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Metal Accumulation Capability by *Platanus acerifolia* (Aiton) Willd., *Ailantus altissima* (Mill.) Swingle, *Robinia pseudoacacia* L. and *Quercus ilex* L., Largely Distributed in the City of Rome

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Abstract

The main of the research was to analyze the leaf metal accumulation capability of *Platanus acerifolia* (Aiton) Willd., *Ailantus altissima* (Mill.) Swingle, *Robinia pseudoacacia* L. and *Quercus ilex* L., largely distributed in Rome. In addition, metal concentration was analyzed in the soil, sampling sites were chosen in historical parks (A sites) and high traffic level sites (B sites). The results highlight significant higher leaf and soil metal concentrations in B than in A sites. The ratio between metal concentration in leaves and soils (Biological Absorption Coefficient, BAC) for all the considered sites was significantly different among the species. Morphological and anatomical leaf traits of the considered species show significant differences in A and B sites in response to traffic level. Overall, the results highlight the importance of the selection of tree species in urban areas for their ability to lower pollution levels.

Keywords

Metals, Leaf, Soil, Urban Areas, Traffic Density, Portable X-Ray Fluorescence

1. Introduction

Atmospheric pollution is one of the most important problems in urban areas worldwide [1] [2] [3] having significant impacts on the population health, particularly in cities. In Europe, more than 85% of people are exposed to air pollution in exceedance of the European standards [4]. Anthropogenic suspended particles in the atmosphere are usually associated with metals in airborne dust generated by traffic or industrial activity [5]. Vehicular traffic is one of the most significant sources of heavy metal emissions in urban areas, which has increased in the last years [6] [7] contributing by 57% - 75% to total emissions [8]. Air particulate is rich in metals, such as chromium, cadmium, copper, iron, nickel, lead, and zinc that can be a health hazard when incorporated into the body through inhalation [9]. Most of the toxic metals in the air are in form of fine particles, ranging from 0.003 μm to 10 μm [10]. [11] identified the association of traffic-related air pollution and the increase in myocardial infarction events, considering that heart disease and stroke are the most common reasons for premature death attributable to air pollution for 80% of the cases [9]. Nevertheless, vegetation that covers comparatively large segments of urban areas (*i.e.* private and public gardens, parks, sport fields, hedges and tree-lined avenues) is a valuable asset for modern cities delivering key functions and benefits [12]. In particular, urban green spaces play an important role from a social perspective by promoting physical activity, increasing people interaction [13] and reducing stress factors. Moreover, plants may reduce metal pollution levels by accumulating particulate matter on their surfaces [14] [15]. Particles can be deposited on plant surfaces and accumulated through sedimentation under the influence of gravity through impaction resulting from wind [16]. A large amount of these particles are accumulated in internal leaf tissues [17] by absorbance via roots. The particles can be transferred from the leaf surface to the soil by rain [18]. The topsoil has a large quantity of metals due to its high amount of organic substance [19]. The extent of metals in urban soils depends also on the deposition time as well as the transport rate [20]. As for plants, in comparison to annuals, trees trap and store more particles due to their larger total leaf surface area [21]. Nevertheless, each species has a different capability of particles capture [22] evergreens being better traps for particles than deciduous species because of their longer leaf longevity, which can accumulate particles throughout the year [17]. Moreover, deposition rates depend on air movement in the crown, transfer through the boundary layer adjacent to surfaces and the absorption capability of leaves. Thus, information on atmospheric pollution can be deduced from the concentration of specific substances in plant tissues offering low-cost information about the environmental quality [8] [23]. Nevertheless, the basic criteria for the selection of plants for biomonitoring are that species should be present in a large number all over the monitoring area and widely spread, thus providing a high density of sampling points.

In such context, the main objective of this research was to analyze the metal accumulation capability by *Platanus acerifolia* (Aiton) Willd., *Ailantus altissima* (Mill.) Swingle, *Robinia pseudoacacia* L. and *Quercus ilex* L., largely distributed in Rome. Rome is among the largest European cities with a large extension of the urbanized area (129.000 ha, of which 43,000 ha of greening and 50,000 ha of agricultural areas), 2,872,800 inhabitants [24], the dominant role of expansion and a large movement of public and private means of transportation (3,505,795; data from Automobile Club Italy 2017) which is a significant source of pollution emissions. The largest pollution emission occurs during the daytime peaking in the first hours of the morning when traffic density is the and from autumn to spring, decreasing in summer when traffic density decreases. Rome is also characterized by a high volume of green areas with important historical parks [25]. The urbanization process in Rome has been increasing during the last years, and many new suburban areas have been built by scaling down free areas surrounding the city, which is changing into a mega city. For such characteristics, Rome may represent an ideal system to study the possibility of improving environmental quality by the selection of plant species that may contribute to lower pollution levels. Our data concerning *P. acerifolia*, *R. pseudoacacia*, *A. altissima* and *Q. ilex* metal accumulation capability can be incorporated into a geographic information system available for urban greening projects and can be exported to other urban areas.

2. Materials and Methods

2.1. Study Area and Climate

The study was carried out in Rome (41˚53'N 12˚29'E) in the period April-July 2019. Different types of sites were selected: historical parks (A sites) and high traffic density sites (B sites) (**Figure 1** and **Table 1**). Traffic density (number of cars min^{-1}) was monitored in the selected streets in June 2019 (mean value of the first 10 days of June) from 7:30 to 10:00 (peak hours, Gratani and Varone, 2013). In all the considered sites were present *P. acerifolia* (a naturalized species), *R. pseudoacacia* and *A. altissima* (two invasive alien species, IAS) and *Q. ilex* (a native species). The territory of Rome is between two distinct volcanic districts: the Albani Hills to southeast of the city and the Sabatini Mountains to northwest [26]. The natural vegetation of the city consists of strips of persistent meadows of the suburban areas, trampled down environments, shrubs, ruderal or nitrophylous vegetation and fragments of deciduous and evergreen woods [27]. Most avenues are characterized by *Quercus ilex*, *Quercus pubescens* Willd., *Platanus acerifolia* and *Pinus pinea* L.

Rome is under a Mediterranean type of climate. The average total annual rainfall was 841 mm most of it distributed in autumn and winter. The average maximum air temperature of the hottest months (July and August) was 31.9˚C ± 0.4˚C and the average minimum air temperature of the coldest month (January) was 4.8˚C ± 1.0˚C. The mean yearly air temperature was 16.7˚C ± 6.6˚C (**Figure 2**). In the year 2019, from January to July, total rainfall was 430.5 mm with a peak in May (101.7 mm), the mean minimum air temperature was in January (3˚C) and the mean maximum air temperature in July (33.6˚C). The mean air humidity was 67.6% (**Figure 3**) (data provided by the Lazio Regional Agency for Development and Agricultural Innovation Meteorological Station of Rome, Lanciani Street, for the period 2008-2018 and for the study period in the year 2019).

