



Pollen viability as a bio-indicator of air quality

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Abstract

Many air pollutants cause plant deterioration. In this study pollen viability was used as bio-indicator of air quality. The study was carried out in the city of Perugia where road traffic is the most important cause of air pollution.

Three areas, with different intensity of road traffic (very high, medium and absent) but all characterized by the presence of the same plant species, were selected.

Eight species were studied: *Hedera helix* L., *Convolvulus sepium* L., *Cynodon dactylon* (L.) Pers., *Quercus ilex* L., *Dactylis glomerata* L., *Parietaria diffusa* M. et K., *Daucus carota* L. and *Tilia cordata* Miller. The pollen of these species was treated with TTC (2, 3, 5 Tryphenil-Tetrazolium-Chloride) staining solution and viability was then estimated by light microscopy. The results showed that the pollen viability was inversely proportioned with pollution. The highest difference in pollen viability between the areas was registered in *Tilia cordata*. *Quercus ilex* instead showed that there was no difference in pollen viability between the three different areas. *Parietaria diffusa* showed a particular behaviour; the highest pollen viability percentage was in polluted areas.

The statistical analysis (ANOVA) showed that the main source of variability of the pollen viability depends on the plant but also the site and the interaction between plant and site were very important with a high significant level ($p < 0.0001$).

Introduction

Recent studies on the effects of air contaminants on vegetation were concerned mainly with the estimation of the effects on vegetative parts, especially leaves. Leaves have a very sensitive structure and, in the presence of some air pollutants, show typical necrotic or chlorotic areas, changes in stomatal apertures, epidermis and mesophyll.

Available information is limited about the effects of air pollutants on the reproductive parts of plants. Mature pollen, ready to be released by the plant, is dry and consequently highly hygroscopic, so it can absorb the humidity of the atmosphere. If the atmosphere contains air pollutants, pollen, by absorbing water, also takes these compounds which can influence its viability (Comtois, 1994).

Sometimes there are also changes in the morpho-biometric parameters of pollen grains (Melati et al., 1997).

However, city environments where pollutants from road traffic are predominant have not been studied as much as natural environments. In order to estimate the relationship between pollen viability and atmospheric pollution, and, therefore, to evaluate the use of pollen viability as an index of pollution, we carried out stratified collections of pollen grains in zones of different pollutant loads and pollen viability was estimated on a sub-sampling of these collections. In order to see how parameters other than air pollution could affect the estimation of viability, different species were used.

Table 1. Species, sampling areas and periods during which the collection of samples took place.

Species	Area	Period
<i>Cynodon dactylon</i>	Collestrada	August 1997
	San Pietro	
	Porta Pesa	
<i>Convolvulus sepium</i>	Collestrada	August 1997
	San Pietro	
	Porta Pesa	
<i>Dactylis glomerata</i>	Collestrada	June 1997
	San Pietro	
	Porta Pesa	
<i>Daucus carota</i>	Collestrada	August 1997
	San Pietro	
	Porta Pesa	
<i>Hedera helix</i>	Collestrada	September 1997
	San Pietro	
	Porta Pesa	
<i>Parietaria diffusa</i>	Collestrada	July 1997
	San Pietro	
	Porta Pesa	
<i>Quercus ilex</i>	Collestrada	May 1998
	San Pietro	
	Porta Pesa	
<i>Tilia cordata</i>	Collestrada	June 1997
	San Pietro	
	Porta Pesa	

Materials and methods

The pollen of five herbaceous spontaneous species: *Convolvulus sepium* L., *Cynodon dactylon* (L.) Pers., *Dactylis glomerata* L., *Parietaria diffusa* M. et K., *Daucus carota* L.; of two arboreal species: *Quercus ilex* L., *Tilia cordata* Miller and of a shrub species: *Hedera helix* L. growing in Perugia, was used. The samples were collected in three different areas (Figure 1): *Porta Pesa*, which is characterized by a high intensity of road traffic, *San Pietro*, which is a typical tourist place, and is characterized by medium intensity road traffic and *Collestrada*, which is suburban with a lack of road traffic (E. Bricchi et al., 1998). Species, sampling areas and periods are listed in Table 1.

