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Trace Element Concentrations Measured in a Biomonitor (Tree Bark) for Assessing Mortality and Morbidity of Urban Population: A New Promising Approach for Exploiting the Potential of Public Health Data

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Abstract: The usefulness of bioindicators to study the state of the environment in different compartments (air, water, and soil) has been demonstrated for a long time. All persistent pollutants can be measured in some form of bioindicator, and numerous organisms are suitable for the biomonitoring purpose. In most of the works on this topic, bioindicators are used to highlight the impact of human activities. Generally, samples collected from polluted areas are compared with samples from an area considered as clean, or samples from areas characterized by different pollution sources are compared with each other. An approach that has not been attempted consists in correlating directly data on environmental quality obtained by bioindicators with parameters measuring the population health. In the present study, the concentrations of As, Cd, Co, Cu, Fe, Mn, Ni, Pb, V, and Zn measured by atomic emission spectrometry (ICP OES) in 56 samples of holm oak bark from trees located in urban parks and along streets in a Northern Italy city were related to the data describing the health status of the citizens. The concentrations of some of the 10 trace elements in the bioindicator were found significantly correlated with mortality and morbidity data regarding cardiac and respiratory diseases. The results, although preliminary, show the potential of this approach for implementing strategies aimed for disease prevention and health promotion in urban areas at risk, with the objective of reducing environmental and health inequalities.

Keywords: air quality; bioindicators; health promotion; morbidity; mortality; urban health

1. Introduction

Since the 1990s, significant correlations between the airborne particulate material (PM) and several adverse health effects have been widely documented, both for short- and long-term exposures [1,2]. In the literature, a large number of studies correlate the presence of PM with mortality all over the world [3,4] and with a series of health problems, i.e., respiratory symptoms, decreased lung function, allergies, exacerbation of chronic respiratory and cardiovascular diseases [5]. These health effects are responsible for an increase of hospital admissions and emergency room visits, especially for the most susceptible subgroups of population, such as children and elderly people [6]. Several studies have also outlined a relationship between maternal PM exposure and adverse birth outcomes, including low birth weight and preterm birth [7,8]. Moreover, environmental pollution has been associated with an increased risk of psychiatric disorders in Europe, Asia, and North America [9–11]. Very recently, a possible relationship between atmospheric pollution and increased risk of transmissible diseases has been an object of study [12–17].

Since PM_{10} has been routinely measured in urban environment, most of epidemiological studies performed in many parts of the world during the past years are based on PM_{10} , which is considered as an exposure indicator. More recently, the number of studies based on $PM_{2.5}$ as a proxy indicator of exposure to air pollution is increased, although the number of cities and towns having the possibility of performing $PM_{2.5}$ measurements is still limited, especially in low-income countries [18].

While the influence of PM and particle size on human health is certain [5], little information is available on the influence of chemical composition and on the effect of trace elements [19–21]. This is also due to the fact that the amount of PM present in the atmosphere is relatively easy to be measured, while it is much more difficult to determine the present trace elements and their levels; in fact, the composition of PM changes very quickly, and it is highly spatially heterogeneous. Therefore, systems able to provide integrated information over time and space are necessary. A help can come from the use of bioindicators allowing obtaining information integrated in time and space in a relatively simple and economic way, at least when trace elements or other persistent pollutants are studied.

In previous works [22,23], the use of tree bark as a bioindicator of atmospheric contaminants provided information on the concentrations of several trace elements in the city of Genoa (NW Italy) and, for comparison, in a small town located 30 km east of Genoa, without important industrial activities [24]. The samples were collected in order to study different urban areas (UAs) with a high spatial density, which could not be achieved using the limited number of monitoring stations available (in Genoa, only two are available for the measurements of Pb, As, Cd, and Ni). This approach was used to draw maps of the city, allowing identifying the most critical areas and indicating the presence of point sources of pollution [22].