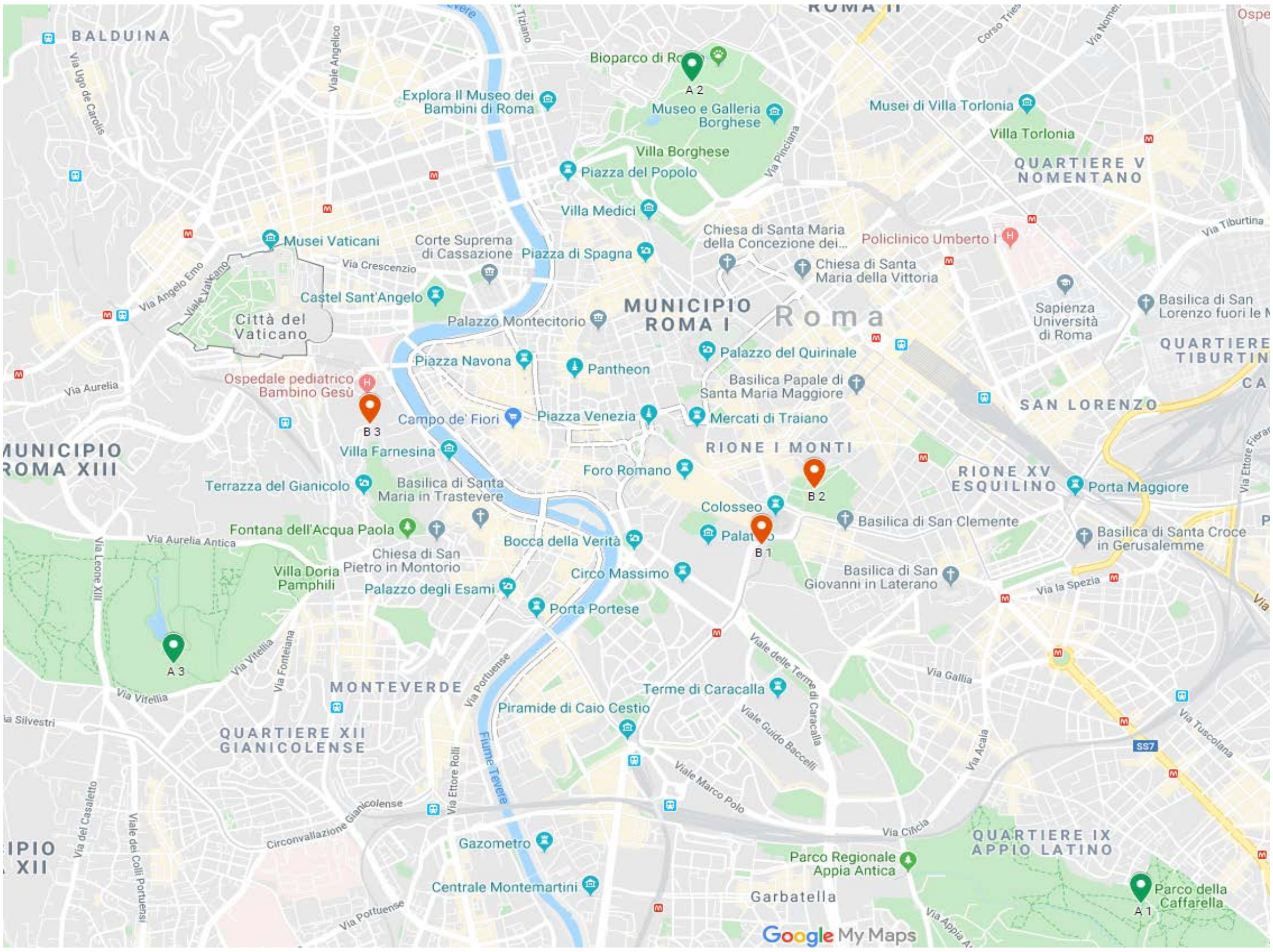

Figure 1. Map of the considered sites in Rome. A sites: historical parks (A1 = Caffarella Valley Park, A2 = Borghese Historical Park, A3 = Doria Pamphjli Historical Park); B sites: high traffic level sites (B1 = Celio Hill, B2 = Oppio Hill, B3 = Passeggiata del Gianicolo Street). From Google My Maps.

Table 1. Description of the considered sites: A sites (historical parks) and B sites (high traffic density streets).

Site	Latitude and Longitude	Short description
Caffarella Valley Park (A1)	(41˚50'N; 12˚33'E)	This site extends in the southeast of the city for 190 ha inside the Appia Antica Archeological Park (3.296 ha); the vegetation is constituted largely by trees. http://www.parcoarcheologicoappiaantica.it/luoghi/caffarella/
Borghese Historical Park (A2)	(41˚54'N; 12˚29'E)	This site extends over 80 ha in the city center; the vegetation covers 71.8% of the total surface of the park of which 55.6 are woods (Gratani et al; 2016).
Doria Pamphilj Historical Park (A3)	(41˚53'N; 12˚27'E)	This site extends for 184 ha in the west of the city; it is one of the largest historical park in the city; the vegetation covers 89.7% of the total surface 70% of it being woods (Gratani et al; 2016).
Celio Hill (B1)	(41˚53'N; 12˚29'E)	This site extends over 2 ha in the city center; it is delimited by streets characterized by an all-day high traffic density (95.8 cars min^{-1}).
Oppio Hill (B2)	(41˚53'N; 12˚29'E)	This site extends for 11 ha in the city center; it is delimited by streets, characterized by an all-day traffic density (59.5 cars min^{-1}).
Passeggiata Gianicolo Street (B3)	(41˚53'N; 12˚27'E)	This site extends from Trastevere district to Garibaldi Square. The selected site run alongside the Bambino Gesu' Hospital and is characterized by an all-day high traffic density (65 cars min^{-1}).

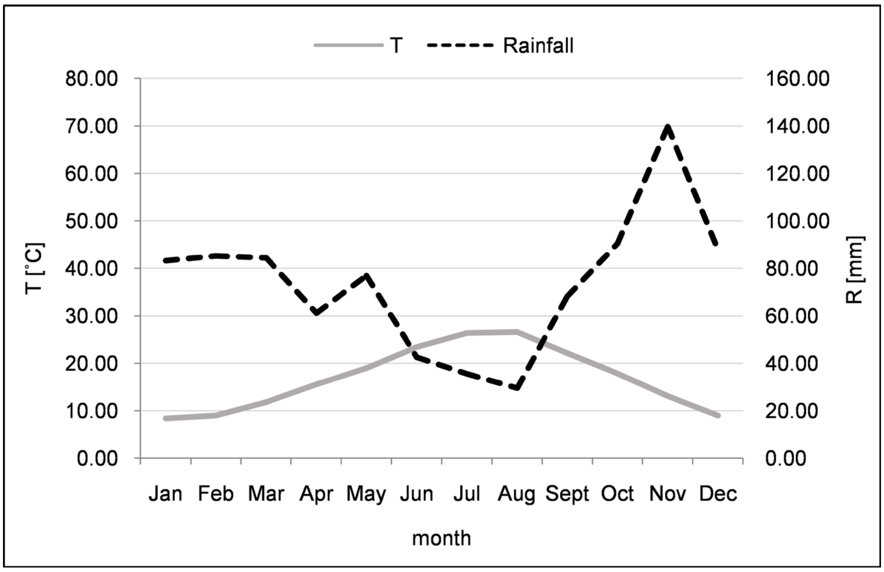

Figure 2. Climatic diagram according to Bagnouls and Gaussen (1953), for the period 2008-2018. The mean monthly air temperature (T˚) and the total monthly rainfall (R) are shown. The area between the two curves shows the period of summer drought. (Data provided by the Lazio Regional Agency for Development and Agricultural Innovation Meteorological Station of Rome, Lanciani Street).

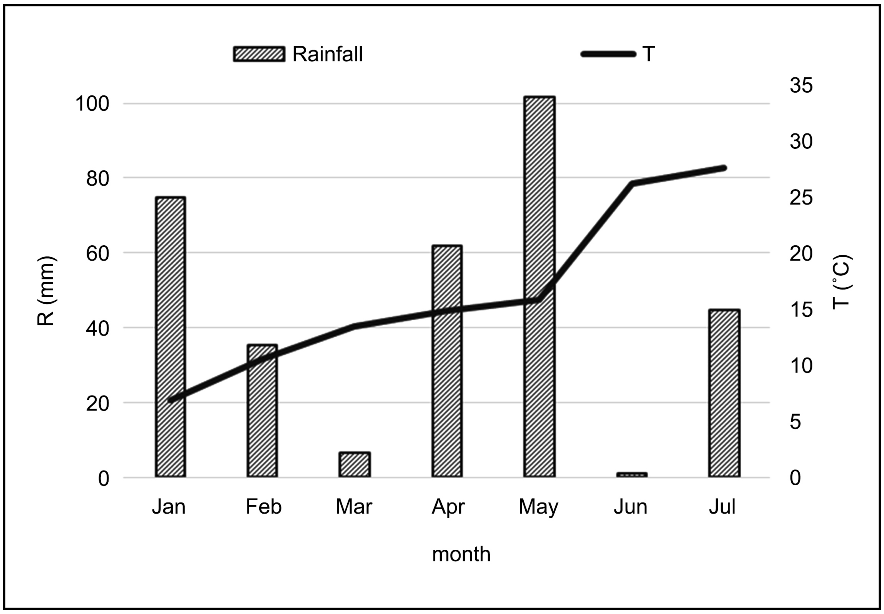

Figure 3. Trend of the total monthly rainfall (R) and the mean air temperature (T) from January to July 2019 (Data provided by the Lazio Regional Agency for Development and Agricultural Innovation Meteorological Station of Rome, Lanciani Street).

2.2. The Studied Species

Platanus acerifolia (Aiton) Willd., *Ailantus altissima* (Mill.) Swingle, *Robinia pseudoacacia* L. and *Quercus ilex* L. were studied.