Pollen was collected from plants in full flowering period which showed maximum anther dehiscence.

Table 2. Pollen viability percentage of the examined plant species.

Species	Mean of pollen viability % and standard deviations		
	Porta Pesa	San Pietro	Collestrada
<i>Cynodon dactylon</i>	91.0 ± 2.9	88.4 ± 3.4	85.0 ± 4.0
<i>Convolvulus sepium</i>	25.0 ± 2.3	30.3 ± 2.5	91.3 ± 4.3
<i>Dactylis glomerata</i>	60.5 ± 3.6	81.5 ± 2.6	94.1 ± 3.2
<i>Daucus carota</i>	39.2 ± 2.7	76.1 ± 4.7	88.0 ± 2.4
<i>Hedera helix</i>	84.0 ± 3.6	85.3 ± 2.9	72.2 ± 4.9
<i>Quercus ilex</i>	83.5 ± 3.2	89.4 ± 2.3	91.0 ± 2.8
<i>Parietaria diffusa</i>	80.4 ± 3.5	21.2 ± 1.8	8.1 ± 0.9
<i>Tilia cordata</i>	12.2 ± 1.5	14.0 ± 1.7	88.1 ± 5.0

Our sampling was carried out in late spring-early summer when pollen viability showed the highest percentage (Franchi et al., 1984).

For each area of the study we chose three plants of the same species and we prepared three slides for each plant. This test was done in the field immediately after sampling.

We used TTC staining technique which is an enzymatic viability test (Sarvella, 1964; as suggested by Comtois, 1991).

The TTC-test is a biological stain that is utilized to detect the respiration of cells. Viable pollen is stained red, while the dead pollen remains yellow/white. Many different techniques exist for pollen viability estimation, TTC is the most widely used specific stain principally in airborne pollutant heavy metal studies, where respiration is a better indication of toxicity than other parameters.

All the samples were treated with the TTC solution and after 24 hours, at room temperature, we estimated the pollen viability.

The data of pollen viability % are mean values obtained by sampling three plants for each area and for several consecutive days.

Results and discussion

The results of the mean pollen viability percentage of the examined species in the three selected areas are reported in Table 2.

The results were contrasting: some species seemed to be influenced by air pollution; the percentage of pollen viability was inversely proportioned to the density of road traffic; some species, such as *Cynodon*

