.314±0.048	0.073±0.011	0.954±0.024	8.64±0.16
82B	Semi-periphery	8	479±21	2.92±0.17	683±20	15.48±0.62	39.3±0.8	0.286±0.029	0.057±0.009	0.675±0.058	7.69±0.28
83A	Periphery	8	583±12	2.97±0.19	658±9	8.27±0.33	22.7±0.9	0.309±0.037	0.092±0.01	0.724±0.026	14.96±0.61
83B	Periphery	8	543±24	2.65±0.29	623±19	11.31±0.47	27.3±1.1	0.268±0.017	0.114±0.012	0.457±0.028	7.69±0.21
91A	Centre	9	689±34	4.64±0.20	996±43	27.54±1.53	41.8±0.7	0.323±0.039	0.075±0.004	0.926±0.031	9.11±0.20
91B	Centre	9	449±6	3.68±0.23	805±0	21.32±0.99	42.3±0.5	0.311±0.044	0.079±0.008	0.741±0.03	7.54±0.16
92A	Semi-periphery	9	670±13	4.79±0.21	1085±13	19.59±0.42	85.7±3.3	0.434±0.025	0.13±0.005	1.714±0.037	27.61±0.98
92B	Semi-periphery	9	766±18	3.01±0.13	657±12	10.80±0.46	22.1±0.6	0.272±0.03	0.057±0.004	0.487±0.024	7.42±0.14
93A	Periphery	9	611±10	3.45±0.10	762±18	20.60±0.83	40.6±1.2	0.296±0.023	0.095±0.007	1.545±0.048	21.54±0.63
93B	Periphery	9	620±23	2.61±0.13	676±30	11.38±0.77	33.8±1.1	0.301±0.038	0.081±0.008	0.47±0.035	11.28±0.31

APPENDIX B: Questionnaire in Italian language



La qualità dell'aria a Milano Cosa ne pensa chi vive o lavora in città?



Grazie per partecipare a questo sondaggio sulla qualità dell'aria di Milano. Il questionario è anonimo. Per cominciare, potrebbe indicare sulla mappa ...

<input type="checkbox"/> ... dove vive a Milano? <input type="checkbox"/> Non vivo a Milano.		<input type="checkbox"/> ... dove lavoro studia a Milano? <input type="checkbox"/> Non lavoro o studio a Milano.	
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Quando sono a Milano posso percepire l'inquinamento dell'aria... (risponda a tutte le domande)

... dagli odori sgradevoli che sento o dalla sensazione di pesantezza dell'aria.	<input type="checkbox"/> Si	<input type="checkbox"/> No
... vedendo la cappa di smog.	<input type="checkbox"/> Si	<input type="checkbox"/> No
... vedendo della polvere scura che ricopre gli oggetti all'aperto.	<input type="checkbox"/> Si	<input type="checkbox"/> No
... associandolo al rumore che fanno le macchine.	<input type="checkbox"/> Si	<input type="checkbox"/> No
... sul mio corpo (es. sulla pelle, sul viso, nei capelli).	<input type="checkbox"/> Si	<input type="checkbox"/> No
Durante una giornata di smog intenso ...		
... evito di aprire le finestre.	<input type="checkbox"/> Si	<input type="checkbox"/> No
... non faccio sport all'aperto.	<input type="checkbox"/> Si	<input type="checkbox"/> No
... vado al lavoro in macchina.	<input type="checkbox"/> Si	<input type="checkbox"/> No
... vado al lavoro con i mezzi pubblici.	<input type="checkbox"/> Si	<input type="checkbox"/> No
Frequentando Milano, ho spesso ...		
... gola secca e arrossata.	<input type="checkbox"/> Si	<input type="checkbox"/> No
... prurito agli occhi.	<input type="checkbox"/> Si	<input type="checkbox"/> No
... problemi respiratori.	<input type="checkbox"/> Si	<input type="checkbox"/> No

Grazie. Adesso, vorremmo sapere quanto è d'accordo con le seguenti affermazioni, riferite alla città di Milano.