Platanus acerifolia is a fertile hybrid, generated spontaneously among *Platanus orientalis* L., native to south-eastern Europe-Asia Minor and *Platanus occi-*

dentalis L., native to North America and introduced in Europe starting from the 16th century [28]. *P. acerifolia* is particularly resistant to stress factors including pollution. It can reach 40 m in height and the crown has an expanded globular-oval shape. The root system is characterized by main roots deepening into the soil with numerous branches, and secondary roots expanding horizontally and more superficially [28]. *P. acerifolia* was introduced in Rome in the late 1800s when the Piemontese Administration, in the ambit of the first regulatory plan of Roma Capitale (1873-1883), planted *P. acerifolia* trees in parks, squares and along avenues, particularly in those of the Lungotevere [29]. *Ailantus altissima* is a deciduous broad-leaved tree species native to North-Eastern China and North Vietnam [30]. It is widely distributed throughout many temperate regions of the world including all continents with the exception of Antarctica [31]. *A. altissima* was introduced in Europe in the late 17th century [32] and it is the most frequent non-native species in Italian cities [33]. *A. altissima* is among the most tolerant species to high pollution levels [34]. It may occur abundantly due to both an efficient sexual reproduction and a clonal growth. It is a shade intolerant species [35] although it can establish a ramet bank that can persist until a gap opens in the forest canopy; moreover, rapid establishment, growth and a vegetative reproduction in high light environments make disturbed areas such as timber harvests particularly prone to invasion by *A. altissima* [36]. Allelopathic compounds in leaves, woods and roots [37] may exacerbate competitive exclusion of native species aiding in the formation of dense, monotypic stands. *Robinia pseudoacacia* is a deciduous broad-leaved species native to north America [38] which is considered one of the most invasive species worldwide [39], especially in Mediterranean countries [40]. Originally introduced as a garden and landscape ornamental plant, this species has been widely planted in Europe [41] in the late 18th and early 19th century. The species was introduced in Italy in 1662 [42]. Allelopathic substances produced in bark and roots [43] and flavonoids isolated from leaves (robinetin, myricetin and quercetin) can inhibit the growth of herbaceous species. It is a light-demanding species, which rapidly colonizes forest gaps from seedlings and by sprouting from stumps and roots, but it tends to be replaced after 15 - 30 years by larger and more competitive tree species for its limited competitive ability [38]. *R. pseudoacacia* nitrogen-fixing capability facilitates the spread in urban areas where elevated CO_2 concentrations lead to a marked increase in nitrogen fixation [44]. *Quercus ilex* is a broadleaved evergreen species native to the Central-Western Mediterranean Basin [45]. Its natural distribution occurs from Portugal and Marocco to the Aegean Islands and Western Turkey, expanding northward up to Italy and France. Its altitudinal range is variable from the coast to 1800 m a.s.l. in southern Spain, and 2900 m a.s.l. in Marocco in the western part of the High Atlas [45]. It is limited in its southern range by the longer summer drought [46] and in latitude by factors associated with low air temperature. *Q. ilex* occurring in mesic through xeric habitats is a shade-tolerant species [47] which is widely used for restoration of heavily deforested areas and abandoned croplands in the Mediterranean Basin. *Q. ilex* has been widely used as ornamental tree in parks, gardens and avenues in the city of Rome since the 16th century.

2.3. Tree Structure

Measurements of tree structural traits were conducted in June 2019. Trees were selected in the considered sites (three plants per each species and per each site). Tree diameter at breast height (DBH, cm) was measured by callipters (Silvanus callipter) and by a DBH tape when the diameter was greater than 65 cm. Tree height and crown height (H and h, respectively) were measured by electronic clinometers (Haglof, Sweden).

2.4. Leaf and Soil Sampling

Fully expanded sun leaves were collected at the beginning of July from the external portion of the tree crown (south-eastern side) for leaf morphological traits (10 leaves per each species and per each site) anatomical traits (10 leaves per each species and per each site) and for mineral content analysis (30 leaves per each species and per each site). Soil samples were collected near the selected trees (500 g per each site) after removing litter, at a depth of 0-15 cm, according to [48]. Leaf and soil samples were stored in polyethylene bags and transferred immediately to the laboratory.

2.5. Leaf Morphology and Anatomy

The following morphological parameters were measured for each site: leaf area excluding the petiole (LA, cm^2), obtained by the Image Analysis System (Delta-T Devices, UK), leaf dry mass (DM, mg), drying at 80˚C to constant mass. Leaf mass per unit of leaf area (LMA, $mg \cdot cm^{-2}$) was calculated by the ratio between DM and LA. Total leaf thickness (LT, μm) was analyzed by a light microscopy using an image analysis system (Axiovision AC software).

2.6. Analytical Determination of Metals in Leaves and Soils

Leaf and soil samples were submitted to a pre-scanning treatment, according to [49]. Leaf samples were oven dry at 70˚ for 4 hours, ground to pass 2 mm sieve. Compressed air was used to clean the grinder and sieve between new samples processing to prevent cross contamination. Soil samples were treated with the same procedure. All leaf and soil samples were placed in hermetic plastic bottles and transferred to the laboratory of the Department of Soil and Plant, Texas Tech University TTU (Lubbock, Texas, USA) for the scanning process by a Portable X-Ray Fluorescence (PXRF) Olympus Vanta M series PXRF on line power (110 VAC) featuring an Rh X-ray tube in a Geochemical mode. Scanning (dwell) time was 45 s per beam, 2 beams ere scanned sequentially such that one scan was complete in 90 s. Samples were massed on a 4 μm prolene thin film, placed on the sample stage in a test stand, covered with a Pb cup and scanned.

The PXRF is a non-destructive proximal sensing instrument for the determination of elements in soil [50]. The iron (Fe), copper (Cu), lead (Pb), zinc (Zn), strontium (Sr), cadmium (Cd), yttirium (Y), rubidium (Rb), thorium (Th), uranium (U), sulfur (S), arsenic (As), potassium (K), calcium (Ca) and manganese (Mn) concentrations were determined in leaf and soil samples from A and B sites. Detection limits for the elements are 5 ppm for Fe, Pb, Zn, Sr, Cd, Y, Rb, Th, As and Mn; 10 ppm for Cu; 25 ppm for K and Ca; 50 ppm for S (https://www.olympus-ims.com/it/vanta/#!cms[tab]=%2Fvanta%2Fresources).

2.7. The Biological Absorption Coefficient (BAC)

The Biological Absorption Coefficient (BAC) was calculated by the ratio between leaf and soil metal concentration) for all the considered species in the selected sites, according to [51].

2.8. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons to detect significance differences among each species collected in different sites. All statistical tests were performed using a statistical software package (Statistica 8.0, Statsoft, USA). The Pearson's correlation coefficient was used to analyzed the relationship between the considered metals in leaves of the selected species.

3. Results

3.1. Tree Structure

The structural traits of the considered trees species per each of the considered sites are shown in **Table 2**. The mean value of DBH, H and h for A and B sites were 1.76 ± 0.52 m, 17.55 ± 3.99 m, 12.93 ± 3.91 m, respectively, for *A. altissima*; 1.81 ± 0.41 m, 16.98 ± 2.98 m, 12.29 ± 2.55 m, respectively, for *R. pseudoacacia*; 1.91 ± 0.38 m, 18.73 ± 2.58 m, 15.74 ± 3.28 m, respectively, for *P. acerifolia* and 1.74 ± 0.06 m, 10.02 ± 0.80 m, 8.03 ± 0.94 m, respectively, for *Q. ilex*.

3.2. Leaf Morphology and Anatomy

The results of leaf morphological and anatomical analysis for all the considered species are shown in **Table 3**. LA was significantly higher for *R. pseudoacacia*, *P. acerifolia*, *A. altissima* in A sites (248.40 ± 77.13 cm^2, 195.96 ± 25.58 cm^2, 939.53 ± 98.21 cm^2, respectively, mean value) than in B sites. *Q. ilex* had an opposite trend with the highest values in B sites (13.31 ± 3.10 cm^2, mean value) than in A sites. DM had significant higher values in A sites (1.08 ± 0.39 g, 1.09 ± 0.27 g, 5.42 ± 1.22 g, mean value) than in B sites for *R. pseudoacacia*, *P. acerifolia* and *A. altissima*, respectively, *Q. ilex* having an opposite trend with the highest value in B sites (0.19 ± 0.06 g, mean value) than in A sites. LMA had significantly higher values for *P. acerifolia* in A sites (5.62 ± 1.38 $mg·cm^{-2}$, mean value) than in B sites, *Q. ilex* having an opposite trend with the highest values in B sites

(14.08 ± 2.61 mg·cm^{-2}). *A. altissima* and *R. pseudoacacia* did not show significant differences in LMA between A and B sites. LT was significantly higher in A sites for *R. pseudoacacia*, *A. altissima* and *P. acerifolia* (135.13 ± 4.00 μm, 214.10 ± 5.24 μm, 215.30 ± 16.75 μm) than in B sites, *Q. ilex* having an opposite trend with a higher values in B sites (192.57 ± 41.20 μm) than in A sites.

