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Bio-monitoring of Airborne Dust Particles Pollutants by Morpho-anatomical Reactions of Urban Tree Leaves Under Dry Climate

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THIS IS A MORO- ANATOMICAL study where *Ficus nitida* tree leaves from dust-affected street are compared with those of control from a pollution free site. Under the impact of dust particles, leaf sizes and specific leaf area are significantly smaller and stomatal density differs. Under pollution circumstances, visual symptoms including chlorosis /yellowish and dark spots on the adaxial surface of the polluted leaves were observed. Thick cuticle, collapsed epidermis, large cystolith, dark phenolic deposits in the spongy parenchyma, relatively thick walls of xylem vessels and slightly alterations in the phloem cells were the anatomical features of the polluted leaves. The degree of these changes varied according to the variations in the intercepted dust mass, the issue which can be use for biomonitoring of air pollution.

Keywords: Stomata, Dust, Biomonitoring, Air pollution, Ficus nitida.

Dust particulates air pollutants due to anthropogenic and natural sources are common in the majority of the 3rd world countries, particularly in dry climate. In Egypt, unpaved roads, Khamasin winds, construction activities, soil erosion, less-manage agriculture practice, and traffic activities are at the main root causes of the problem (El-Khatib *et al*., 2007). These particles are known to have toxic effects not only in plants and animals but also in humans.

The reaction of different tree species to the particulate air pollutants is strongly correlated with their structural and functional features. Uptake and accumulation of these pollutants at higher concentration can be cytotoxic in some plant species, causing structural and ultra structural changes, hence affect the growth, anatomy and physiology of plant (Barcelo *et al*., 1988; Han *et al*., 2004; Silva *et al.,* 2005; Verma *et al*., 2006). The uptake of dust particles and improve of air quality by the trees of urban environment have been studied (Beckett *et al.,* 1998; Free-Smith *et al*., 2005; El-Khatib *et al*., 2007). Dust interception capacity of tree plants depends on their surface geometry, phyllotaxy, and leaf external characteristics such as hairs, cuticle etc., height, and canopy of trees (Nowak, 1994; Neinhuis and Barthlott, 1998, Pal *et al*., 2002). Although, the using of tree leaves as accumulative biomonitors of air pollutants

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has attained great ecological importance (Bargagli, 1998; WHO, 2000), where they act as pollution receptors and biological absorbers or filters of pollutants (Wittig, 1993; Free-Smith *et al*., 2004), less attention has been given to morphological and anatomical parameters of plants as indicators of long-term responses to changing (urban) habitat quality (Balasooriyaa *et al*., 2009). Parameters as specific leaf area, stomatal density and pore surface were recognized to vary depending on microclimatic conditions (Barber *et al.,* 2004). Moreover, sampling and analysis of these parameters is relatively easy and inexpensive. Therefore, the aim of this paper was to follow the influence of the dust particulate pollutants upon the morpho-anatomical features of leaves of urban trees in order to assess their potential as effective parameters for biomonitoring of air particulate pollution.

Materials and Methods

Plant materials and sampling

samples of *Ficus nitida* tree leaves were collected, during the spring season of the physical year 2007, from four urban sites with high dust particulates pollutants and an unpolluted control site, which all located at Sohag city, Upper Egypt (31° – 32° E, 26° – 27° N), where the dry climate is prevailing. Except the control site (far away from any pollution source), the study sites were selected near the center of the city and on the sides of the main city roads with high traffic density, where a large volume of traffic exists. Thirty six leaves /site were detached from the trees at 1.5-2.0 m above the ground (9 leaves/ space direction), kept in plastic bags and transferred within icebox to the laboratory for the further examinations.

Determination of dust particulate mass

Surface dust was removed by washing of 15 of the fleshy leaves of the collected samples of each site using 100 dist. H_2O several times using an ultrasonic shaker. The washing solution was filtrated using 0.22 µm pore size filter papers. The weight of deposit (g/m^2 leaf area) per site was then determined as the difference of filter weights divided by the total leaf area (cm^2).

Morphological examinations

The individual leaf area (in cm^2) was calculated by tracing out the leaves on graph paper according to the method of Vora and Bhatnagar, (1986). The method of Sarijeva *et al.* (2006) was used for Specific Leaf Area (SLA), which expressed as $cm^2\ g^{-1}$ dw.

For morphological examination, eye lens and scanning electron microscopy (SEM) were used. For SEM examination of abaxial and adaxial leaf surface, discs of one cm^2 of *F.nitida* were dissected from an unwashed leaf, between the second and third lateral veins from the leaf base of each leaf. The discs were fixed on specific stubs using a carbon double Sticker, (Tomašević *et al.*, 2005;

Kim, 2008), so that both abaxial and adaxial surfaces could be studied. For imaging, the stubs were sputter coated with gold palladium under vacuum using sputter coater JFC-1100E machine. Coated specimens were examined for stomatal density, size, and shape on the leaf surface. Samples were photographed by SEM (JSM-5300LV; JEOL) at magnifications 500 x and 3500 x, while the electron beam energy was fixed at 30 KeV.