	Per niente d'accordo	Poco d'accordo	Né d'accordo né in d'accordo	Abbastanza d'accordo	Completamente d'accordo
È più probabile avere problemi di salute legati all'inquinamento dell'aria, che non essere coinvolti in un incidente stradale.	1	2	3	4	5
L'inquinamento dell'aria è un problema serio, per chi vive o lavora qui.	1	2	3	4	5

	Per niente d'accordo	Poco d'accordo	Né d'accordo, né in disaccordo	Abbastanza d'accordo	Completamente d'accordo
I bambini sono più danneggiati dall'inquinamento dell'aria, rispetto agli adulti.	1	2	3	4	5
Le aree verdi, come i parchi pubblici ed i viali alberati, migliorano la qualità dell'aria qui in città.	1	2	3	4	5
Rispetto al resto di Milano, la zona dove abito ha una qualità dell'aria migliore.	1	2	3	4	5
Rispetto al resto di Milano, la zona dove lavoro/studio (per la maggior parte del tempo) ha una qualità dell'aria migliore.	1	2	3	4	5
Studiando le piante, si può capire meglio quanto è inquinata l'aria in città.	1	2	3	4	5

A Milano attualmente è in corso uno studio dell'inquinamento dell'aria basato sull'analisi delle piante.

	Poco importante			Molto importante	
Secondo lei quanto è importante per chi vive a Milano?	1	2	3	4	5
Quanto sarebbe interessato a saperne di più su uno studio del genere?	Per nulla			Molto	
	1	2	3	4	5

Ci potrebbe dire quanto spesso sente parlare dell'inquinamento dell'aria a Milano, sui seguenti mezzi di informazione?

Televisione e radio.	<input type="checkbox"/> Non li utilizzo	<input type="checkbox"/> Mai	<input type="checkbox"/> Raramente	<input type="checkbox"/> Regolarmente	<input type="checkbox"/> Spesso
Giornali e riviste.	<input type="checkbox"/> Non li leggo	<input type="checkbox"/> Mai	<input type="checkbox"/> Raramente	<input type="checkbox"/> Regolarmente	<input type="checkbox"/> Spesso
Siti internet (es. Comune di Milano).	<input type="checkbox"/> Non li utilizzo	<input type="checkbox"/> Mai	<input type="checkbox"/> Raramente	<input type="checkbox"/> Regolarmente	<input type="checkbox"/> Spesso
Social network (es. Facebook, Instagram).	<input type="checkbox"/> Non li utilizzo	<input type="checkbox"/> Mai	<input type="checkbox"/> Raramente	<input type="checkbox"/> Regolarmente	<input type="checkbox"/> Spesso

Ci potrebbe dire, quanto spesso utilizza i seguenti mezzi di trasporto per spostarsi a Milano?

Automobile o motorino.	<input type="checkbox"/> Mai	<input type="checkbox"/> Raramente	<input type="checkbox"/> Regolarmente	<input type="checkbox"/> Spesso	<input type="checkbox"/> Sempre
Bicicletta.	<input type="checkbox"/> Mai	<input type="checkbox"/> Raramente	<input type="checkbox"/> Regolarmente	<input type="checkbox"/> Spesso	<input type="checkbox"/> Sempre
Mezzi pubblici (bus, metro, tram).	<input type="checkbox"/> Mai	<input type="checkbox"/> Raramente	<input type="checkbox"/> Regolarmente	<input type="checkbox"/> Spesso	<input type="checkbox"/> Sempre
A piedi.	<input type="checkbox"/> Mai	<input type="checkbox"/> Raramente	<input type="checkbox"/> Regolarmente	<input type="checkbox"/> Spesso	<input type="checkbox"/> Sempre

Lei è ... Uomo Donna Titolo di studio: Elementari Medie Superiori Laurea

Età (anni): 18-25 26-40 41-55 56-65 più di 65