3.3. Metal Concentration in Leaves

Leaf metal concentrations of the considered trees species in the selected sites are shown in Table 4. The leaf metals concentration related to traffic density was significantly higher in B sites for all the considered species. In particular, *Q. ilex* had a significant higher concentration of Fe, Cu, Pb, Zn, Sr and S in B sites decreasing by 36.5%, 51.5%, 40.0%, 29.9%, 54.3% and 43.0%, respectively, in A sites (mean value). *P. acerifolia* has significantly higher Fe, Pb, Cu and Cd values in B sites decreasing by 53.1%, 30.8%, 31.1% and 31.6%, respectively, in A sites, while Rb concentration was significantly lower in B sites increasing by 95.5% in A sites. *R. pseudoacacia* had significantly higher Fe, Cu, Sr and S values in B sites decreasing by 34.6%, 47.4%, 38.8% and 24.1%, respectively, in A sites (mean value). *A. altissima* had a significant higher Fe, Cu and Pb in B sites decreasing by 37.9%, 44.9% and 42.9% in A sites (mean value), while Mn was significantly lower in B sites increasing by 345.5% in A sites.

Table 2. Structural traits of the considered trees (mean value for each A and B sites): DBH = diameter at breast height (m); H = tree height (m); h = crown height (m) in the selected sites (mean value). A sites: historical parks (A1 = Caffarella Valley Park, A2 = Borghese Historical Park, A3 = Doria Pamphjli Historical Park), B sites: high traffic level sites (B1 = Celio Hill, B2 = Oppio Hill, B3 = Passeggiata del Gianicolo Street).

SPECIES	SITE	DBH	H	h	SPECIES	SITE	DBH	H	h
A. altissima	A1	1.63 ± 0.38	15.43 ± 1.10	10.56 ± 1.07	*P. acerifolia*	A1	1.95 ± 1.13	22.00 ± 2.92	20.08 ± 3.22
	A2	1.83 ± 0.35	25.50 ± 1.80	20.17 ± 1.89		A2	1.96 ± 0.40	16.38 ± 1.17	13.20 ± 1.79
	A3	1.54 ± 0.01	16.03 ± 1.66	10.98 ± 0.81		A3	1.75 ± 0.25	17.82 ± 1.33	15.24 ± 1.53
	B1	1.37 ± 0.06	15.00 ± 1.00	10.30 ± 0.62		B1	1.30 ± 0.12	15.65 ± 0.92	11.19 ± 1.81
	B2	2.77 ± 0.07	17.50 ± 1.50	14.73 ± 1.54		B2	2.03 ± 0.69	19.20 ± 1.61	16.31 ± 2.03
	B3	1.43 ± 0.06	15.83 ± 2.72	10.84 ± 1.65		B3	2.45 ± 0.71	21.31 ± 2.73	18.43 ± 2.41
	mean value	1.76 ± 0.52	17.55 ± 3.99	12.93 ± 3.91		mean value	1.91 ± 0.38	18.73 ± 2.58	15.74 ± 3.28
R. pseudoacacia	A1	1.90 ± 0.17	16.73 ± 1.78	11.46 ± 1.25	*Q. ilex*	A1	1.71 ± 0.07	9.23 ± 0.59	6.76 ± 0.64
	A2	1.82 ± 0.18	17.43 ± 0.75	13.63 ± 0.80		A2	1.78 ± 0.08	11.30 ± 1.01	9.52 ± 1.06
	A3	1.27 ± 0.24	14.97 ± 1.47	10.25 ± 1.00		A3	1.81 ± 0.10	9.70 ± 0.74	8.04 ± 0.88
	B1	2.50 ± 0.30	20.50 ± 2.05	14.23 ± 1.45		B1	1.65 ± 0.05	10.52 ± 0.80	8.30 ± 0.93
	B2	1.53 ± 0.08	12.50 ± 1.19	8.75 ± 1.55		B2	1.69 ± 0.08	9.28 ± 0.63	7.33 ± 0.78
	B3	1.82 ± 0.33	19.73 ± 3.41	15.40 ± 2.38		B3	1.79 ± 0.06	10.09 ± 0.78	8.25 ± 0.90
	mean value	1.81 ± 0.41	16.98 ± 2.98	12.29 ± 2.55		mean value	1.74 ± 0.06	10.02 ± 0.80	8.03 ± 0.94

Table 3. Anatomical and morphological leaf traits for *A. altissima, R. pseudacacia, P. acerifolia* and *Q. ilex* in the considered A sites (A1 = Caffarella Valley Park; A2 = Borghese Historical Park; A3 = Doria Pamphjli Historical Park) and B sites (B1 = Celio Hill; B2 = Oppio Hill; B3 = Passeggiata del Gianicolo Street). Leaf Area = LA (cm^2); Total leaf thickness = LT (μm); Dry mass = DM (g); Leaf mass per unit of leaf area = LMA (gm^{-2}). Different capital letters indicate significant differences for the considered species between A and B sites. Different lowercase letters indicate significant differences among A and B sites (*= $p < 0.05$; **= $p < 0.01$). Mean values ± standard deviation are shown.

SPECIES	LA	DM	LMA	LT	SITE	LA	DM	LMA	LT	SPECIES
A. altissima	906.97 ± 92.23a	4.91 ± 0.89a	5.48 ± 1.26a	220.00 ± 4.30a	A1	181.91 ± 15.93a	1.02 ± 0.19a	5.64 ± 1.20a	228.30 ± 2.70a	*P.acerifolia*
	982.67 ± 111.11a	5.86 ± 1.53a	5.91 ± 1.01a	212.28 ± 6.30ab	A2	181.63 ± 20.06ab	1.13 ± 0.31a	6.13 ± 1.01a	221.20 ± 6.70b	
	915.93 ± 58.81a	5.28 ± 1.11a	5.81 ± 1.45a	210.02 ± 4.40b	A3	211.66 ± 25.57b	1.13 ± 0.32a	5.44 ± 1.68a	196.40 ± 17.30a	
	939.53 ± 89.21A	5.42 ± 1.22A	5.77 ± 1.16A	214.10 ± 5.24A	mean value	195.96 ± 25.58A	1.09 ± 0.27A	5.62 ± 1.38A	215.30 ± 16.75A	
	991.64 ± 26.39b	5.59 ± 0.45a	5.64 ± 0.30a	198.20 ± 3.20b	B1	152.57 ± 30.86ab	0.80 ± 0.13a	5.35 ± 1.03a	165.80 ± 5.20a	
	543.96 ± 95.27a	3.77 ± 1.19a	6.83 ± 1.65a	201.90 ± 1.80b	B2	155.64 ± 8.54a	0.74 ± 0.11b	4.80 ± 0.92a	163.30 ± 3.70a	
	737.50 ± 25.49ab	4.05 ± 2.14a	5.43 ± 2.76a	181.20 ± 6.00a	B3	184.38 ± 17.24b	0.76 ± 0.21b	4.20 ± 1.40a	178.90 ± 4.10b	
	666.96 ± 187.84B*	4.14 ± 1.45B*	6.28 ± 1.83A	193.77 ± 11.04B*	mean value	168.44 ± 22.89B**	0.76 ± 0.16B**	4.63 ± 1.20B*	169.33 ± 8.38B**	
R. pseudoacacia	244.46 ± 70.42a	1.13 ± 0.26a	4.72 ± 0.71a	139.42 ± 3.70b	A1	9.81 ± 2.26a	0.13 ± 0.01a	13.30 ± 2.17a	81.30 ± 3.50a	*Q.ilex*
	262.80 ± 71.24a	1.09 ± 0.34a	4.16 ± 0.79a	134.48 ± 2.60a	A2	9.97 ± 2.34a	0.11 ± 0.03a	11.58 ± 2.57a	127.50 ± 4.20b	
	236.37 ± 99.21a	1.04 ± 0.56a	4.29 ± 0.52a	131.50 ± 4.70a	A3	8.82 ± 2.10a	0.11 ± 0.04a	12.87 ± 1.73a	102.40 ± 4.50c	
	248.40 ± 77.13A	1.08 ± 0.39A	4.34 ± 0.66A	135.13 ± 4A	mean value	9.34 ± 2.19A	0.12 ± 0.03A	12.6 ± 2.11A	103.73 ± 23.10A	
	223.02 ± 15.32b	1.02 ± 0.25b	4.52 ± 0.82a	111.71 ± 1.20b	B1	16.39 ± 3.21a	0.25 ± 0.07b	14.92 ± 3.05a	223.90 ± 5.10a	
	193.10 ± 26.80b	0.79 ± 0.14ab	4.09 ± 0.34a	112.47 ± 3.80b	B2	11.05 ± 1.81b	0.16 ± 0.03a	14.82 ± 2.23a	207.90 ± 1.30b	
	128.08 ± 22.52a	0.52 ± 0.14a	4.15 ± 1.34a	103.12 ± 3.70a	B3	13.25 ± 1.34ab	0.16 ± 0.03a	12.25 ± 1.60a	145.90 ± 3.90c	
	171.65 ± 45.98B*	0.72 ± 0.25B*	4.21 ± 0.93A	109.10 ± 4.84B**	mean value	13.31 ± 3.10B**	0.19 ± 0.06B**	14.08 ± 2.61B*	192.57 ± 41.20B*	