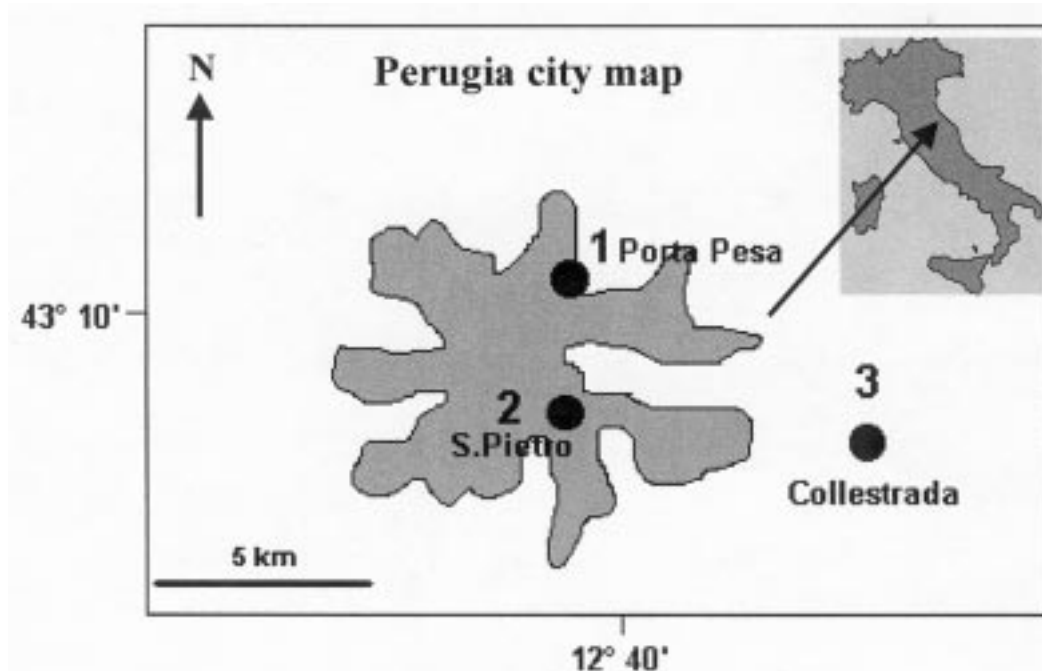


Figure 1. Map of the pollen sampling areas.

dactylon and *Quercus ilex*, according to Bellani et al., 1998, did not seem to be very damaged by atmospheric pollution. Other species, however, such as *Parietaria diffusa* M. et K., seem to be well adapted to air pollution and showed their maximum pollen viability in the area of the city where road traffic is highest (Figure 2). This species has probably developed a particular metabolism to control air pollutants (Melati et al., 1997). In central Italy, *Parietaria diffusa* has a long flowering period corresponding to meteorological conditions which also influence pollen viability. Its long flowering period determines a high production of seeds, and therefore, of different genotypes, which undergo the natural selection to adverse environmental conditions. It is probable that in urban environments, those individuals which best resisted atmospheric pollutants were gradually selected, and *Parietaria*, adapted to the microclimate of the city walls, confirms this hypothesis.

Hedera helix and *Cynodon dactylon* did not seem to be influenced by air pollution. However, *Daucus carota*, *Convolvulus sepium* and *Dactylis glomerata* were damaged. The pollen viability percentage of the area of Porta Pesa was about half that of Collestrada. These species seem to have a poor capacity of adaptation.

Cynodon dactylon and *Dactylis glomerata* are two Gramineae but they have a different appropriateness to the areas characterized by a low air quality. *C. dactylon* seems to be more resistant than *D. glomerata*.

Cynodon dactylon flowers at the end of August/beginning of September, when the relative humidity of the air is lower than the percentage of humidity recorded in the flowering period of *Dactylis glomerata*, which occurs in spring. The particles in the atmosphere remain suspended in the droplets of humidity which are easily absorbed by the pollen. It is natural to think that a plant which emits its pollen at the same time as high percentages of relative humidity should undergo greater stress deriving from airborne pollutants than a plant which has its flowering period in the summer.

Hedera helix has a viability trend (Figure 2) which is similar to that of *Cynodon dactylon*, for the same reasons hypothesised for it.

Cynodon dactylon, like *Parietaria*, has a high seed production during the year. Therefore, the high pollen viability percentage in environments with a high traffic density could be due to natural selection.

In general, *Quercus ilex* and *Tilia cordata* are used as ornamental plants on road sides. Our research has shown that *Quercus ilex* does not seem particularly influenced by the degree of environmental pollution,

