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Biomonitoring Air Pollution in Carob Leaves †

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Abstract: The optical properties and ecophysiological parameters of leaves of *Ceratonia siliqua* L. (carob), expanded in more and less polluted habitats, were compared, in order to evaluate the effect of air quality in leaf development. The accumulation of pigments (chlorophylls *a* and *b*, and carotenoids) and specific leaf area (SLA, $cm^2 g^{-1}$) were seasonally determined during leaf development (i.e., in nine successively grown leaves along shoots). Leaf transmittance (T) and reflectance (R) spectra for both adaxial and abaxial leaf surfaces were measured between 250 and 2500 nm wavelengths, using a UV-VIS spectrophotometer and leaf absorptance (Abs) [(Abs = 100 – (R + T)] is used to assess the effect of environmental quality of more and less polluted habitats in Athens, according to the files of the Hellenic Ministry of Environment and Energy, on carob leaf physiology. An increase, in the studied leaf parameters, was observed, for carob trees grown in the urban site. There was an increase in SLA from spring to late summer and a decrease in late autumn. Leaves of the less polluted site in the bush, regardless of the developmental stage exhibited greater water absorption, while the adaxial surface absorbed more radiation in both categories of plants. It seems likely that differences in optical properties and pigment accumulation have important implications for model simulation purposes and may be used for air pollution biomonitoring .

Keywords: Air pollution; biomonitoring; chlorophyll; *Ceratonia siliqua;* climate change; leaf optical properties; model simulation; pigment accumulation; SLA

1. Introduction

The urban environment does not usually offer to trees ideal living conditions (ground impermeable, less water available, lack of soil nutrients, toxic products and atmospheric pollutants). Air pollutants lead to a variety of adverse effects and visible injury symptoms in plant leaves. Various studies show that different plant species elicit the environmental quality in which they grow by changing their leaf anatomical and physiological properties, and thus changes in leaf properties can be used to provide a reasonably accurate assessment of habitat quality [1–4]. Pollution can directly affect plants' physiology either via leaves exposed to air-polluted conditions or indirectly via soil acidification. Pollutants absorbed by the leaves cause changes in stomatal opening, photosynthesis and the concentration of chlorophylls, which directly affects the plant productivity [5]. The effect of the air pollutants on plant structure and function has been in the focus of interest for many investigators. It is difficult to estimate the effects of air pollutants because organisms are concomitantly exposed to a wide range of uncontrolled abiotic and biotic variables (parasites, weather conditions and complex mixture of pollutants). On the physiological and morphological point of view, the plants from polluted sites possess important phenotypical alterations changes especially regarding their colors, shapes, leaf length, width, area and petiole length. As leaves represent the main surfaces of plant canopies where energy and gases are exchanged they are the most sensitive parts to be affected by air pollution; therefore, at various stages of leaf development, they may serve as sensors of air pollutants, indicating that plants do survive in polluted environments [6–9].

Biomonitoring is useful for the assessment of environmental impacts of pollution on living organisms including plants. The benefit of using plants as a bio-sensors is their uncomplicated deployment in field campaigns. Moreover, monitoring based bio-sensors are cheap compared to the costly physico-chemical monitoring [10–12].

Carob tree (*Ceratonia siliqua* L.) is being investigated as a potential bio-monitor plant for urban habitats. It is a common tree, native in the Mediterranean Basin [13], appearing in urban and suburban areas, exhibiting great morphogenetic plasticity and tolerance to drought stress conditions [14]. It requires little if any cultivation, tolerates poor soils and is long lived [15,16]. Carob tree has a great potential as a tree crop for restoring vegetation, reforestation and improving the productivity of marginal drylands. It is widely planted as an ornamental tree on the streets, considering that it reflects sunlight and reduces noise pollution. The sclerophyll carob leaves are characterized by a very thick, unilayered adaxial epidermis, while stomata are present only on the abaxial surface [17–19]. The compound leaves of carob expand within a 3-months period; then, they cease growing, and are exposed to the environmental conditions for approximately 20 months [20–22].

