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Allergenic risk assessment of urban parks: Towards a standard index

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ABSTRACT

Allergenicity indices are a powerful tool to assess the health hazard posed by urban parks to pollen allergic subjects. Nonetheless, only few indices have been developed and applied to urban vegetation in the last decade, and they were never compared nor standardised over the same dataset. To address this issue, in this paper the two best-known allergenicity indices, the Urban Green Zones Allergenicity Index (I_{UGZA}) and the Specific Allergenicity Index (SAI), have been calculated for the same park (the Botanical Garden of Bologna), collecting vegetation data through both systematic sampling and arboreal census. The results obtained with the two data collection methods were comparable for both indices, indicating systematic sampling as a reliable approximation of the total census. Besides, the allergenic risk resulted moderate to high according to SAI, and very low according to I_{UGZA}. Since SAI does not consider the total volume of the vegetation, it was deemed less reliable than I_{UGZA} in evaluating the allergenicity of an enclosed green space.

1. Introduction

Pollen is a major source of airborne allergens. It causes seasonal allergic rhinitis (pollinosis) in a significant share of the human population, and it can occasionally trigger allergic asthma. According to the latest broad epidemiological studies, the prevalence of pollen allergy in Europe is up to 40% (D'Amato et al., 2007), and it seems to be raising over time. Hence, understanding airborne pollen trends is of great importance for the high prevalence and the socio-economic impact that pollen-related respiratory diseases have on a global scale and several approaches have been developed for pollen allergy risk assessment, as recently reviewed(Suanno et al., 2021a,b). The progressive worsening of pollen allergy burden is partly a consequence of modern environmental problems such as air pollution and climate change, that can impact morbidity, mortality, incidence, and prevalence of the disease (D'Amato et al., 2016). In fact, air pollutants have been proven to exacerbate rhinitis symptoms by direct interaction with pollen allergens and the respiratory mucosa. Particulate matter also appears to affect human exposure to pollen allergens, possibly acting as carrier and keeping them airborne even outside the pollen season (Aloisi et al., 2018; Cecchi, 2013; D'Amato, 2001). On the other hand, air pollution also contributes to climate change. Higher mean temperatures, heat waves and heavy rainfalls associated to climate change tend to alter spatial and temporal distribution of airborne pollen, potentially anticipating the pollen season and extending its duration (D'Amato et al., 2016). Moreover, all these abiotic stressors can also modify the pollen potency, by enhancing the expression of allergenic proteins (Cecchi, 2013; Fernández-González et al., 2010, 2011).

All these dynamics play an important role in the urban environment, where air pollution and climate change effects are heavier than in less anthropogenic environments. The health of pollinosis sufferers in urban centres is also threatened by gardening choices that enhance the potential allergenicity of green spaces, such as the plant species selection, association, and maintenance (Capotorti et al., 2020; Cariñanos and Casares-Porcel, 2011). This picture is worsened by the increased likelihood for city inhabitants to develop pollen allergies compared to people living in the countryside (Patel et al., 2018).

While the ecosystem disservice provided by allergenic ornamental species has been taken into account in the UE environmental policies (Science for Environment Policy, 2012) and in some European national

Abbreviations: SAI, Specific Allergenicity Index; I_{UGZA}, Urban Green Zones Allergenicity Index; AIROT, Aerobiological Index of Risk for Ornamental Trees; NP, nano-phanerophytes; P scap, scapose phanerophytes; P caesp, cespitose phanerophytes; H-Index, Shannon-Wiener diversity index; ARPAE, Regional Agency for Prevention and Environment of Emilia-Romagna; WAO, World Allergy Organisation.

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regulations, guidelines for hypoallergenic trees selection can only be applied to future green infrastructures. In fact, the substitution of existing allergenic trees in parks and streets would not be convenient from an economic and ecological point of view (Cariñanos and Casares-Porcel, 2011). Hence, it is important to assess the allergenicity of existing urban vegetation in order to plan an appropriate maintenance, and to alert the allergic subjects of the risk it poses (Suanno et al., 2021). For this purpose, to our knowledge three allergenicity indices have been proposed in the last decade: the Specific Allergenicity Index (SAI) (Hruska, 2003), the Urban Green Zones Allergenicity Index (I_{UGZA}) (Cariñanos et al., 2014) and the Aerobiological Index of Risk for Ornamental Trees (AIROT) (Pecero-Casimiro et al., 2019). AIROT calculates the allergenicity potential of a single plant species over large a study area, combining LiDAR remote sensing and Kriging interpolation. Its estimation involves biological features of the species and aspects of the surrounding environment that may influence pollen dispersal. SAI and I_{UGZA} instead are applied to smaller patches of urban vegetation, but they include all the allergenic species present in the area, providing a complete picture of the allergic risk. These two indices take into account different biological and biometric parameters of the vegetation that are related to pollen production, dispersal an allergenicity. While SAI has been employed only few times and mainly on anthropogenic, spontaneous vegetation, I_{UGZA} had a considerable success after its publication and it has been applied to many urban parks in several European cities (Cariñanos et al., 2016, 2019, 2019; Kasprzyk et al., 2019). However, the rapid spread of this index was not preceded by a method standardisation, especially for the sampling design, making it difficult to compare results obtained from incomparable datasets. In this work, the two indices SAI and I_{UGZA} were calculated for the same urban park (Botanical Garden of Bologna, Italy) using different sampling methods and inclusion criteria. One aim of this study is to evaluate the consistency between the two indices, and, in case they results are in disagreement, to indicate which one is more adequate to describe the allergenicity of a circumscribed green area. This kind of comparison, to our knowledge, has never been performed, and its results would be very helpful in the choice of the appropriate metrics to apply to the urban vegetation. The other aim of the present work is to test the comparability between different data collection approaches, in order to corroborate the results from previous studies applying the indices, and to suggest a standard sampling method that is both time efficient and reliable. Thus, this study will provide an example of sampling design choice and validation for allergenicity indices, to give them a greater ecological value, and to ensure their reproducibility and the comparability between different datasets.