Table 4. Metals content (ppm) in *A. altissima*, *R. pseudacacia*, *P. acerifolia* and *Q. ilex* leaves in the considered A sites (A1 = Caffarella Valley Park, A2 = Borghese Historical Park, A3 = Doria Pamphjli Historical Park) and B sites (B1= Celio Hill, B2 = Oppio Hill, B3 = Passeggiata del Gianicolo Street). Different letters indicate significant differences for the considered species between A and B sites (*= p < 0.05; **= p < 0.01). Mean values for A and B sites ± standard deviation are shown. The As and Ni concentration is below the limit of detection of the portable X-ray fluorescence Vanta Olympus M series (<5 ppm).

SPECIES	SITE	Fe	Cu	Pb	Zn	Sr	Cd	Y	Rb	Th	U	S	As	Ni	K	Ca	Mn
A. altissima	A1	533	24	5	35	70	19	10	18	33	15	4258	--	--	41,573	43,596	143
	A2	556	23	6	39	87	16	5	326	37	11	5096	--	--	48,635	38,520	101
	A3	766	18	6	52	88	19	11	30	37	16	5309	--	--	53,160	97,752	246
	mean value	618.33 ± 128.40a	22.67 ± 3.21a	5.67 ± 0.58a	42.00 ± 8.89a	81.67 ± 10.12a	18.00 ± 1.73a	8.67 ± 3.21a	124.67 ± 174.46a	35.67 ± 2.31a	14.00 ± 2.65a	4887.67 ± 556.61a	--	--	47,789.33 ± 5840.61a	59,956.00 ± 32,830.54a	163.33 ± 74.61a
	B1	1018	29	9	38	180	17	9	22	35	12	6706	--	--	57,518	38,317	5
	B2	1099	47	7	37	101	17	10	21	40	13	4158	--	--	39,548	52,157	38
	B3	870	42	7	73	71	18	11	25	38	14	10243	--	--	41,582	55,397	67
	mean value	995.67 ± 116.12b*	37.33 ± 9.29b*	7.67 ± 1.15b*	49.33 ± 20.50a	117.33 ± 56.31a	17.33 ± 0.58a	10.00 ± 1.00a	22.67 ± 2.08a	37.67 ± 2.52a	13.00 ± 1.00a	7036.67 ± 3056.87a	--	--	46,216.00 ± 9840.51a	48,623.67 ± 9071.66a	36.67 ± 31.02b*
R. pseudacacia	A1	360	15	5	51	78	20	9	29	37	13	4776	--	--	53724	20205	5
	A2	558	10	6	47	69	21	8	38	44	14	4126	--	--	34729	55486	5
	A3	380	15	5	95	77	15	10	40	42	15	4682	--	--	59872	50814	64
	mean value	432.67 ± 109.00a	13.33 ± 2.89a	5.33 ± 0.58a	64.33 ± 26.63a	74.67 ± 4.93a	18.67 ± 3.21a	9.00 ± 1.00a	35.67 ± 5.86a	41.00 ± 3.61a	14.00 ± 1.00a	4528.00 ± 351.30a	--	--	49,441.67 ± 13,107.11a	42,168.33 ± 19,163.71a	24.67 ± 34.06a
	B1	626	31	6	92	118	18	10	42	41	13	6949	--	--	45,366	50,540	58
	B2	606	25	6	72	138	16	8	103	40	13	5117	--	--	51,102	38,286	22
	B3	754	20	6	81	110	21	10	45	35	13	5825	--	--	48,131	28,957	53
	mean value	662.00 ± 80.30b*	25.33 ± 5.51b*	6.00 ± 0.00a	81.67 ± 10.02a	122.00 ± 14.42b**	18.33 ± 2.52a	9.33 ± 1.15a	63.33 ± 34.39a	38.67 ± 3.21a	13.00 ± 0.00a	5963.67 ± 923.84b*	--	--	48,199.67 ± 2868.62a	39,261.00 ± 10,824.48a	44.33 ± 19.50a
P. acerifolia	A1	490	19	6	62	49	12	10	51	45	16	5692	--	--	62,505	39,806	198
	A2	763	18	6	51	74	13	10	49	40	14	6541	--	--	44,233	51,636	225
	A3	965	25	6	60	100	14	11	29	46	16	6784	--	--	29,678	79,026	632
	mean value	739.33 ± 238.38a	20.67 ± 3.79a	6.00 ± 0.00a	57.67 ± 5.86a	74.33 ± 25.50a	13.00 ± 1.00a	10.33 ± 0.58a	43.00 ± 12.17a	43.67 ± 3.21a	15.33 ± 1.15a	6339.00 ± 573.34a	--	--	45,472.00 ± 16,448.54a	56,822.67 ± 20,117.86a	351.67 ± 243.15a
	B1	1329	30	8	50	112	20	12	21	41	15	7743	--	--	47,538	39,814	137
	B2	2100	34	7	63	100	19	11	22	42	15	8302	--	--	37,553	64,562	125
	B3	1300	26	7	52	91	18	9	23	40	13	5692	--	--	39,963	46,801	121
	mean value	1576.33 ± 453.74b*	30.00 ± 4.00a	7.33 ± 0.58b*	55.00 ± 7.00a	101.00 ± 10.54a	19.00 ± 1.00b**	10.67 ± 1.53a	22.00 ± 1.00b*	41.00 ± 1.00a	14.33 ± 1.15a	7245.67 ± 1374.24a	--	--	41,684.67 ± 5210.39a	50,392.33 ± 12,758.88a	127.67 ± 8.33a
Q. ilex	A1	730	25	6	47	27	19	11	46	40	15	2604	--	--	60,158	24,580	294
	A2	534	23	6	62	52	21	12	35	39	15	2878	--	--	41,105	41,171	37
	A3	719	17	6	60	38	15	12	34	34	15	2861	--	--	40,914	30,095	139
	mean value	661.00 ± 110.12a	21.67 ± 4.16a	6.00 ± 0.00a	56.33 ± 8.14a	39.00 ± 12.53a	18.33 ± 3.06a	11.67 ± 0.58a	38.33 ± 6.66a	37.67 ± 3.21a	15.00 ± 0.00a	2781.00 ± 153.52a	--	--	47,392.33 ± 11,055.80a	31,948.67 ± 8449.40a	156.67 ± 129.41a
	B1	1058	38	8	89	59	20	11	16	37	14	3872	--	--	19,251	36,810	273
	B2	1177	44	9	67	103	20	11	18	44	15	4698	--	--	24,146	50,552	148
	B3	888	52	7	85	94	17	10	39	48	17	6068	--	--	70,784	38,099	110
	mean value	1041.00 ± 145.25b*	44.67 ± 7.02b**	8.00 ± 1.00b*	80.33 ± 11.72b*	85.33 ± 23.25b*	19.00 ± 1.73a	10.67 ± 1.73a	24.33 ± 12.74a	43.00 ± 5.57a	15.33 ± 1.53a	4879.33 ± 1109.17b*	--	--	38,060.33 ± 28,445.02a	41,820.00 ± 7589.26a	177.00 ± 85.28a

Table 5. Pearson's correlation coefficients matrix of the analyzed metals for *R. pseudacacia, P. acerifolia, A. altissima* and *Q. Ilex* leaf samples in the selected sites. The values of Pearson coefficients in bold are statistically significant ($p < 0.05$). The *p*-values are shown in italic.