The objective of this research is to understand the effect of air pollution on the optical properties and on the chlorophyll content of carob leaves and develop a model that classifies an area whether it is polluted or not, by using this plant species as a bio-monitor.

2. Experiments

Compound leaves of two carob trees (approximately 60–70 years old), without any watering or fertilizing treatment, grown at two sites with different air quality (more polluted urban area 37°58'17.85''N, 23°45'28.24''E, and less polluted suburban area 37° 57'34.35''N, 23° 47'56.25''E) were collected throughout a year. Concentrations of air pollutants were measured by the Hellenic Ministry of Environment and Energy [Table 1] [23]. The accumulation of photosynthetic pigments (chlorophylls *a* and *b*, and carotenoids) was seasonally determined during the leaf development (i.e., in nine successively grown leaves along shoots). Leaf area, dry weight and specific leaf area were also estimated. Transmittance (T), reflectance (R) and absorptance (A) spectra for both the adaxial and the abaxial leaf surfaces were measured between 250 and 2500 nm wavelength (bandwidth 2 nm), using a UV-Vis spectrophotometer.

Table 1. Mean PM_{10} (particulate matter with a diameter less than 10 μm), NO, NO_2 and O_3 (μg m^{-3}) and CO (mg m^{-3}) values at the two experimental sites of Athens metropolitan area.

2018	Less polluted (suburban area)					More polluted (urban area)				
Months	PM_{10}	CO	NO	NO_2	O_3	PM_{10}	CO	NO	NO_2	O_3
Apr	27	-	2	18	106	38	0.5	10	41	60
May	3	-	1	14	96	32	0.4	5	28	76
Jun	20	-	1	13	101	29	0.3	3	25	81
Jul	18	-	1	11	101	28	0.4	4	28	80
Aug	19	-	1	6	103	26	0.2	1	16	85
Sep	17	-	1	14	96	5	0.3	4	7	79
Oct	23	-	2	13	73	31	0.4	9	3	63
Nov	15	-	2	14	53	25	0.5	11	29	42
Dec	12	-	1	12	52	33	0.9	23	30	42
Jan	13	-	2	14	59	33	0.8	24	35	44
Feb	13	-	2	13	66	27	1.5	11	34	59
Mar	37	-	1	15	79	44	0.7	6	28	59

2.1. Estimating Specific Leaf Area and Chlorophyll Content

Nine successively leaves grown along shoots were collected early in the morning. Following harvest (within 1 h), the leaves were scanned in a flatbed scanner to calculate the fresh area using ImageJ Pro then they dried at 60 °C for 48 h to a constant mass and weighed to the nearest 0.001g. Specific leaf area (SLA) was calculated by the ratio of fresh leaf area per dry leaf mass ($cm^2 g^{-1}$). The dried material was then powdered, using a MFC mill (Janke and Kunkel GMBH & Co, Germany) and stored in tightly sealed containers, in a cool dry and dark environment. The total chlorophyll (Chl) content was spectrophotometrically determined in leaf samples according to a modified acetone method [24]. Chlorophyll concentration was extracted from dried, grounded leaf samples mixed and homogenized with acetone (80% *v*/*v*) using china pestle and mortar, and filtered through Whatman # 2 filter paper. The chlorophyll content was measured in aliquots of the leaf extracts using a spectrophotometer (Pharmacia Biotech Novaspec II) at A663.2, A646.8, A470 and the absorbance readings were applied to relevant equations, in order to determine the chlorophyll content [24].