Starting from those results, in the present work, an explorative study was performed in order to verify if the information provided by bioindicators was significantly correlated with the available data on the health status of the population living in the areas, where the bioindicators were sampled.

While many studies regarding the effects of environmental pollution on health are based on pollution data (PM , NO_x) obtained by simulation and mathematical models [25,26], the approach here proposed is based on experimental measurements combining the advantages of high spatial density and low cost. Linking directly chemical and epidemiological data may help to plan ad hoc sampling in urban environments and to implement effective health programmes and services.

2. Experiments

The bioindicator chosen was the bark of a tree species, holm oak (*Quercus ilex* L.), which, due to its finely squared fissured structure and its persistence for years, has proven to be capable of providing information about the presence of metals in the atmosphere [24,27,28]. A total of 56 bark samples were collected in 2014–2015 in the city of Genoa (North Western Italy) from UAs characterized by different types of pollution (vehicular traffic, domestic heating, maritime and port activities, and industries) [22]. The trees selected were located in urban parks or along streets (within 5 m from the edge): the bark was sampled at a height of 1.50–1.80 m from the ground. For comparison, 10 samples were collected in a small seaside town (approximately 30,000 inhabitants) located 30 km east of Genoa, without important industrial activities [24].

After freeze-drying and homogenization in 25 mL Teflon grinding jars containing zirconium oxide grinding balls by a MM2 mixer mill (Retsch GmbH, Haan, Germany), amounts of 0.10–0.15 g of each sample were mineralized with 65% (m/m) nitric acid (for trace metal analysis, from Scharlau, Barcelona, Spain) in closed Teflon PFA vessels heated in a microwave digestion system MDS 2000 (CEM Corporation, Matthews, NC, USA). After cooling, the solutions, transferred into 25 mL volumetric flasks, were diluted to 25 mL using ultrapure (>18 MOhm cm) water (Elgastat UHQ, Elga Ltd., High Wycombe, UK). The chemical data used in the present study were the concentrations of 10 trace elements (As, Cd, Co, Cu, Fe, Mn, Ni, Pb, V, and Zn), measured in 66 bark samples by atomic emission spectrometry with a plasma source (ICP OES, iCAP 7000 Series, Thermo Scientific, Cambridge, UK) and an axial plasma view. Calibration was carried out with aqueous standard solutions in 3 M nitric acid, using scandium

at 4 µg/mL as an internal standard. Three blanks for each run of nine samples were analyzed in order to check any possible contamination. The accuracy was assessed by analyzing a certified reference material (CRM 482 (Lichen) certified by the European Commission's Joint Research Centre—Institute for Reference Materials and Measurements), and satisfactory results were obtained [22]. More details on tree species, sampling, sample treatment, instrumentation, and analysis were reported in previous papers [22,24].

Genoa, the capital of the Liguria region, is the sixth largest Italian city, with a population of 578,000 inhabitants [29]. The city is subdivided into nine “municipi” (administrative districts), with a population in the range between 58,000 and 90,000 inhabitants (9.5–14.8% of the total) and including a different number of UAs, called “circonscrizioni”. Each UA (25 in total with each having approximately 25,000 inhabitants and a range of 10,000–40,000) corresponds to a traditional territorial subdivision, with certain characteristics in terms of population distribution, urban environment, and productive activities. Regarding the local structure of health care, Genoa is divided into six Social Health Districts (SHDs), operational structures locally ensuring health promotion and protection, according to integrated social and health policies.

In the present study, the health status of the citizens was measured in terms of mortality and morbidity, using indicators selected from the internationally recognized ECHI list [30]. The values of these indicators are collected at the national level by the surveillance system “PASSI”, established in Italy in 2007 to monitor health behavior and associated risk factors [31]. These data are publicly available and provide a health profile of the population of the sampled area and yearly updated [32].