	Fe	Cu	Pb	Zn	Sr	Cd	Y	Rb	Th	U	S	K	Ca	Mn
Fe		0.49	**0.90**	0.21	0.64	0.34	0.07	0.34	−0.27	−0.51	0.55	−0.49	0.05	0.25
		0.33	***0.01***	*0.69*	*0.18*	*0.51*	*0.90*	*0.51*	*0.60*	*0.30*	*0.26*	*0.32*	*0.93*	*0.63*
Cu			0.44	0.60	**0.87**	−0.33	0.31	0.44	−0.19	−0.56	**0.90**	0.11	0.00	0.47
			0.39	*0.21*	***0.03***	*0.52*	*0.55*	*0.38*	*0.71*	*0.25*	***0.02***	*0.84*	*1.00*	*0.34*
Pb				0.00	0.58	0.30	−0.26	0.43	0.08	−0.46	0.40	−0.73	0.29	0.00
				1.00	*0.22*	*0.57*	*0.62*	*0.39*	*0.88*	*0.36*	*0.43*	*0.10*	*0.58*	*1.00*
Zn					0.42	−0.62	0.76	0.11	−0.05	0.21	0.63	0.49	0.27	**0.98**
					0.41	*0.19*	*0.08*	*0.83*	*0.93*	*0.69*	*0.18*	*0.32*	*0.61*	***0.00***
Sr						−0.30	0.00	0.78	−0.30	−0.62	0.65	0.08	−0.14	0.27
						0.56	*1.00*	*0.07*	*0.56*	*0.19*	*0.17*	*0.88*	*0.79*	*0.60*
Cd							−0.12	−0.48	−0.34	−0.42	−0.05	−0.66	−0.32	−0.44
							0.82	*0.33*	*0.51*	*0.41*	*0.93*	*0.16*	*0.54*	*0.38*
Y								−0.48	−0.36	0.12	0.60	0.45	−0.06	**0.85**
								0.33	*0.49*	*0.82*	*0.21*	*0.37*	*0.91*	***0.03***
Rb									0.03	−0.27	0.04	0.09	0.00	−0.07
									0.96	*0.60*	*0.94*	*0.86*	*1.00*	*0.89*
Th										0.61	−0.32	−0.35	**0.90**	−0.09
										0.20	*0.53*	*0.50*	***0.01***	*0.87*
U											−0.54	0.23	0.60	0.26
											0.27	*0.67*	*0.21*	*0.62*
S												0.02	−0.03	0.61
												0.98	*0.95*	*0.20*
K													−0.38	0.41
													0.45	*0.42*
Ca	*R.pseudacacia*													0.27
														0.61
Mn														

	Fe	Cu	Pb	Zn	Sr	Cd	Y	Rb	Th	U	S	K	Ca	Mn
Fe		0.70	0.79	0.11	0.57	−0.22	0.41	−0.52	0.57	−0.15	0.23	0.06	0.05	−0.58
		0.12	*0.06*	*0.83*	*0.24*	*0.67*	*0.43*	*0.29*	*0.24*	*0.78*	*0.66*	*0.91*	*0.93*	*0.23*
Cu			0.38	0.26	0.00	−0.27	0.29	−0.33	0.65	−0.21	0.34	−0.61	−0.28	−0.68
			0.46	*0.61*	*1.00*	*0.60*	*0.57*	*0.53*	*0.17*	*0.69*	*0.52*	*0.20*	*0.60*	*0.14*
Pb				0.06	**0.81**	−0.44	0.04	−0.24	0.20	−0.47	0.41	0.48	−0.25	−0.72
				0.91	***0.03***	*0.38*	*0.94*	*0.65*	*0.70*	*0.35*	*0.42*	*0.33*	*0.63*	*0.11*
Zn					−0.36	0.29	0.45	−0.20	0.33	0.34	**0.87**	−0.15	0.40	0.13
					0.49	*0.58*	*0.37*	*0.70*	*0.52*	*0.52*	***0.02***	*0.78*	*0.43*	*0.81*
Sr						−0.39	−0.13	−0.15	−0.14	−0.46	0.01	0.71	−0.28	−0.55
						0.44	*0.81*	*0.77*	*0.80*	*0.36*	*0.99*	*0.12*	*0.59*	*0.26*
Cd							0.78	−0.67	−0.39	**0.97**	0.03	−0.13	0.61	0.66
							0.07	*0.15*	*0.45*	***0.00***	*0.95*	*0.81*	*0.20*	*0.15*
Y								**−0.94**	0.06	**0.81**	0.28	−0.22	0.55	0.21
								0.01	*0.91*	*0.05*	*0.59*	*0.68*	*0.26*	*0.69*
Rb									0.08	−0.64	−0.17	0.12	−0.32	0.02
									0.88	*0.17*	*0.74*	*0.82*	*0.54*	*0.96*
Th										−0.18	0.15	−0.31	0.26	−0.19
										0.74	*0.78*	*0.55*	*0.63*	*0.72*
U											−0.01	−0.17	0.75	0.73
											0.99	*0.75*	*0.09*	*0.10*
S												0.02	0.00	−0.27
												0.97	*1.00*	*0.60*
K													0.17	0.10
													0.74	*0.85*
Ca	*A.altissima*													0.78
														0.07
Mn														

Continued

	Fe	Cu	Pb	Zn	Sr	Cd	Y	Rb	Th	U	S	K	Ca	Mn
Fe		**0.94**	0.62	0.13	0.71	**0.84**	0.32	**−0.81**	−0.34	−0.23	**0.75**	−0.50	0.28	−0.33
		0.01	*0.19*	*0.80*	*0.12*	***0.04***	*0.53*	*0.05*	*0.51*	*0.66*	***0.08***	*0.31*	*0.59*	*0.52*
Cu			**0.74**	0.15	**0.80**	**0.89**	0.52	**−0.91**	−0.15	−0.02	0.78	−0.46	0.25	−0.22
			0.09	*0.78*	*0.06*	***0.02***	*0.29*	*0.01*	*0.78*	*0.97*	*0.07*	*0.37*	*0.63*	*0.68*
Pb				−0.43	0.69	**0.94**	0.47	−0.76	−0.51	−0.28	0.53	−0.04	−0.37	−0.55
				0.40	*0.13*	***0.01***	*0.35*	*0.08*	*0.31*	*0.59*	*0.28*	*0.94*	*0.47*	*0.26*
Zn					−0.28	−0.28	0.06	0.12	0.75	0.67	0.12	0.01	0.47	0.29
					0.60	*0.60*	*0.90*	*0.83*	*0.09*	*0.15*	*0.82*	*0.99*	*0.35*	*0.58*
Sr						0.81	0.59	**−0.90**	−0.24	−0.15	0.69	−0.71	0.37	0.11
						0.05	*0.22*	***0.01***	*0.65*	*0.78*	*0.13*	*0.11*	*0.47*	*0.84*
Cd							0.39	**−0.90**	−0.50	−0.35	0.62	−0.31	−0.10	−0.47
							0.44	***0.01***	*0.31*	*0.49*	*0.19*	*0.55*	*0.86*	*0.35*
Y								−0.38	0.22	0.57	0.81	−0.14	0.19	0.19
								0.46	*0.67*	*0.24*	*0.05*	*0.79*	*0.72*	*0.73*
Rb									0.22	0.22	−0.55	0.60	−0.25	0.08
									0.68	*0.68*	*0.26*	*0.21*	*0.63*	*0.88*
Th										**0.88**	−0.12	0.02	0.46	0.71
										0.02	*0.83*	*0.97*	*0.36*	*0.11*
U											0.22	0.18	0.30	0.52
											0.68	*0.74*	*0.56*	*0.29*
S												−0.38	0.32	−0.09
												0.46	*0.54*	*0.87*
K													**−0.83**	−0.51
													0.04	*0.31*
Ca		*P.acerifolia*												0.76
														0.08
Mn														