2. Materials and methods

2.1. Study area

This research was conducted in the metropolitan city of Bologna (44°29′N 11°20′E), capital of the Emilia-Romagna region, Italy. Bologna is situated at the foot of the Tuscan-Emilian Apennine, and it extends for 140.7 km^2 along the southern edge of the Po plain, where Reno and Savena valleys merge. This location entails a humid temperate climate, with the average annual minimum temperature of 10 °C and maximum temperature of 19 °C, and mean annual rainfalls of 768.7 mm (data from the Regional Agency for Prevention and Environment of Emilia-Romagna (ARPAE, 2019), measured over the period 1991–2015).

Bologna is densely populated, hosting 384,202 inhabitants. Since municipal vegetation covers around 9% of the city surface, there are 29 m^2 of public greenery per person. Public parks are mainly distributed in the more densely inhabited areas, but they are scarce in the city centre (data from the National Statistic Institution of Italy, ISTAT, relative to the year 2016 (ISTAT, 2016)).

The urban green area chosen for this study is the Botanical Garden of Bologna (44°30′00′N 11°21′14″E). This park belongs to the museum system of the University of Bologna (SMA), and it is one of the oldest Botanical Gardens in Italy, founded in 1568 and then moved into its current location in 1803 (Fig. 1). The park extends for 1.8 ha, and it gathers a great diversity of both native and exotic plant species.

Since it is one of the few public parks located in the city centre, and a tourist attraction with free access, it is frequently visited by city dwellers and foreigners, with 55,338 visitors counted during the year 2019. It is therefore important to assess its allergenicity risk. Moreover, this park offers a good model for method standardisation, being relatively small and allowing to correctly identify plant species and to keep track of their maintenance. These aspects allowed to carry on a detailed and complete census of the arboreal species, that would not have been possible in wider areas with unknown and unmaintained vegetation. While the presence of exotic and uncommon plant species implies that the Botanical Garden is not representative of common urban parks, the plant collection is mainly composed by native species, indigenous of the region, and some areas of the Botanical Garden even recreate the local natural habitats. In fact, the vegetation is organised in different habitats and exhibitions: a wooded garden at the entrance, with the prevalence of evergreen species; a wooded hill frequently pruned and managed, with the prevalence of indigenous herbs, trees and shrubs; two small orchards with edible and medical species, surrounded by spontaneous weeds and grasses; vast areas with a frequently mowed lawn and individual trees spaced apart; patches with unmaintained weeds and grasses, for conservation and experimental purposes; the recreation of a local riparian forest, with the prevalence of old silver poplars (*Populus alba* L.); a system of small lakes with freshwater plants and algae; an exhibition of rocky vegetation; and the recreation of a local continental forest, with low levels of maintenance and characterised by the presence of indigenous species of the region. Greenhouses and temporary exhibitions are also present, but they were not considered in this study.

2.2. Data collection

Vegetation data were collected in 2019 and 2020, form spring to summer. The vegetation considered in this study included not only the ornamental species displayed in the Garden, but also the spontaneous flora present therein, in form of grass, weeds, or shrubs. In fact, for educational and scientific purposes, some areas of the Botanical Garden are subject to low levels of maintenance, allowing spontaneous grass es and weeds to grow and bloom, and local trees to reproduce from seeds.

In 2019 the vegetation was sampled by systematic sampling, considered by the authors the fittest objective method to apply because of the highly heterogeneous structure of the park vegetation. The whole surface of the park was divided with a 30 × 30 m grid in Quantum Geographic Information System (QGIS) (QGIS Association, 2021), selecting the centre of each square as centre of the 10 × 10 m plots (Fig. S1). Plots falling outside the park perimeter or over the buildings were excluded from the sampling. Eventually, the vegetation was sampled in 18 plots representing 10% of the whole garden surface. For each plot, all the spermatophytes were identified using the Botanical Garden inventory and monographic flora, and their volume was measured. Data on herbaceous and arboreal vegetation were collected separately, as shown in Table 1.

In 2020 a census of the "arboreal species" (trees and shrubs) present in the Botanical Garden was carried out. To attain an objective definition of arboreal species, only the plant species included in the Raunkiær classifications of nano-phanerophytes (NP), scapose phanerophytes (P scap) and cespitose phanerophytes (P caesp) (Raunkiaer, 1934) were considered. Woody climbers (P lian) were excluded from the census, due to difficulties in defining their shape and height. Since no woody climber produces a pollen considered allergenic in Emilia Romagna, their exclusion avoided overestimations of the crown volume without compromising the allergenic indices results. For all the arboreal species, only individuals taller than 1 m were censed, in order to simplify the task and to exclude spontaneous young seedlings that would have been