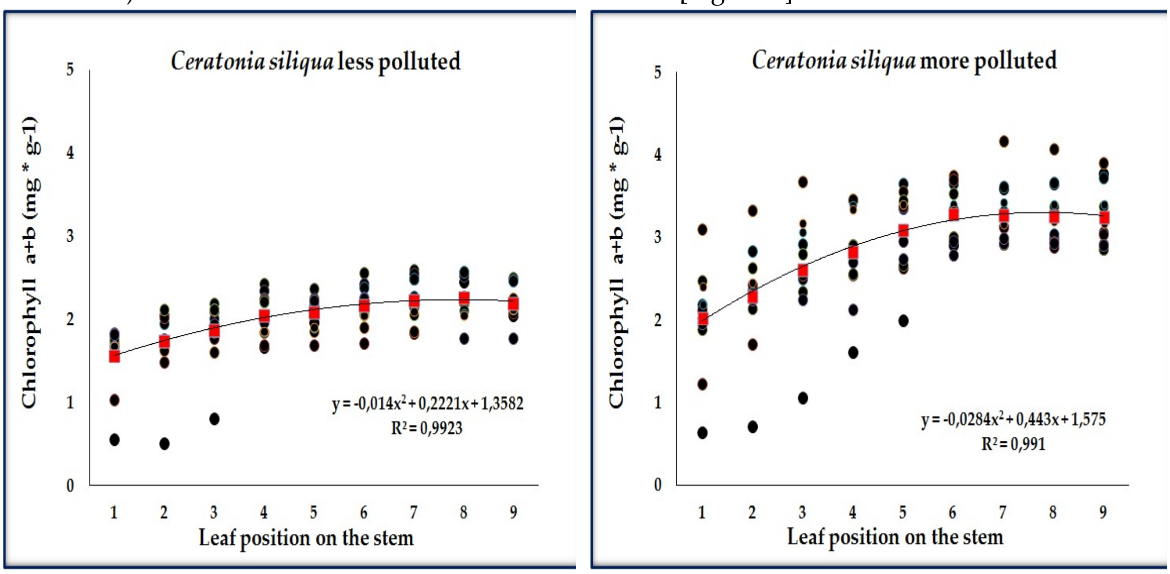
2.2. In Situ Measurements of Optical Properties of Fresh Leaves

Leaf reflectance (R) and transmittance (T), for both adaxial and abaxial fresh carob leaf surfaces was measured between 250 and 2500 nm wavelength [25] (bandwidth 2 nm), using a UV/VIS spectrophotometer (Perkin Elmer Lambda-950), equipped with an integrating sphere and glassfibre tubes [26]. The calculated leaf absorptance (pigments, water, dry matter) at a range of wavelengths from 250 to 2500 nm [A = 100 – (R + T)] was used to assess the effect of environmental quality of the contrasting habitats in Athens for the carob tree. Statistical significance of the differences in optical properties will be tested for model simulation purposes.

3. Results

3.1. Chlorophyll Content

An increase, in the studied leaf parameters, was observed, for carob trees grown in the urban site. Leaf chlorophyll content was found much higher at the more polluted site [Figure 1,2] in comparison with that of the less polluted area; in young leaves a relatively high carotenoid content was estimated. Leaf chlorophyll *a+b* concentration increased up to the 6th leaf (counting from the top of the shoot) for both habitats and then remained constant [Figure 1].