	Fe	Cu	Pb	Zn	Sr	Cd	Y	Rb	Th	U	S	K	Ca	Mn
Fe		0.72	**0.95**	0.54	0.68	0.13	−0.54	−0.74	0.35	−0.11	0.59	−0.47	0.52	0.37
		0.11	***0.00***	*0.27*	*0.14*	*0.80*	*0.27*	*0.09*	*0.50*	*0.83*	*0.22*	*0.34*	*0.30*	*0.47*
Cu			0.71	0.71	**0.87**	0.15	**−0.88**	−0.34	**0.84**	0.49	**0.95**	0.09	0.55	0.00
			0.12	*0.11*	***0.02***	*0.78*	***0.02***	*0.51*	***0.04***	*0.32*	***0.00***	*0.87*	*0.26*	*0.99*
Pb				0.54	0.77	0.35	−0.42	**−0.84**	0.38	−0.16	0.58	−0.59	0.74	0.16
				0.27	*0.07*	*0.50*	*0.41*	***0.04***	*0.46*	*0.76*	*0.23*	*0.22*	*0.09*	*0.76*
Zn					0.59	0.04	−0.52	−0.56	0.31	0.16	0.72	−0.21	0.41	−0.05
					0.22	*0.94*	*0.29*	*0.25*	*0.56*	*0.76*	*0.11*	*0.69*	*0.42*	*0.93*
Sr						0.15	−0.58	−0.48	0.77	0.45	**0.89**	−0.11	**0.84**	−0.36
						0.78	*0.23*	*0.33*	*0.08*	*0.37*	***0.02***	*0.83*	***0.04***	*0.48*
Cd							0.04	−0.35	0.14	−0.42	−0.11	−0.42	0.46	0.05
							0.94	*0.49*	*0.80*	*0.41*	*0.84*	*0.41*	*0.36*	*0.93*
Y								−0.03	**−0.81**	−0.59	**−0.82**	−0.42	−0.11	−0.27
								0.96	***0.05***	*0.22*	***0.05***	*0.41*	*0.84*	*0.60*
Rb									0.12	0.52	−0.24	**0.90**	−0.66	−0.09
									0.83	*0.29*	*0.65*	***0.02***	*0.15*	*0.87*
Th										**0.76**	**0.83**	0.47	0.45	−0.22
										0.08	***0.04***	*0.35*	*0.37*	*0.67*
U											0.67	**0.81**	0.06	−0.45
											0.15	***0.05***	*0.92*	*0.37*
S												0.21	0.53	−0.22
												0.70	*0.28*	*0.68*
K													−0.48	−0.13
													0.34	*0.81*
Ca		*Q.ilex*												−0.51
														0.30
Mn														

Table 6. Metals content (ppm) in soils of the considered A sites (A1 = Caffarella Valley Park, A2 = Borghese Historical Park, A3 = Doria Pamphjli Historical Park) and B sites (B1 = Celio Hill, B2 = Oppio Hill, B3 = Passeggiata del Gianicolo Street) sites. Different letters indicate significant differences for the considered species between A and B sites (*= $p < 0.05$; **= $p < 0.01$). Mean values for A and B sites ± standard deviation are shown. The Cd concentration is below the limit of detection of the portable X-Ray Fluorescence Vanta Olympus M series (<5 ppm).

SITE	Fe	Cu	Pb	Zn	Sr	Cd	Y	Rb	Th	U	S	As	Ni	K	Ca	Mn
A1	44,140	105	176	151	621	--	33	205	44	5	805	33	21	15,283	35,708	1300
A2	35,666	94	170	179	441	--	24	217	53	5	1081	32	19	10,679	31,650	1031
A3	35,624	108	195	188	758	--	33	220	43	6	1257	29	19	13,011	33,499	1189
mean value	38,476.67 ± 4904.64a	102.33 ± 7.37a	172.67 ± 13.05a	172.67 ± 19.30a	607.67 ± 158.99a	--	30.00 ± 5.20a	214.00 ± 7.94a	46.67 ± 5.51a	5.33 ± 0.58a	1047.67 ± 227.84a	31.33 ± 2.08a	19.67 ± 1.15a	12991.00 ± 2302.07a	33,619.00 ± 2031.66a	1173.33 ± 135.18a
B1	46119	255	828	252	1049	--	30	288	66	7	1561	49	31	16,481	60,060	1163
B2	40891	210	359	283	1096	--	29	290	71	6	1069	45	31	15,008	82,244	1076
B3	40025	215	309	249	1065	--	26	275	62	5	1240	41	28	13,265	48,927	1023
mean value	42,345.00 ± 3296.94a	226 ± 24.66b**	498.67 ± 286.30a	261.33 ± 18.82b**	1070.00 ± 23.90b**	--	28.33 ± 2.08a	284.33 ± 8.14ab**	66.33 ± 4.51b**	6.00 ± 1.00a	1290.00 ± 249.78a	45.00 ± 4.00b*	30.00 ± 1.73b**	14,918.00 ± 1609.89a	63,743.67 ± 16961.21b*	1087.33 ± 70.68a

Table 7. Ratio between leaf and soil metal concentration (Biological Absorption Coefficient, BAC) calculated for *A. altissima*; *R. pseudacacia*; *P. acerifolia* and *Q. ilex*. Mean values of the considered sites (A and B sites) for each species ± standard deviation are shown. Different letters indicate significant differences among the considered species ($p < 0.05$).

SPECIES	SITES	Fe	Cu	Pb	Zn	Sr	Y	Rb	Th	U	S	K	Ca	Mn
A. altissima	A1	0.012	0.028	0.028	0.232	0.113	0.303	0.088	0.750	3.000	5.289	2.720	1.221	0.110
	A2	0.016	0.027	0.027	0.218	0.197	0.208	1.502	0.698	2.200	4.714	4.554	1.217	0.098
	A3	0.022	0.031	0.031	0.277	0.116	0.333	0.136	0.860	2.667	4.224	4.086	2.918	0.207
	B1	0.022	0.114	0.011	0.151	0.172	0.300	0.076	0.530	1.714	4.296	3.490	0.638	0.004
	B2	0.027	0.324	0.019	0.131	0.092	0.345	0.072	0.563	2.167	3.890	2.635	0.634	0.035
	B3	0.022	0.302	0.023	0.293	0.067	0.423	0.091	0.613	2.800	8.260	3.135	1.132	0.065
	mean value	0.02 ± 0.005ab	0.23 ± 0.007b	0.023 ± 0.007a	0.217 ± 0.065a	0.126 ± 0.049b	0.319 ± 0.07ab	0.328 ± 0.576a	0.669 ± 0.125a	2.425 ± 0.480a	5.112 ± 1.616ab	3.437 ± 0.764a	1.293 ± 0.842a	0.087 ± 0.071ac
R. pseudacacia	A1	0.008	0.143	0.028	0.338	0.126	0.273	0.141	0.841	2.600	5.933	3.515	0.566	0.004
	A2	0.016	0.106	0.027	0.263	0.156	0.333	0.175	0.830	2.800	3.817	3.252	1.753	0.005
	A3	0.016	0.139	0.026	0.505	0.102	0.303	0.182	0.977	2.500	3.725	4.602	1.517	0.054
	B1	0.014	0.122	0.007	0.365	0.112	0.333	0.146	0.621	1.857	4.452	2.753	0.841	0.050
	B2	0.015	0.172	0.017	0.254	0.126	0.276	0.355	0.563	2.167	4.787	3.405	0.466	0.020
	B3	0.019	0.144	0.019	0.325	0.103	0.385	0.164	0.565	2.600	4.698	3.628	0.592	0.052
	mean value	0.014 ± 0.004a	0.138 ± 0.022a	0.021 ± 0.008a	0.342 ± 0.091b	0.121 ± 0.02b	0.317 ± 0.042a	0.194 ± 0.081a	0.733 ± 0.173a	2.421 ± 0.345a	4.568 ± 0.802ab	3.526 ± 0.609a	0.956 ± 0.546a	0.031 ± 0.024a
P. acerifolia	A1	0.011	0.181	0.034	0.411	0.079	0.303	0.249	1.023	3.200	7.071	4.090	1.115	0.152
	A2	0.021	0.191	0.027	0.285	0.168	0.417	0.226	0.755	2.800	6.051	4.142	1.631	0.218
	A3	0.027	0.306	0.031	0.319	0.132	0.333	0.132	1.070	2.667	5.397	2.281	2.359	0.532
	B1	0.029	0.118	0.010	0.198	0.107	0.400	0.073	0.621	2.143	4.960	2.884	0.663	0.118
	B2	0.065	0.234	0.019	0.223	0.091	0.379	0.076	0.592	2.500	7.766	2.502	0.785	0.116
	B3	0.016	0.187	0.023	0.209	0.085	0.346	0.084	0.645	2.600	4.590	3.013	0.957	0.118
	mean value	0.028 ± 0.019b	0.203 ± 0.063ab	0.023 ± 0.008a	0.274 ± 0.082ab	0.11 ± 0.034ab	0.363 ± 0.043ab	0.14 ± 0.079a	0.784 ± 0.211a	2.652 ± 0.349a	5.973 ± 1.241b	3.152 ± 0.791a	1.252 ± 0.639a	0.209 ± 0.163b
Q. ilex	A1	0.017	0.238	0.034	0.311	0.043	0.333	0.224	0.909	3.000	3.235	3.936	0.688	0.226
	A2	0.015	0.245	0.027	0.346	0.118	0.500	0.161	0.736	3.000	2.662	3.849	1.301	0.036
	A3	0.020	0.157	0.031	0.319	0.050	0.364	0.155	0.791	2.500	2.276	3.145	0.898	0.117
	B1	0.023	0.149	0.010	0.353	0.056	0.367	0.056	0.561	2.000	2.480	1.168	0.613	0.235
	B2	0.029	0.303	0.025	0.237	0.094	0.379	0.062	0.620	2.500	4.395	1.609	0.615	0.138
	B3	0.022	0.374	0.023	0.341	0.088	0.385	0.142	0.774	3.400	4.894	5.336	0.779	0.108
	mean value	0.021 ± 0.005b	0.244 ± 0.086b	0.024 ± 0.008a	0.318 ± 0.043b	0.075 ± 0.029a	0.388 ± 0.058b	0.133 ± 0.064a	0.732 ± 0.125a	2.733 ± 0.497a	3.324 ± 1.083a	3.174 ± 1.561a	0.816 ± 0.261a	0.143 ± 0.076cb