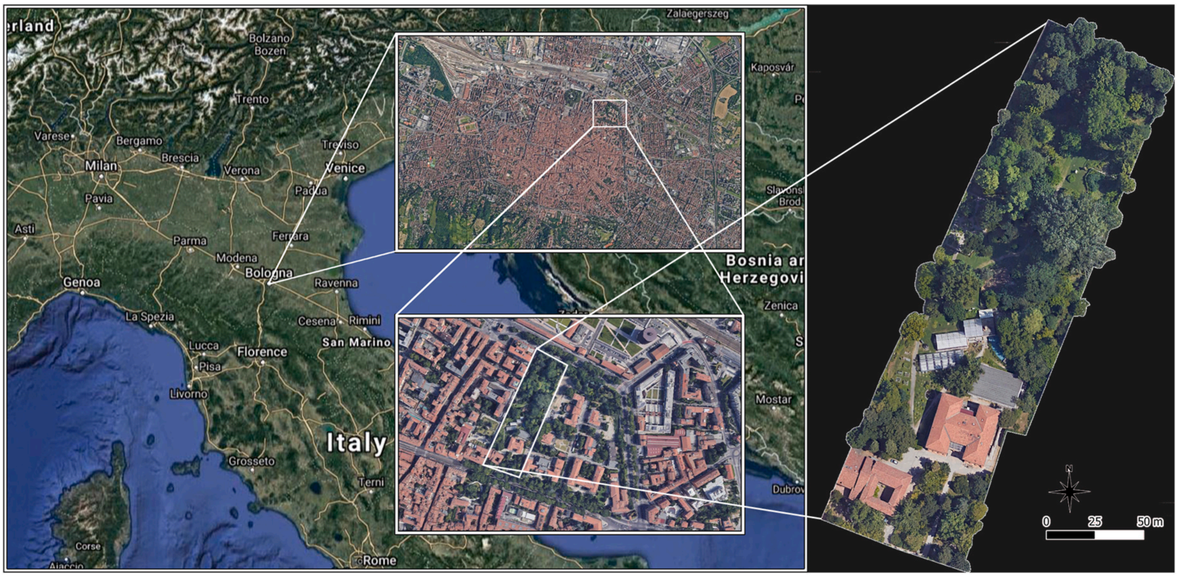

Fig. 1. "Botanical Garden of Bologna" – Map of the Botanical Garden of Bologna and its geographic context, created using satellite images in QGIS and Adobe Photoshop.

Table 1
Scheme for data collection in the field to calculate SAI and I_{UGZA}.

	PARAMETERS COLLECTED IN THE FIELD	
	Parameter	Calculation
Herbaceous species	Maximum height	Average height of the taller plants for each species.
	Relative abundance	Measured as percent surface cover.
Arboreal species	Crown volume	Estimated approximating the crown shape to the geometrical figure of parallelepipedon, sphere, cone, and cylinder (Cariñanos et al., 2014; Kasprzyk et al., 2019).
	Crown base	Calculated as the crown projection on the ground, measuring its diameters or sides with a metric tape.
	Crown height	Measured by clinometer or telemeter. Height was always considered the highest point of the crown, even when it did not fall inside the survey area.
	Sex	Female, male, dioecious/hermaphrodite. Evaluated by analysing the flowers.
	Fertility	Presence/absence of flowers and fruits throughout the year.

removed during the ordinary maintenance of the park. All the specimens that met these requirements were identified up to the species level, using the Botanical Garden plant inventory and monographic flora, and measured as indicated in Table 1.

Additional information on maintenance and vegetation structure was also collected, but it was not used for indices calculation.

The other parameters required by allergenicity indices were drawn from literature, as indicated by their authors (Cariñanos et al., 2014; Hruska, 2003), and are reported in Table 2.

2.3. Data analysis

Species richness, Shannon-Wiener diversity index (H-Index) (Shannon, 1948), and Pielou's evenness (Pielou, 1966) were calculated for both data collections. For the systematic sampling, analyses were carried out separately on either all the spermatophytes, or "arboreal species" (NP, P scap, P caesp) only.

Table 2
Scheme of parameters drawn from literature to calculate SAI and I_{UGZA}.

PARAMETERS DRAWN FROM LITERATURE	
Parameter	Sources
Allergenicity	ARPAE list of allergenic species in the region (ARPAE, 2020a), WAO list of allergenic plants in Italy (WAO, 2012), systematic reviews on allergenic species in Italy (Ortolani et al., 2015), allergen databases (Allergome Team and Collaborators, 2021), books (Oh, 2018).
Cross-allergenicity	Published literature on cross-reactivity among pollen allergens (Cancelliere et al., 2020; Gadermaier et al., 2014; Gangl et al., 2015; Gastaminza et al., 2009; Lombardero et al., 2002; López-Matas et al., 2016; Moraes et al., 2018; Mothes et al., 2004; Panzani et al., 1986; Schwietz et al., 2000; Weber, 2003).
Pollination strategy	Published literature on individual species pollination, when it was not apparent from the flower structure (Anderson, 1976; Meeuse, 1984; Ortolani et al., 2015).
Duration of pollination period/ phenanthesic period	ARPAE pollen calendar for Bologna (ARPAE, 2020b), monographic flora (Conti et al., 2005; Pignatti, 2017).
Life cycle	Monographic flora (Conti et al., 2005; Pignatti, 2017).

Individual plants were included in the allergenicity indices calculations if they were fertile (flowers/fruits present), pollen-producing (males, hermaphrodites, or monoecious), and belonging to an allergenic species. Species were considered allergenic if they met one of the following criteria: (I) listed as allergenic in Emilia Romagna by the ARPAE website www.arpae.it (ARPAE, 2020a); (II) listed as allergenic in Italy by the World Allergy Organisation (WAO) website www.worldallergy.org (WAO, 2012); (III) listed as allergenic in Italy by systematic reviews on the matter (Ortolani et al., 2015); (IV) not reported as allergenic in Italy, but showing cross-reactivity with pollen allergens that are clinically relevant in Italy (Table 2). The workflow followed to evaluate the allergenicity of each plant species is illustrated in Fig. S2.

When the taxonomic determination was not achievable to the species or genus level, the individual was considered allergenic if its genus or family included species that are allergenic in Emilia Romagna.