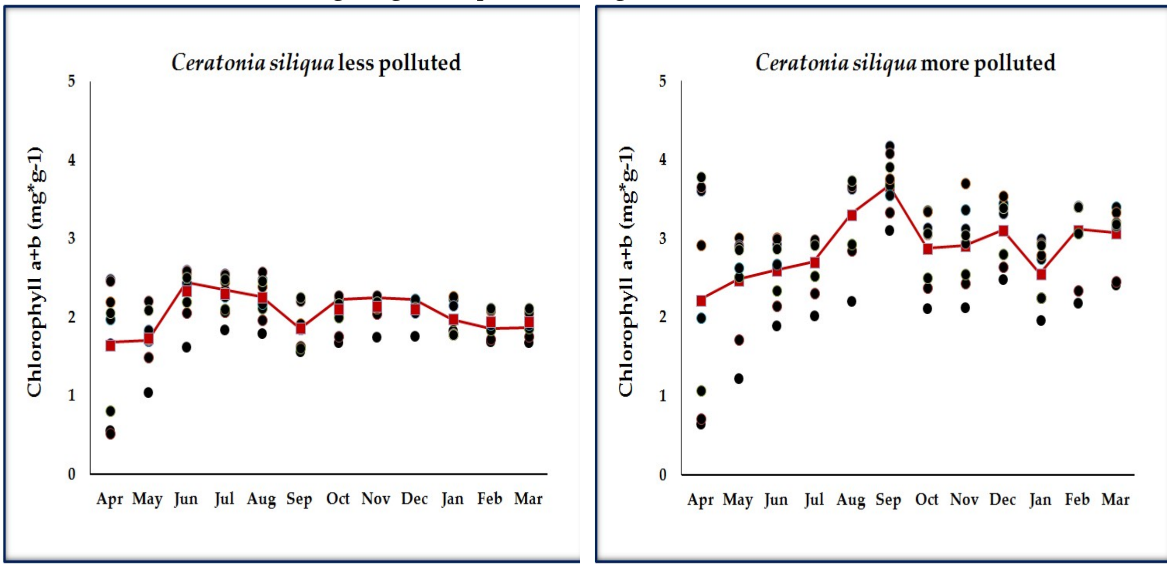


(**a**) (**b**)

Figure 1. Chlorophyll content in relation to the leaf position on the stem (nine successively growing leaves, counting from the top of the shoot) during a twelve-month period: (**a**) less polluted site; (**b**)

more polluted site. The red dots refer to the mean value throughout a year. The equation of the polynomial regression line and its coefficient (R^2-value) are given in the figure.

A significant increase of the concentration of chlorophyll *a* + *b*, was observed during June–July in leaves grown in the suburban site in the bush, whereas in leaves grown in the urban site the maxima were obtained during August-September [Figure 2].



(**a**) (**b**)

Figure 2. Chlorophyll content throughout a year: (**a**) less polluted area; (**b**) more polluted area. The red dots refer to the mean value of nine successively growing leaves, counting from the top of the shoot.

3.2. Specific Leaf Area (SLA) (Leaf Area/Dry Weight cm^2g^{-1})

The specific leaf area (SLA) was measured throughout the year. The SLA values varied with leaf position on stem and in their responsiveness to environmental stimuli. Younger leaves exhibit lower values of SLA due to smaller leaf area and decreased dry weight. Significant difference was observed between the two research sites; suburban carob leaves possessed lower SLA in comparison with leaves growing in the urban area. There was an increase in SLA from spring to late summer and a decrease in late autumn. Additionally, a decrease of SLA was observed in mature leaves from both urdan and sub-urban sites [Figure 3]. A high SLA indicates a low dry matter investment per unit of leaf area.

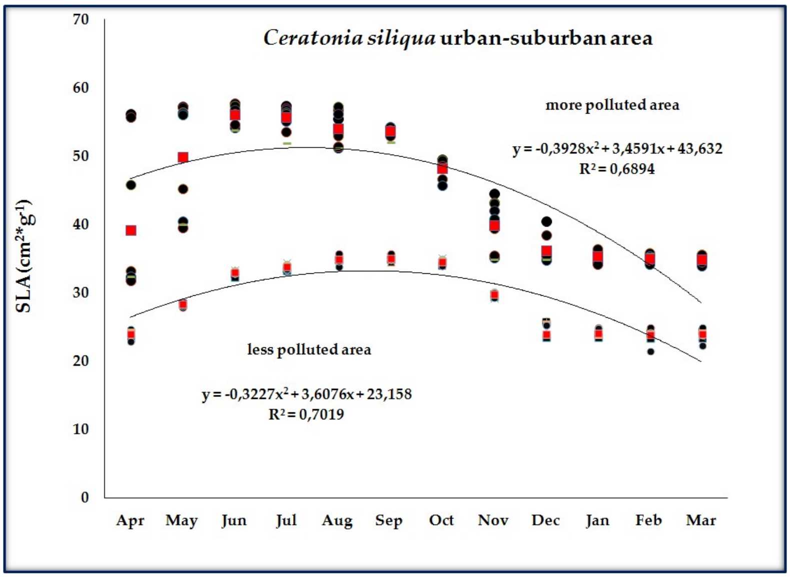


Figure 3. SLA values for two carob trees growing in different habitats throughout a year. The red dotsrefer to the mean value of nine successively growing leaves, counting from the top of the shoot. The equation of the polynomial regression line and its coefficient of determination (R^2-value) are given in the figure.