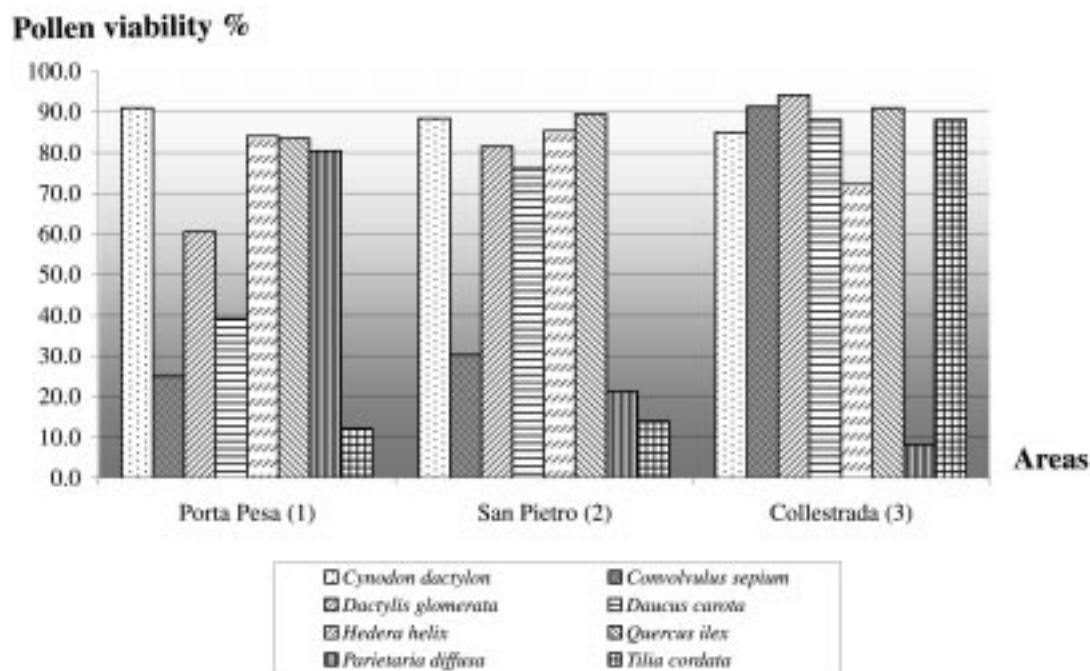


Figure 2. A comparison of the mean pollen viability percentage of the examined plant species. In general the pollen viability is inversely proportioned to the density of road traffic.

even if the percentage of pollen viability registered in the area of Collestrada is higher than that in Porta Pesa. *Quercus ilex* was not strongly influenced by the different air conditions probably because it is a typical plant of the Mediterranean evergreen maquis so it is more appropriate to the urban environment.

Tilia cordata, on the other hand, shows, in urban environment, a very low pollen viability percentage. This result explains a low capacity of adaptation to the city because *Tilia* is a typical plant of mountain places.

In general, the highest pollen viability percentage is in unpolluted areas such as in Collestrada, but sometimes some vegetable species have unusual behaviour. In *Parietaria diffusa*, the pollen viability percentage is higher in Porta Pesa than in Collestrada.

In Table 3 we present the results of two factors ANOVA where the percentage of variability is segregated by sites (i.e. contamination) and plant species.

We can see that these two factors have a significant (-0.0001) influence on pollen viability and that for any future investigations using viability as a bio-indicator the choice of the plant species will be more determinant than the site of the study.

Moreover, there is a significant interaction between plant species and site, an unexpected result because

Table 3. Statistical analysis ANOVA.

Sources of variability	F	P
Main Effect	1233.36	< 0.0001
Site	789.79	< 0.0001
Plant	1357.90	< 0.0001
2 way interaction	661.26	< 0.0001
Plant/Site		
Explained	884.604	< 0.0001

these species have different ecological requirements. Therefore, it will always be difficult to compare different species growing in different environments.

Conclusions

Concluding, we could define, on the basis of the pollen viability test results, *Cynodon dactylon* and *Parietaria diffusa* as bio-accumulators, are tolerant species, while *Daucus carota*, *Convolvulus sepium*, *Tilia cordata* and *Dactylis glomerata* as bio-indicators, are sensitive species to pollutants; *Hedera helix* and *Quercus ilex*

instead, were uninfluenced by environmental conditions.

This research presents data of only eight species, which are insufficient to give a specific conclusion. Nevertheless, based on these first results, we can say that pollen viability can be a significant parameter to see which plant species can adapt to an urban environment.

To understand how pollen viability evolves, it is necessary to know, other than the percentage referred to at the moment of its release, the length of the viability itself in the subsequent days. This kind of study is currently the object of our research.

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