Hence, allergenicity indices were calculated for both data sets using Formulae (1) (Hruska, 2003) and (2) (Cariñanos et al., 2014).

$$SAI = \frac{\sum_{i=1}^{n} lc_i + pp_i + cr_i + a_i}{N} \quad (1)$$

In (1): **i** = i-species, **N** = total number of allergenic species, **lc** = life cycle, **pp** = phenanthesic period, **cr** = cross reactivity, **a** = abundance. Parameters calculation is explained in Table 3.

SAI ranges between 2 and 10. Values below 4 are associated with a low allergic risk, values from 4 to 6 with a moderate risk, and values above 6 with a high risk (Hruska, 2003). This index is calculated considering allergenic species only.

$$I_{UGZA} = \frac{1}{H_{max} * PAV_{max} * S_T} * \sum_{i=1}^{k} n_i * PAV_i * V_i \quad (2)$$

In (2): $\mathbf{H_{max}}$ = maximum height reached by vegetation; **PAV** = allergenicity potential value (Table 4); $\mathbf{S_T}$ = total surface of the green area; ***i*** = i-species; **n** = total number of individuals; **V** = average vegetation volume.

I_{UGZA} ranges from 0 to 1, with values lower than 0.3 indicating a low allergic risk, from 0.3 to 0.5 a moderate risk, and a high risk above 0.5 (Cariñanos et al., 2014).

In this work, the maximum value assigned to Ap is 3, and it is not attributed an exceptional value of Ap = 4 to the main local allergens, differently from what suggested by I_{UGZA}authors (Cariñanos et al., 2014, 2016). In fact, to assume Ap = 4 while keeping and PAV_{max} = 27 would imply that I_{UGZA} can theoretically exceed the value of 1. On the other hand, using Ap = 4 and assuming PAV_{max} = 36 would significantly lower the final I_{UGZA} result, because the number of main local allergens is usually very small. However, calculations using both of these Ap and PAV_{max} combinations were carried on for comparison, and are reported in Table S3.

For both systematic sampling and census datasets, SAI and I_{UGZA} were calculated on (I) all the allergenic species; (II) arboreal species only.

Since during the census only arboreal species were measured, some approximations were made to extend data of herbaceous species from the sampling to the whole park surface, in order to obtain a complete, albeit rough picture of the park allergenicity. This was realised by extending the percent surface cover of herbaceous species from the total sampled surface of the plots, to the entire vegetated area of the park, as explained by Kasprzyk and collaborators (Kasprzyk et al., 2019). The vegetated area in this case was estimated in QGIS by subtracting the surface occupied by buildings, paths, and the lake, from the total park area. This approach however implies an extreme simplification of the herbaceous vegetation diversity and distribution. In particular, it assumes plants to be evenly distributed along the Garden surface, ignoring that spontaneous plants tend to be aggregated in plant communities. Nonetheless, this simplification appeared acceptable for the purposes of the present study, and it avoided the introduction of subjectivity in the sampling.

Table 3
Estimation of SAI parameters according to Hruska (2003). Abundance is expressed as percent surface cover.

Life cycle (lc)	Phenanthesic period (pp)	Cross reactivity (cr)	Abundance (a)
Annual = 1	Less than 1 month = 0.5	None = 0	<1% = 0.5
Biennial = 2	More than 1 month = 2	Present = 1	1–25% = 1
Perennial = 3			25–50% = 2
			50–75% = 3
			75–100% = 4

Table 4
Allergenicity Potential Value (PAV) parameters for IUGZA calculation, according to Cariñanos and collaborators (Cariñanos et al., 2014), with minor modifications.

Parameter	Definition	Arbitrary values
PAV (or VPA)	Allergenicity Potential Value of each species. PAV = tp*ap*dpp	PAV_{max} = 27
Tp	Type of pollination.	Sterile, cleistogamous or female = 0
		Entomophilous = 1
		Amphiphilic = 2
		Anemophilous = 3
Ap	Allergenicity potential of the plant species relative to the study area.	Nonallergenic = 0
		Low = 1
		Moderate = 2
		High = 3
Dpp	Duration of pollination period. Pollen grains belonging to the same pollen type are considered as a single pollination event.	1–3 weeks = 1
		4–6 weeks = 2
		>6 weeks = 3

On the systematic sampling dataset, species richness, H-index and allergenicity indices were also calculated per plot, to test the linear regression between species richness and allergenic species, and between species diversity and allergenicity indices, using the "lm" function in RStudio (RStudio Team, 2020).

3. Results

During the systematic sampling of 2019, 328 different species were identified, belonging to 86 plant families. They showed high H-index and evenness, as expected for a Botanical Garden (Table 5). Of the species sampled, around 69% were herbaceous, 30% were arboreal, and the remaining 1% were woody climbers (Table 5, Fig. 2A). Among these species, only 46 were allergenic, equally divided between arboreal and herbaceous (Table 5, Fig. 2B), and 21 of them are considered major allergens in the region (Tab. S1, S2).

The arboreal species census of 2020 also revealed a high level of diversity and evenness, comparable with the systematic sampling. This approach detected an arboreal species richness of 226, more than double the one recorded by systematic sampling, divided among 56 plant families. Only 19% of the species censed were classified as allergenic (Table 5, Fig. 3).