3.3. Leaf Optical Properties

The leaf absorptance (A) was calculated [A = 100 – (R + T)] by measuring Transmitance (T) and Reflectance (R) using a UV/VIS spectrophotometer (Perkin Elmer Lambda-950), in the range between 300 nm and 2500 nm assessing pigments concentration, water content, dry matter etc. The absorption of light by photosynthetic pigments dominates the optical properties of green leaves in the visible spectrum (400–700 nm). Chlorophyll *a* (the most abundant plant pigment) absorbs light with wavelengths of 430 nm (blue) and 662 nm (red), chlorophyll b (increases the range of light) absorbs light of 453 nm and 642 nm, and carotenoids (accessory pigment) absorb light maximally between 460 nm and 550 nm. A great amount of phenolic compounds were found in the plant tissue that absorb in the UV region (260–350 nm). Anthocyanins (flavonoid pigments not associated with photosynthesis) strongly absorb light between 450 nm and 550 nm (blue and green light), with a peak at about 520 nm [Table 2]. However, foliar reflection in the near-infrared plateau (NIR, 700 nm–1100 nm) is affected by multiple scattering of photons within the leaf, and it is related to the internal structure, fraction of air spaces, and air-water interfaces that refract light within leaves.

Water is almost transparent to visible light, whereas in the shortwave-infrared one observes two major water absorption peaks centered near 1470 nm and 1900 nm, and two minor absorption peaks centered near 970 nm and 1200 nm.

The organic compounds (e.g., cellulose, hemicellulose, lignin, structural proteins) that comprise the dry matter of plant cell walls form complex assemblages, that actually strongly absorb radiation in the UV ($\lambda \leq 0.4$ μm) and in the middle-infrared ($\lambda \geq 2.5$ μm) region [25].

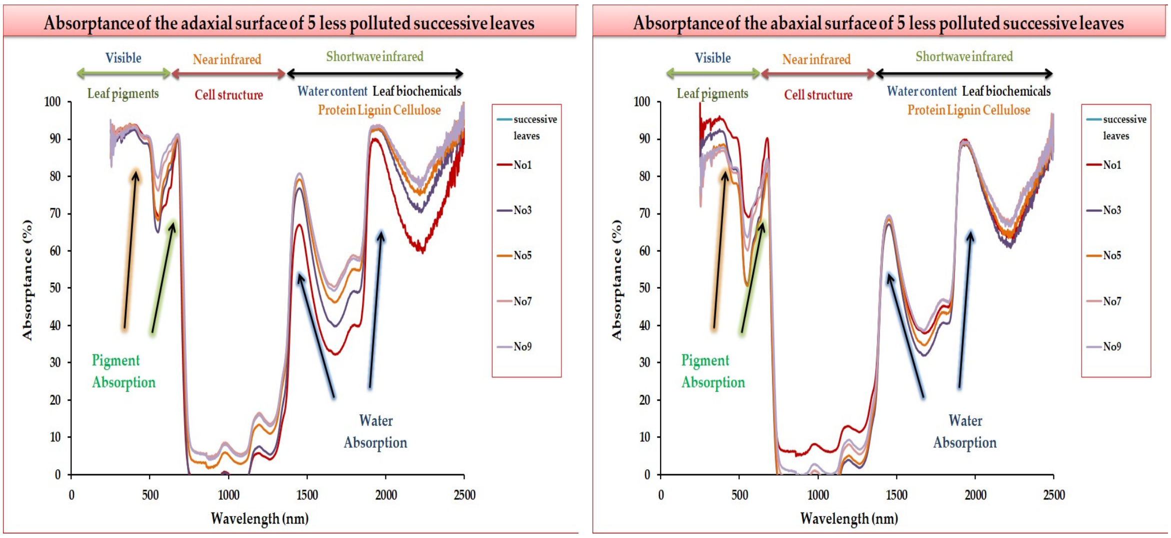
Table 2. Peak absorption of the most common plant pigments, biochemical compounds, water.