The Pearson's coefficient analysis highlighted significant ($p < 0.05$) linear correlation among some of the considered metals (**Table 5**) in the selected species. In particular, in *P. pseudoacacia* Fe was positively related with Pb; Cu with S and Sr; Zn with Mn; Y with Mn; Th with Ca. In *A. altissima* Pb was positively related with Sr; Zn with S; Cd with U while Y was negatively related with Rb. In *P. acerifolia* Fe was positively related with Cu, Cd and S; Cu with Cd; Pb with Cd while Rb was negatively related with Fe, Cu, Sr and Cd; Th with U, and S with Ca. In *Q. ilex*, Fe was positively related with Pb; Cu with Sr, Th and S; Sr with S and Ca; Th with S; Rb with K while Cu was negatively related with Y; Pb with Rb; Y with Th and S.

3.4. Metals Concentration in Soil and BAC

The metal concentration in soil samples for the considered sites is shown in **Table 6**. Significant higher concentrations of Cu, Zn, Sr, Rb, Th, Ni, As, K and Ca were monitored in B sites decreasing by 54.8%, 33.9%, 43.3%, 24.7%, 29.7%, 34.4%, 30.4%, 12.9% and 47.3% in A sites. In particular, Celio hill had the highest soil metal concentration for Pb (828 ppm), Ni (31 ppm), Fe (46.119), Cu (255), S (1561) and As (49 ppm), and Celio hill and Oppio hill a higher concentration for Sr (1049 ppm and 1096 ppm, respectively).

The Biological Absorption Coefficient (BAC) was calculated for all the considered sites (**Table 7**) and it was significantly different among the species for metals related to traffic density (Fe, Cu, Pb, Zn, Sr and S) and for natural metals (Mn and Y). Moreover, among the species, *Q. ilex* had the highest BAC for Cu (0.244), Pb (0.014), Y (0.388) and U (3.347), followed by *P. acerifolia* for Fe (0.028), Th (0.784), S (5.973) and Mn (0.209), by *A. altissima* for Sr (0.126), Rb (0.328) and Ca (1.293) and by *R. pseudoacacia* for Zn (0.342) and K (3.526).

4. Discussion

Contamination of aquatic and terrestrial ecosystems with metals is a major environmental problem. Although metals are naturally present in soils, contamination comes from local sources, mostly industry, agriculture, sewage sludge, waste incinerations and road traffic [8] [9]. Heavy metals have been seen as a key marker since they are not degradable by natural processes [52]. The contribution from different emission source sectors to air metals content depends not only on the amount of pollutants emitted, but also on the proximity to the emission source [4]. Heavy metals accumulation in plant tissues causes various morphological, physiological and biochemical responses; however, these changes vary among species, which underscores that the response of plants to pollution is species-specific. Plants may be used as bioindicators of environmental pollution having the advantage of high temporal and spatial resolution [9] and leaves are the most sensitive plant organ to be affected by pollutants [17]. The accumulation of contaminants in plants reflects the cumulative effects of environmental pollution from both soil and atmosphere [9]. In particular, Zn is a natural element of soil but an excess of it is mainly due to vehicular traffic, losses of oil and cooling liquids, corrosion of galvanized steel safety fences [53]. Fe and Cu are natural constituents of soil nevertheless, their source in street dust has been ascribed to corrosion of the metallic parts of cars like engine wear, thrust bearing and brush wear [54]. Pb is a natural but minor component of the soil and is directly related to emissions from motor vehicles [55]. For the last 40 years, road transport emissions were considered the principal source of Pb in urban areas being produced by combustion of petrol in the engine. In the eighties, Pb concentration in the atmosphere decreased because of the restriction in the use as an additive in gasoline in many Countries [56], and in 1989 in Italy. Cd is an additive in lubricating oils; Ni occurs both in organic and inorganic form in the soil [57], and in anthropogenic sources by its use in car brake discs and emission from cars with catalytic mufflers are the result of particles detached from the Al supporting substrate [58]. The contribution to ambient S derives from sulphur-containing fossil fuels and biofuels used for domestic heating, stationary power generation and transport. Regarding As, Cd, Ni and Hg there was a decrease in their emission in Europe between the years 2000 and 2015 [4]. Some effects on plants not directly caused by pollution but of which polluting agents are a predisposing factors can be observed, *i.e.* aphids grow more in polluted environments because pollutants increase the content of free amino acids [59] which represents a nutritional value for insects, consequently, plants are not able to activate mechanisms of defense [60].

Our results highlight differences in metals concentration in soil and leaf samples as well as in leaf morphological and anatomical traits for the considered species and among the considered sites. Leaf metals concentration in B sites (848, 26 ppm, mean values of all the metals for all the considered species) exceeds the concentration in A sites (601, 37 ppm mean value of all the metals for all the considered species). On average, the considered species show higher leaf concentration for Fe, Cu, Pb, Zn, Sr and S at Celio Hill and Oppio Hill (B sites) justified by the highest traffic density all day long. A lower leaf metal concentration at Passeggiata del Gianicolo Street, than the other considered B sites, can be justified by the larger open area of this B site, considering that pollutants are released at ground level and their upward movement is restricted due to tall buildings and congested thoroughfares, according to the results of [61] Among the considered species, *Q. ilex* and *P. acerifolia* have the highest concentration of metals determined by vehicular traffic than the other species. As regards to the soil, the highest concentration of Fe, Cu, Pb, Zn, Sr, Rb, S, Ni, Th, Ca and As were monitored at Celio Hill and Oppio Hill. The BAC, which reveals a variable and sometimes specificity of plants to absorb elements from the soil (51 Kabata-Pendias and Pendias, 2001) is the highest for metals related to vehicular traffic in *Q. ilex* for Cu (0.244) and Pb (0.014); in *P. acerifolia* for Fe (0.028) and S (5.973); in *A. altissima* for Sr (0.126), and in *R. pseudoacacia* for Zn (0.342) reflecting the different species ability to accumulate metals. The positive correlation among some metals confirms a common origin from vehicular traffic, according to [25]. In particular, in *P pseudoacacia* Fe is related with Pb, Cu with S and Sr; in *A. altissima* Pb is related with Sr, and Zn with S; in *P. acerifolia* Fe is related with Cu, S and Cd, Cu is related with Cd, and Pb with Cd; in *Q. ilex* Fe is related with Pb, Cu with Sr and S, Sr with S. Moreover, leaves of the considered species which are exposed to traffic emissions show changes in some leaf morphological and anatomical traits, according to [62]. In particular, *P. acerifolia* has significantly lower LA, DM, LMA and LT in B sites than in A sites, while *Q. ilex* shows an opposite trend, according to [17]. *A. altissima* and *R. pseudoacacia* have significantly lower LA, DM and LT in B than in A sites. These results highlight the adaptive strategies of the considered species in response to environmental factors changing. In *Q. ilex* the increased LA, LT and LMA in response to pollution in B sites may be considered a compensatory response to the lower leaf longevity in these sites [17]. Among the invasive alien species (IAS), *A. altissima* shows a lower leaf traits variability between A and B sites being one of the most tolerant species to high pollution levels [34].

The results as a whole underline the importance of tree species in urban areas for their ability to metals accumulation in leaves. Among the considered species, *Q. ilex* shows the highest metal accumulation capability. However, the large presence in Rome of invasive alien species (IAS), as *A. altissima* and *R. pseudoacacia* [27] Lucchese and Pignatti, 2009) negatively affects the presence of native species. In this case, the competent Authorities should intervene, in compliance with the Regulation EU n. 1143/2014 issued by the European Commission which included restrictions to the introduced IAS and an obligation to establish a framework of early detect and rapid removal of IAS, allowing the recolonization of the autochthonous species. In this case, *Q. ilex* is an autochthonous species that borders numerous avenues and historical parks in Rome contributing to improving urban air quality. Our data concerning tree structure, leaf morphological and anatomical traits and heavy metal accumulation capability could be used to realize an inventory available for urban tree planting programs to ameliorate air quality and to carry out a long-time monitoring of metal pollution for the city of Rome.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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