Overall, both methods revealed a share of allergenic species lower than a quarter of the total richness. The complete list of allergenic species identified in the Botanical Garden is reported in Tables S1 and S2. The most abundant allergenic species in the park, in terms of percent surface cover, are rough meadow-grass (*Poa trivialis* L., 19.6%), perennial ryegrass (*Lolium perenne* L., 9.8%), upright pellitory (*Parietaria officinalis* L., 1.7%), annual meadow-grass (*Poa annua* L., 0.8%), and mouse barley (*Hordeum murinum* L., 0.5%) for the herbaceous species (Tab. S2); and silver poplar (*Populus alba* L., 10.9%), hazel (*Corylus avellana* L., 6%), paper mulberry (*Broussonetia papyrifera* (L.) L'Hér. ex Vent., 3.6%), box elder (*Acer negundo* L., 3.4%), and field maple (*Acer campestre* L., 3.3%) for the arboreal species (Tab. S1). Hazel and field maple have the habitus of small trees and shrubs, thus their high surface cover corresponds to a high number of individuals (56 and 48 respectively). On the contrary, the other arboreal species with high surface cover are represented by a small number of individuals. Allergenic arboreal species with an high number of individuals are instead broadleaf privet (*Ligustrum lucidum* W.T.Aiton, 173), common privet (*Ligustrum vulgare* L., 119), and field elm (*Ulmus minor* Mill., 30). However, it

Table 5
Species richness, species diversity, evenness, I_{UGZA}, and SAI values for the Botanical Garden of Bologna, according to the systematic sampling of 2019 and the arboreal species census of 2020.

Parameter	Plant type	Systematic sampling	Census
Species richness	All spermatophytes	328	–
	Arboreal species	99	226
	Herbaceous species	225	–
	Woody climbers	4	–
Allergenic species richness	All spermatophytes	46	–
	Arboreal species	23	43
	Herbaceous species	23	–
Shannon-Wiener index (H-index)	All spermatophytes	4.21	–
	Arboreal species	3.65	4.45
Evenness	All spermatophytes	0.73	–
	Arboreal species	0.79	0.82
I_{UGZA}	All spermatophytes	0.07	0.07
	Arboreal species	0.06	0.07
SAI	All spermatophytes	5.98	6.12
	Arboreal species	6.17	6.30

was not possible to assess whether the plants counted as different individuals could be ramets of a clonal colony, hence the percent surface cover was used as measure of abundance in all the calculations, instead of the number of individuals.

The areas of the Botanical Garden with a higher number of allergenic species, according to the systematic sampling, were a wooded hill (Fig. S1, plot 8), hosting 13 allergenic species in 100 m^2, both herbaceous and arboreal; the recreation of a local forest (Fig. S1, plot 16), with 12 herbaceous and arboreal allergenic species; and the orchard of edible and medicinal plants (Fig. S1, plot 14), that harboured 11 allergenic herbaceous species, 8 of which were spontaneous grasses and weeds.

The linear regression calculated on the number of species per plot from the systematic sampling, suggests a significant positive correlation ($p\text{-value}<0.05$) between total species and allergenic species richness, and between non-allergenic and allergenic species richness. However, in both cases the dispersion was too high (adjusted $R^2 < 0.4$) to confirm the relationship (Fig. S3).

Allergenicity potential estimations were consistent between systematic sampling and census, for both I_{UGZA} and SAI. Moreover, the results of the two indices did not change significantly when considering all the spermatophytes, or arboreal species only. Nonetheless, it is worth

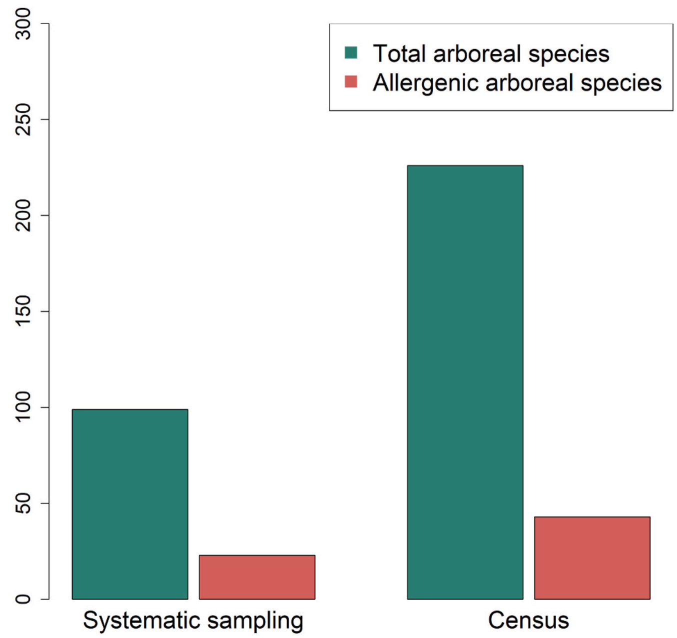

Fig. 3. "Allergenic species" - Comparison between total and allergenic arboreal species for the Botanical Garden of Bologna, according to the systematic sampling of 2019 and the census of 2020.