Compound	Absorption Peaks, Wavelengths (nm)
Phenolic compounds	260–370
Chlorophyll *a*	430 and 662
Chlorophyll *b*	453 and 642
Carotenoids	460–550
Anthocyanins	450–550 (maximum at 520)
Water	970 nm, 1200 nm, 1470 nm and 1900 nm (maximum)

Cellulose - Lignin	1400–2000 and 2000–2500 (maximum)
Protein – Starch - Sugar	1400 and 2000–2500 (maximum)

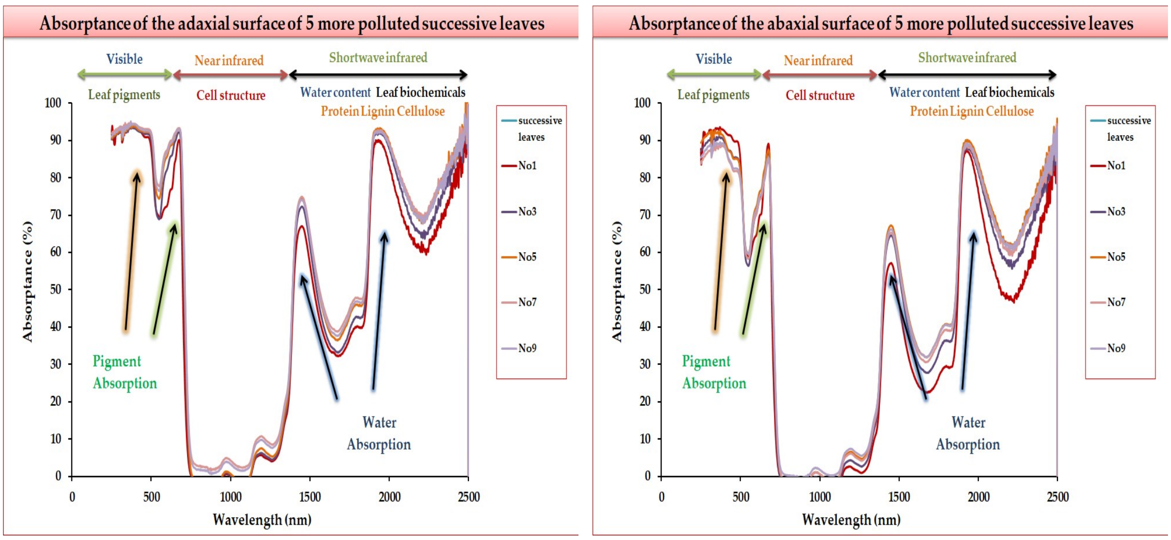
Leaf chlorophyll contents were found higher at the more polluted site, in comparison with that of the less polluted area [Figure 6]. Absorptance spectra showed higher reflectance efficiency in mature leaves than in young leaves and was significantly higher in more polluted sites compared to less polluted. In addition, the abaxial surfaces reflected more than the adaxial surfaces in the visible portion of the spectrum and absorb less light in both plants [Figure 4,5].

A stronger absorptance is noticed at the near infrared and shortwave infrared spectra (water absorptance) for young and mature carob leaves of the urban site. The spectral response is highest in shortwave infrared near 1950 nm [Figure 6]. Leaves of the less polluted site, regardless of the development stage, exhibit greater water absorption, while the adaxial surface absorbes more radiation in both categories of plants [Figure 6].