In detail, mortality data were expressed in terms of standardized mortality ratio (SMR), i.e., the ratio between the number of the observed deaths in the study group and the number of the expected deaths in the general population. The SMR data for the 25 UAs of the city of Genoa in the period 2009–2017 and for the small town located 30 km from Genoa were obtained from the reports of Russo and Gennaro [33,34]. The incidence of different noncommunicable diseases was also considered: chronic respiratory disease (obstructive chronic bronchitis, asthma, and respiratory failure), hypertensive disease (essential and secondary hypertension), heart disease (hypertensive and ischemic heart diseases), and heart failure. Moreover, the number of regular smokers and a composite deprivation index were considered as a determinant of health and an indicator of the socioeconomic situation, respectively. The data used for this study, grouped by SHD, refer to the 2016 data and are available from the website of the Ligurian Health Agency [35], where each indicator is explained in detail.

The Systat software for Windows version 13 (Systat Software Inc., Chicago, IL, USA) was used for performing statistical analysis and producing graphs. The correlations between chemical and health variables were computed using Pearson correlation coefficients (r) and Spearman rank-order correlation coefficients (ρ) as a nonparametric measure of the association between variables; taking into account the kind and number of data available, statistical significance was set at a p -value of <0.1 in order not to discard results, which might deserve further evaluations.

3. Results and Discussion

In order to highlight the relationships, if any, between element concentrations in the bioindicator and SMR data, the samples of tree bark were grouped by UA, and the median value of the concentration of each element was computed. Since the samples were collected in 14 of the 25 Genoa UAs, the data set studied contained 15 areas, i.e., the small town and the 14 UAs of the city. Each area was described by the SMR value and by the 10 median values, one for each element, computed from 3 to 5 trees (10 in the small town) collected in that area.

The SMR values were found significantly correlated with the concentrations of Fe ($r = 0.591$; $p = 0.020$) and Mn ($r = 0.494$; $p = 0.061$). Although the correlations and the associated statistical tests can be considered convincing, one data point showed a high leverage. It corresponded to the UA characterized by the highest mortality and by levels of several elements (Fe, As, and Zn), which were nearly twice with respect to the other UAs and were related to the activities of a steel plant in the past

years [22]. The measurements of the Fe concentration in the bioindicator reflect the mortality in the various areas of the city with satisfying approximation.

Besides SMR, a number of indicators of the health status of the population were also taken into account, reporting the incidence of a certain disease per 1000 inhabitants on the basis of hospital discharge forms and drug prescriptions [35]. This part of the study was made difficult by the small number of data available: in fact, these indicators, although they had potentially richer information than the mortality data, were publicly available as aggregated by SHD ($n = 6$ in Genoa), and not by UA ($n = 25$), with a consequent reduction of dimensionality. It is well-known that the analysis of the relationships between environmental and health data is strongly affected by the level of spatial resolution [36,37]. Considering that for one of the six SHDs of Genoa, no samples were collected, the tree bark data were divided into six groups (one for the small town and five for the city), and the median values were computed. Despite the poor spatial resolution of the health data available, significant correlations were found between the Pb concentration in the bioindicator and the incidence of heart diseases in the population (Figure 1), i.e., heart disease without decompensation ($r = 0.723$; $p = 0.100$) and decompensated heart failure ($r = 0.919$; $p = 0.010$). The study of the relationships between variables was also performed using the nonparametric Spearman rank-order correlation coefficients (ρ): significant correlations were found between the concentrations of Cd and Pb and the decompensated heart failure (for both elements, $\rho = 0.829$; $p = 0.025$).

Moreover, as shown in Figure 2, the Pb concentration was also found significantly correlated with the incidence, among urban residents, of chronic respiratory disease, i.e., chronic obstructive pulmonary disease (COPD), chronic bronchitis, asthma and respiratory failure ($r = 0.930$; $p = 0.022$; $\rho = 0.700$; $p = 0.100$), and V concentration with heart failure ($r = 0.821$; $p = 0.089$; $\rho = 0.800$; $p = 0.050$).