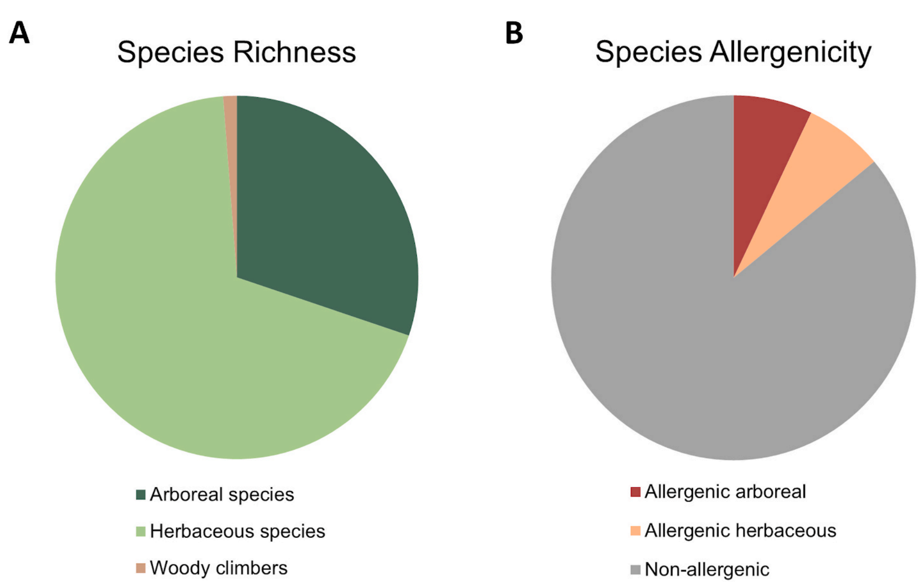

Fig. 2. "Species richness" - Distribution of the species richness among three groups of vegetation (A), and partition of allergenic and non-allergenic species (B) in the Botanical Garden of Bologna, according to the systematic sampling of 2019.

noting that I_{UGZA} was slightly lower when considering only the arboreal species of the systematic sampling, while SAI was slightly higher for the arboreal species of both the systematic sampling and the census (Table 5, Fig. 4). However, the major difference between the two indices is the estimated risk level. In fact, while SAI pointed towards a moderate to high allergenic risk for the park, with values around 6, I_{UGZA} values lower than 0.1 suggested a very low allergenicity potential, well under the threshold of 0.3 indicated by I_{UGZA} authors as a trigger for allergic reactions (Table 5). Results of the alternative formulations of I_{UGZA} with $ap = 4$ for the main allergens can be found in Table S3 and agree with this allergenicity level. In fact, I_{UGZA} resulted 0.01 units lower when using $PAVmax = 36$, and 0.01 units higher when using $PAVmax = 27$, for all the datasets.

When focusing on the most problematic allergenic species of the park, results are less clear. In fact, while systematic sampling and census led to the same allergenicity indices values, they notably differed in identifying the main plant species responsible for it. This discrepancy is present in both indices (examples in Tables 6 and 7), but it is more pronounced in I_{UGZA}, where systematic sampling overestimated the importance of some allergenic species such as European hop-hornbeam (*Ostrya carpinifolia* Scop.) and field elm (*Ulmus minor* Mill.), that resulted less influent on the allergenicity when considering the whole arboreal vegetation. In fact, the census revealed that the most problematic species for the allergenicity of the Botanical Garden are silver poplar (*Populus alba* L.) and hazel (*Corylus avellana* L.). On the other hand, both data collection methods and allergenicity indices agreed in indicating the narrow-leafed ash (*Fraxinus angustifolia* Vahl), a major allergen of the region, as one of the main contributors to the park allergenicity. These differences are explained by the lower species richness detected by the systematic sampling, and the different relative abundances reported for the same species between the sampling and the census.

H-index, I_{UGZA}, and SAI were also calculated for each plot from the systematic sampling dataset. A linear regression test was performed to further investigate the relationship between plant diversity and allergenicity (Fig. S4), obtaining no significant correlation between H-Index and the two allergenicity indices (p-value>0.1).

4. Discussion

This study-case is peculiar due to the nature of the park, that is conceived to host a great diversity of plant species in a relatively narrow space, and to reproduce various habitats. While the Botanical Garden was otherwise a good model for method standardisation, these characteristics complicated the sampling design, that needed to be optimised to be suitable for both the dense and variable vegetation of the Garden, and the more sparse and uniform vegetation of other urban parks. A common feature of all these green areas however is their anthropogenic nature, that implies an artificial distribution of the individual ornamental plants, creating aggregations of some species in structures such as living screens, groves, and rows. While the distribution of spontaneous plants like weeds tends to be random, ornamental species distribution in urban parks is usually planned, and a random sampling would probably miss most of the plant diversity. Hence, the systematic sampling was chosen as fittest method to reduce the time and effort needed to analyse the vegetation, while still obtaining objective data suitable for statistical analysis. Nonetheless, possible sampling issues might occur when the systematic sampling grid overlaps with geometric features of the park design. This can be avoided with a careful placement of the grid in the

Table 6
List of the three main arboreal and herbaceous species contributing to I_{UGZA} for the Botanical Garden of Bologna with their percent cover on the total park surface (% cover). Species are ordered from higher to lower I_{UGZA} values.

Importance	Family	Species	% cover
Arboreal species from census			
1	Salicaceae	*Populus alba* L. **	10.9
2	Betulaceae	*Corylus avellana* L. ***	6.0
3	Oleaceae	*Fraxinus angustifolia* Vahl ***	2.2
Arboreal species from systematic sampling			
1	Oleaceae	*Fraxinus angustifolia* Vahl ***	2.2
2	Betulaceae	*Ostrya carpinifolia* Scop. *	1.4
3	Ulmaceae	*Ulmus minor* Mill. *	2.0
Herbaceous species from systematic sampling			
1	Poaceae	*Poa trivialis* L. ***	19.6
2	Poaceae	*Lolium perenne* L. ***	9.8
3	Urticaceae	*Parietaria officinalis* L. ***	1.7

Allergenic levels for the Emilia Romagna region: * slightly allergenic; ** moderately allergenic; *** extremely allergenic.