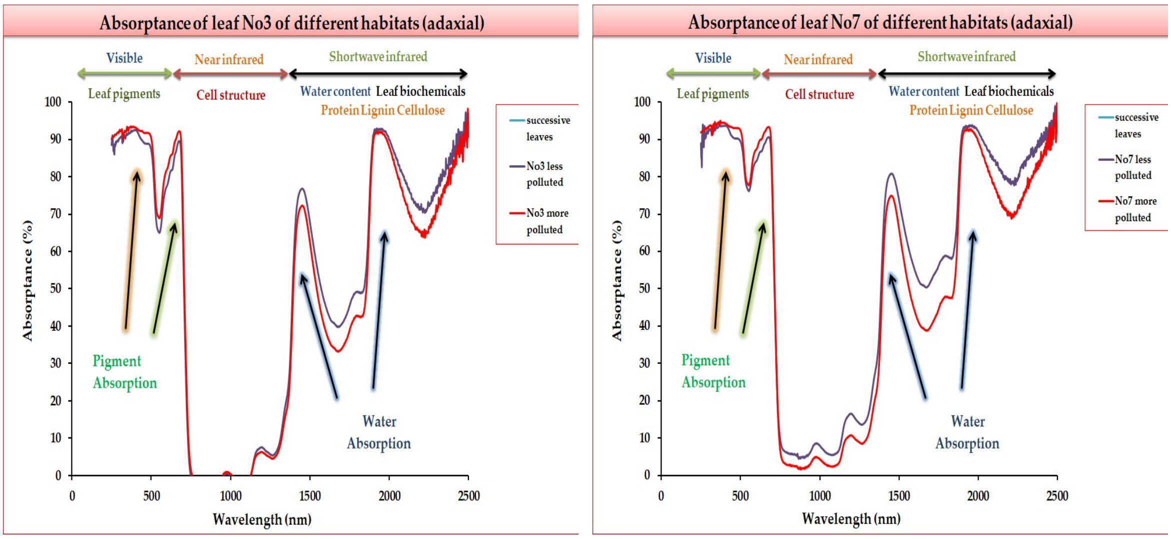
(a) (b)

Figure 4. Absorptance spectrum of 5 fresh leaves [the number (No) refers to leaf position on the stem counting from the top] collected from a less polluted site: (**a**) the adaxial leaf surface; (**b**) the abaxial leaf surface.



(a) (b)

Figure 5. Absorptance spectrum of 5 fresh leaves [(the number (No)refers to leaf position on the stem counting from the top] collected from a more polluted site: (**a**) the adaxial leaf surface; (**b**) the abaxial leaf surface.



(**a**) (**b**)

Figure 6. Absorptance profile for adaxial leaves with different stem position growing in a suburban and urban site: (**a**) absorptance of the 3rd leaf (No3); (**b**) absorptance of the 7th leaf (No7), counting from the top of the stem.

4. Discussion

Over the past few decades industrialization and anthropogenic activities affect the increasing concentrations of atmospheric pollutants, especially atmospheric CO_2 and tropospheric O_3, which play significant roles in the functioning of ecosystems. Air pollution problems are primarily gathered near urban and industrial areas and mostly have a negative impact on plants as foliar surface undergoes different structural and functional changes. Leaf construction involves a stoichiometric balance among biophysically and environmentally dependent metabolites (chlorophyll, nitrogen, water) and SLA (specific leaf area) and varies according to the environmental conditions [25]. Although high stress inhibits the synthesis and accumulation of chlorophylls, pigments seem to be stimulated by low-level stress. Increased chlorophyll concentration in response to low-level stress may equip the leaf-system with an enhanced capacity for defense against high-level (health-threatening) challenges (pigment hormesis) [27].

In this study, we assess the potential of carob tree (*Ceratonia siliqua* L.) as a bioindicator and/or a biosensor for monitoring air pollution as it is a commonly distributed species, it can be samples easily and shows a physiological response to differences in habitat quality. The accumulation of pigments and specific leaf area, which were seasonally determined during leaf development for carob trees of two different habitats (urban, suburban) as well as leaf specular behavior, indicate a significant increase, in the studied leaf parameters for carob trees grown in the urban site. It seems likely that differences in optical properties and pigment accumulation have important implications for model simulation purposes and may be used for air pollution biomonitoring.

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Conflicts of Interest: The authors declare no conflict of interest.

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