The observed relationships were confirmed when the element concentrations were related to mortality data for specific causes; significant correlations were found between the Pb concentration and the ischemic SMR ($r = 0.853$; $p = 0.031$; $\rho = 0.696$; $p = 0.100$) and between the V concentration and the SMR from circulatory diseases ($r = 0.731$; $p = 0.099$; $\rho = 0.754$; $p = 0.030$) (Figure 3).

These findings are in agreement with previous studies [24] highlighting that Pb was the element with the greatest enrichment factor in the UAs of Genoa, with concentrations which are seven times higher with respect to that in the small town. Moreover, shipping emissions are responsible for high atmospheric levels of V in important port cities [38,39]. In Genoa, the maritime traffic takes place mainly in the western part of the city, characterized by V concentrations twice that in the eastern part. A correlation between the level of V in atmospheric PM and cardiovascular hospitalization was found in several studies [40,41].

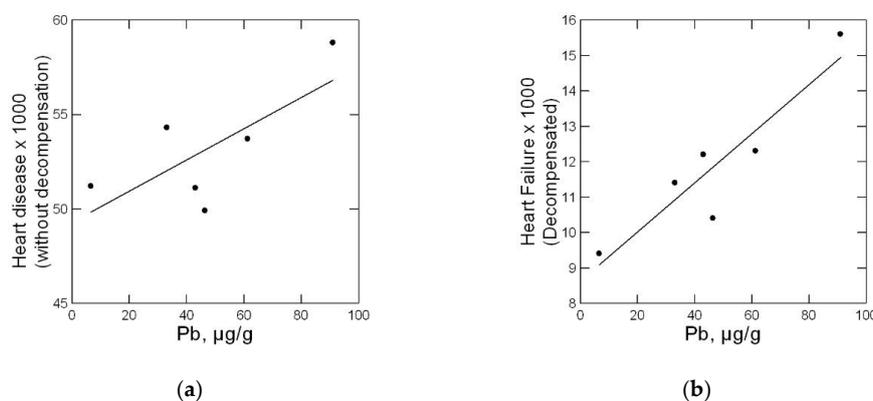


Figure 1. Correlation between the median value of the Pb concentration (as $\mu\text{g/g}$) measured in samples of tree bark and the incidence of heart diseases in six Socio Health Districts: (a) heart disease without decompensation $\times 1000$ inhabitants ($r = 0.723$; $p = 0.100$); (b) decompensated heart failure $\times 1000$ inhabitants ($r = 0.919$; $p = 0.010$).

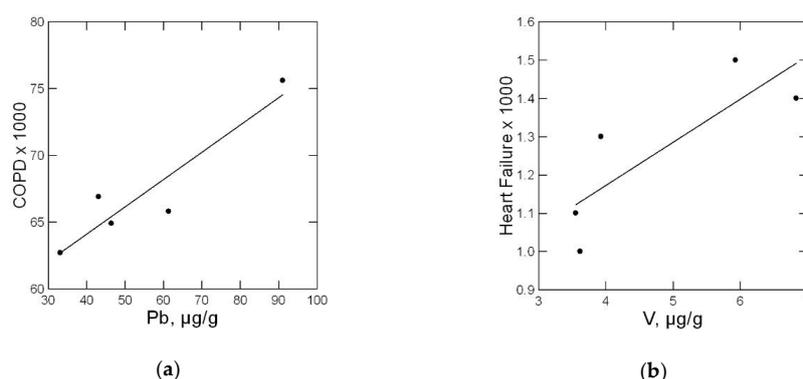


Figure 2. Correlation between the element concentrations and the health indicators of the population living in the five Socio Health Districts of the city of Genoa: **(a)** the relationship between the median value of the Pb concentration (as µg/g) measured in the samples of tree bark and the incidence of chronic respiratory disease (COPD) × 1000 inhabitants ($r = 0.930$; $p = 0.022$); **(b)** the relationship between the median value of the V concentration (as µg/g) measured in the samples of tree bark and the incidence of heart failure × 1000 inhabitants ($r = 0.821$; $p = 0.089$).