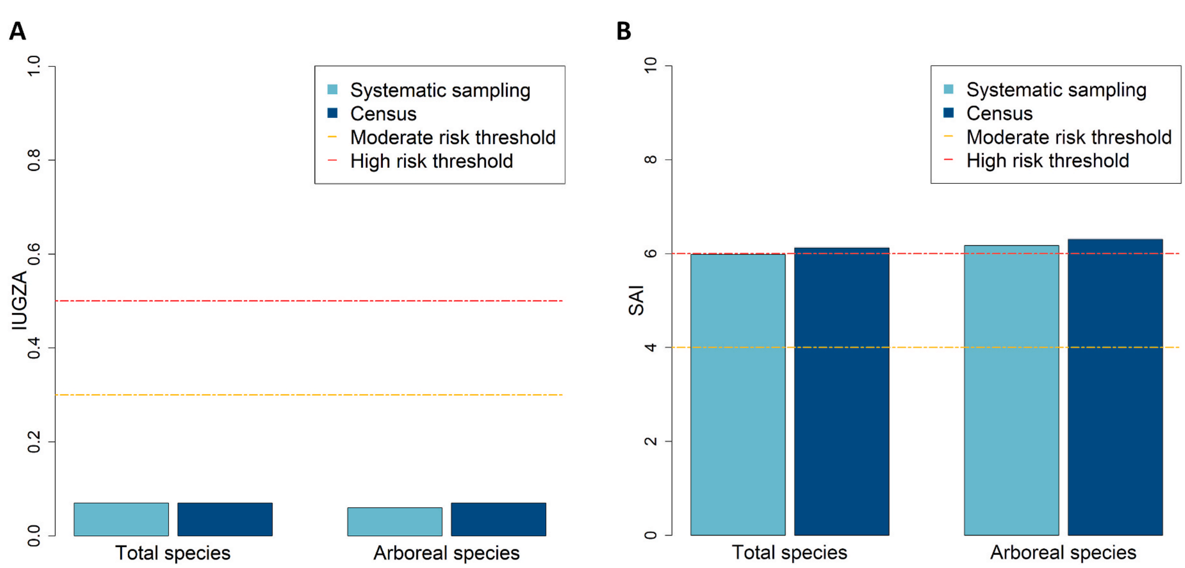

Fig. 4. "Allergenicity indices" - Comparison of IUGZA (A) and SAI (B) values for the Botanical Garden of Bologna, calculated on different datasets and vegetation groups. Light blue bars: data from systematic sampling of 2019; dark blue bars: data from arboreal census of 2020. Dotted lines indicate the risk thresholds: yellow for moderate and red for high risk. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 7
List of the plant species with the highest SAI value (SAI = 7) for the Botanical Garden of Bologna, with their percent cover on the total park surface (% cover).

Family	Species	% cover
Systematic sampling		
Betulaceae	*Corylus avellana* L. ***	6.0
Betulaceae	*Ostrya carpinifolia* Scop. *	1.4
Cupressaceae	*Cupressus sempervirens* L. ***	0.5
Oleaceae	*Fraxinus angustifolia* Vahl ***	2.2
Pinaceae	*Pinus nigra* J.F.Arnold *	0.7
Poaceae	*Poa trivialis* L. ***	19.6
Poaceae	*Lolium perenne* L. ***	9.8
Urticaceae	*Parietaria officinalis* L. ***	1.7
Census		
Betulaceae	*Corylus avellana* L. ***	6.0
Betulaceae	*Ostrya carpinifolia* Scop. *	1.4
Cupressaceae	*Sequoia sempervirens* (D.Don) Endl. *	1.1
Fagaceae	*Quercus ilex* L. **	1.6
Fagaceae	*Quercus robur* L. *	1.5
Oleaceae	*Fraxinus angustifolia* Vahl ***	2.2
Pinaceae	*Pinus nigra* J.F.Arnold *	1.2
Taxaceae	*Taxus baccata* L. *	0.4

Allergenic levels for the Emilia Romagna region: * slightly allergenic; ** moderately allergenic; *** extremely allergenic.

GIS environment, considering vegetation structure and paths distribution, or by modifying the mesh dimension.

In this study, systematic sampling covered only 10% of the park total surface. Nevertheless, it provided half of the arboreal species richness recorded by the complete census. It also allowed to estimate species diversity and evenness values comparable to those of the census, and compatible with the heterogeneity of the park (Table 5). Most importantly, the systematic sampling allowed to estimate not only the same allergenic risk level, but almost the same allergenicity potential values than those calculated on the complete census dataset (Table 5, Fig. 3). This suggests that the sampling method hereby proposed could offer a reliable approximation of the park allergenicity, by analysing as little as 10% of the park extension. However, it is important to reproduce this study in other urban parks, to verify and corroborate the consistency between the results obtained by systematic sampling and census. If these results will be confirmed, this approach could offer a quick way to obtain reliable and comparable allergenic levels in future studies.

The main problem of systematic sampling revealed by this study is the misidentification of the main allergenic plant species. This can be explained by the fact that individuals of the same species are not evenly or randomly distributed along the park, but instead they tend to be aggregated in the recreation of small habitats. Hence, the higher surface cover of a species does not always correspond to a higher probability to be sampled. This caused the systematic sampling to miss entirely some allergenic species that were instead identified as the main drivers of the Botanical Garden allergenicity by the census. This problem could be avoided increasing the percentage of the park surface sampled.

The two allergenicity indices consistently pointed towards two different allergenicity levels for the park, for all the datasets considered (Table 5). While SAI indicated a moderate to high allergenicity potential, I_{UGZA} reported allergenicity values that are lower than most of those published for Mediterranean parks (Cariñanos et al., 2017; Kasprzyk et al., 2019). The extremely low values of I_{UGZA} could depend on the fact that this study employed the first version of the index (Cariñanos et al., 2014), keeping the maximum height in the numerator (2), and considering it to be the height of the tallest individual plant in the dataset. In recent papers, I_{UGZA} formula is presented without the maximum height in the numerator (Cariñanos et al., 2017; Velasco-Jiménez et al., 2020), but this would impair the index reliability since the maximum I_{UGZA} value could exceed 1, and the index would have the unit of measurement of the height (m). Another difference of the I_{UGZA} formula employed in this work with those employed in literature was the choice to not consider extreme values of Ap = 4, but this did not have a significant impact on the results (Tab. S3).

Assuming a comparability between the results of the present study with those published in literature for other green areas, I_{UGZA} values for the Botanical Garden are consistent with those obtained for other public and private parks evaluated in Central Italy (Rome), such as Villa Sciarra, Parco Centrale del Lago, and Parco San Sebastiano (Cariñanos et al., 2019), and with the Spanish historic park Parque de los Pinos, that also has a similar tree density (Cariñanos et al., 2017). On the other hand, the allergenicity potential of the Garden is lower than other Mediterranean urban green areas of comparable extension, like the Spanish parks Campus Norte (Orense), Jardín de Ajora (Valencia), and La Alamedilla (Salamanca) (Cariñanos et al., 2017).

It is apparent that the Botanical Garden has a low number of allergenic species, none of which shows a spatial dominance since Pielou's evenness is very high, despite the presence of clustered habitats. While this justifies the low allergenicity level detected by I_{UGZA}, it disagrees with SAI results. This inconsistency does not seem to be linked to the peculiar heterogeneity the study area, since no correlation between the plant diversity and the results of the allergenic indices has been detected in this study (Fig. S4). Moreover, in this study, the behaviour of the two indices was not affected by the species richness considered (Table 5, Fig. 4). Hence, the difference between I_{UGZA} and SAI results is likely explained by the different parameters they consider. In order to choose which index is to be trusted, it is important to notice that some of these parameters might not be strictly related to the allergenicity potential, such as the duration of the life cycle (lc) in SAI; while others, like the local allergenicity (ap) in I_{UGZA}, can be considered more accurate (Tables 3 and 4). SAI in fact lacks such parameter, and thus it assumes all the species to be equally allergenic, even though the allergenic species of the Botanical Garden displayed a wide range of allergenicity levels. Another limit of SAI is that being an average value, it can overestimate the allergenicity of a green area when considering only a subset of allergenic species that are perennial or have higher surface cover. This explains why the allergenicity of the park appears higher according to SAI when considering arboreal species only, compared to the allergenicity calculated on all spermatophytes (Table 5, Fig. 3B). In conclusion, I_{UGZA} seems more reliable than SAI in estimating the allergenicity potential of a green area, hence the Botanical Garden of Bologna may be considered safe for pollen-allergic visitors, based on the local vegetation. However, neither of the two allergenicity indices takes into account the extra-local component of airborne pollen, that could affect the air quality of the park. Hence, to thoroughly assess the allergenicity risk of the area, airborne pollen sampling at ground and roof level should be carried out as well.

Another interesting finding of this research is that the allergenicity potential of the Botanical Garden is mostly driven by trees and shrubs, even though herbaceous species are more than double the arboreal species sampled, and they cover almost the same surface. This finding is in agreement with previous statements by Cariñanos and collaborators (Cariñanos et al., 2016, 2017), and it suggests that the systematic sampling of only arboreal species may be an accurate way to simplify and speed up the data collection. However, this hypothesis needs to be tested on other parks that have a sparser tree canopy.

Finally, the systematic sampling allowed to test the relationship between plant diversity and allergenicity. A possible positive linear relationship between species richness and number of allergenic species is not supported by the adjusted R^2, while there is no correlation between H-Index and I_{UGZA} values. This was expected since the distribution of plant species in urban parks is intentional and driven by aesthetical or practical motives, hence the presence of allergenic species is aleatory. Moreover, with equal plant diversity, the allergenicity potential of an area can change according to the number and the volume of the allergenic species present in the area. Vice versa, if the whole volume of the vegetation is made of plants having the same PAV (Table 4), the allergenicity potential would be the same whether all the individuals belong to the same species, or if each one of them belongs to a different species. These hypotheses however need to be supported by further studies on wider areas.

5. Conclusions

Testing two data collection methods and two allergenicity indices on the same urban park allows to validate the sampling approach and the reliability of the indices. The present work shows that systematic sampling and complete census of selected phanerophytes provide comparable results, hence the systematic sampling could be a valid and rapid option for data collection when calculating allergenicity indices. Moreover, vegetation sampling to calculate allergenicity indices could be limited to trees and shrubs, since they seem to drive the allergenicity of the park. However, these hypotheses need to be confirmed by further studies on different urban parks. Nonetheless, plant checklists and inventories should be compiled for the park, in order to detect the allergenic species possibly missed by the sampling.

While the data collection methods are comparable, the two indices led to opposite risk evaluations. According to I_{UGZA}, the allergenicity of the Botanical Garden of Bologna is very low, while according to SAI it ranges from moderate to high. Since I_{UGZA} behaviour on different datasets appeared more consistent than that of SAI, it was hereby considered more reliable. To corroborate this hypothesis, allergenicity indices should be compared to the allergic symptomatology reported by the Botanical Garden visitors in future studies.

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Author contributions

CS, IA, LP, DFG, and SDD contributed to the design of the work as well as drafting the work and revising it critically for important intellectual content; then they made the final approval of the version to be published. They agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. In details, the idea to write an article about allergenicity indices was proposed by CS, DFG and SDD. CS performed the thorough literature search, designed and performed data collection and analysis with the contribution of IA and LP. CS wrote the article, with the contribution of all authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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