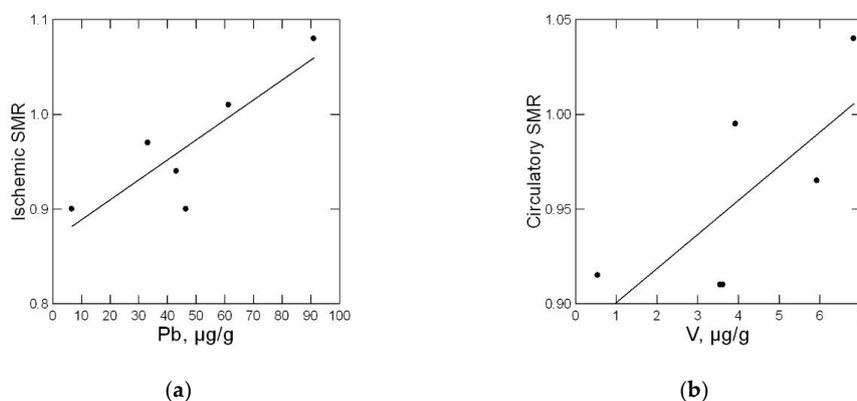


Figure 3. Correlation between the element concentrations and the cause-specific mortality in six Socio Health Districts: **(a)** the relationship between the median value of the Pb concentration (as µg/g) measured in the samples of tree bark and the ischemic standardized mortality ratio (SMR) ($r = 0.853$; $p = 0.031$); **(b)** the relationship between the median value of the V concentration (as µg/g) measured in the samples of tree bark and the SMR from circulatory diseases ($r = 0.731$; $p = 0.099$).

Actually, the division of the territory in SHDs does not provide a sufficient spatial resolution, and the data aggregated by UA would be more useful. However, the results of the present study, although preliminary, point out the feasibility of an investigation that would permit obtaining information on the health status of urban populations, combining high spatial density and low cost. Furthermore, recent investigations on other types of bioindicators have shown that there are measurements able to provide information on atmospheric metal pollution by means of other analytical techniques, even simpler and cheaper than atomic emission spectrometry, such as X-ray fluorescence [42–44] and magnetic analysis [45–49]. This would make the approach accessible to a large number of laboratories in terms of purchase and operating costs of equipment.

4. Conclusions

The data obtained from bioindicators that were used in this study do not represent, in an optimal way, the data of mortality (by UA) and morbidity (by SHD), since sampling was planned to highlight the different anthropic sources of metal pollution. However, the indications that can be drawn from the data analysis carried out show that bioindicators can be useful to plan an ad hoc study aimed to assess the health status of the population in urban environments. The advantages of this approach

are numerous for the involved stakeholders, citizens, and public authorities. In fact, a bioindicator, when properly chosen, allows getting information with a spatial resolution, which cannot be obtained with traditional monitoring stations. In addition, bioindicators add the temporal dimension to the data collected; in fact, they are not limited to the observation at the moment of sampling, but reflect the exposition during a more or less long period of time, since they are collected in streets, where people live. The possibility of measuring different parameters in each bioindicator sample by means of a simple technique at affordable costs can be useful to identify the source of a health problem and therefore may help in implementing actions designed to limit the negative impact of environmental pollution on human health or improving the effectiveness of the existing interventions. The inclusion of sociodemographic information offers the possibility of reducing inequalities by addressing health risks.

On the basis of these indications, the present research might proceed in two directions: (1) exploring UAs not yet covered by the initial sampling plan; (2) better integrating data from bioindicators and records from the systems of healthcare information in higher spatial detail, always in compliance with the privacy laws applied to the processing of confidential data.

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