Anatomical examination

For Light microscopy, the leaf discs ($1cm^2$) were fixed using the methods adopted by karnovsky (1965) and Spurr (1969). Then, they moved to rubber block and incubated at 60°C for 48 hrs. Semi thin sections with a glass knife on an ultra microtome were made at 400 nm. Sections directly stained by general stain of toludine blue (1% (w/v) at 50 °C, then they moved to cleaned slides, dried and examined for parameters including cuticle, epidermis, plasid tissue, mesophyll tissue. Stomatal density (number of stomata/mm^2 leaf area) was determined using fifteen randomly chosen microscopic fields of the surfaces of each leaf disc. The photos were taken with a Premiere digital eyepiece MA88 camera, using an Olympus CH-2 microscope under 100 x, 400 x magnifications.

Statistical analysis

The variations among mean (three replicates) were calculated using Minitab statistical package, ANOVA T-test was used to calculate the significant differences among means at $P<0.05$, $p<0.01$.

Results and Discussion

Pollution stress altered the structure of leaves of the investigated species. Nevertheless, this species appear to be sensitive to air pollutants (El-Khatib & El-Swaf, 2001) and despite the observed modifications they continue to grow and reach maturity (flowering stage). The results of the present study showed that there are site specific changes observed on the dust masses collected by the surface of the leaves (Table 1), during the study period, where significance difference ($P<0.05$) was recorded in between. In comparison with control, clearly morphological alterations in polluted leaves were observed as visible signs of pollution injuries, including chlorosis /yellowish and dark spots on the adaxial leaf surface. These morphological signs are best regarded as evidence of biochemical response to air pollutants, but they don't necessarily indicate sensitivity in terms of growth inhibition (Madkour and Lourence 2002; El-Khatib 2003, El-khatib *et al*. 2004). Other morphological features of the leaf samples are reported in Table (1). The leaf area is lower in trees growing in the polluted area than those of control one; significant difference ($P<0.05$) was recorded in between. The specific leaf area showed the same trend of leaf area, where maximum values were recorded in the control samples. This is in coincidence with the results of Vassilev *et al.* (1998) who reported the positive correlation between SLA value and those of leaf area. Abaxial stomatal frequency increased significantly ($P<0.05$) and reached its maximum value in the polluted leaf samples as compared with those of control.

TABLE 1. Dust deposit mass, leaf area, specific leaf area and adaxial stomatal frequency values of leaf samples of *F.nitida* tree collected from different sites. All values are Mean ± SD, n= 3.

Site	Deposit mass (g/m^2)	Leaf area (LA) (cm^2)	Specific leaf area (SLA) (g/cm^2)	Abaxial stomatal density ($number/mm^2$)
control	0.616 ± 0.12	0.0700 ± 0.008	0.01807 ± 0.0067	250 ± 30
Polluted	4.414 ± 0.34	0.0373 ± 0.006	0.0116 ± 0.0002	302 ± 21
Significance levels	$P<0.01$	$P<0.05$	$P<0.05$	$P<0.05$

Electron microscopy investigation showed that leaf samples of the polluted site (Plate. 1A) appeared with a rough adaxial surface, when compared with those of unpolluted control site (plate. 1B). Polluted leaves were found to have higher stomatal frequency, smaller size and narrower aperture occluded by dust particles (DP) on the abaxial leaf surface (Plate. 1C) than those of control (Plate. 1 D). Also, stomata destruction and great damages of guard cells were recorded on abaxial surface of the polluted leaves (Plate. 1E). Meanwhile, stomata of normal shape, healthy state, and clear structure of guard cells were observed in those of control samples (Plate, 1F). Higher stomatal frequency and destruction of guard cells were reported by Pal *et al*. (2002), under air pollution stress. Also, they considered by Iqbal *et al*. (1996) as an avoidance mechanism against the inhibitory effect of pollutant on physiological activities.

In the leaf anatomical investigation, control samples appeared with thin cuticle and epidermal cell with thin wall (Plate. 2A), small size cystolith and distinguishable transition zone (Plate. 2B), regular palisade parenchyma arranged in double layers with plastids occupied almost of palisade parenchyma cells (Plate, 2C) and spongy parenchyma with few intercellular spaces (Plate, 2D). Concerning the vascular bundles, (Plate, 2E), unpolluted leaves have a clear conductive elements structure; the xylem vessels (XV) appeared with thin wall and the phloem cells (Ph) were appeared with normal shape and thin walls.

In contrast to the control samples, polluted leaves exhibited adaxial collapsed epidermal cells and a thick cuticle, dark phenolic deposits in the mesophyll cells (Plate, 3A) and large size cystolith (Plate. 3B). In our opinion that, such changes are not as mechanisms to avoid the attack of pollutants because the changes not seem to be response to avoid stress, but to the effects resulting from the stress. Anyhow, they considered by Reig-Armiñana *et al*. (2004) as mechanisms to avoid pollutants attack and withstand the unfavorable conditions. Barcelo *et al*. (1988) reported the increase of secondary products within the plant tissues under stress conditions; this may explain the observed increase in the size of the

cystolith. The presence of dark deposits (Plate, 3A) indicates that long-term exposure to air pollutants leads to enhance the accumulation of phenolic compounds, which considered by Wild and Schmitt (1995 as the most common reactions of plants to stress.

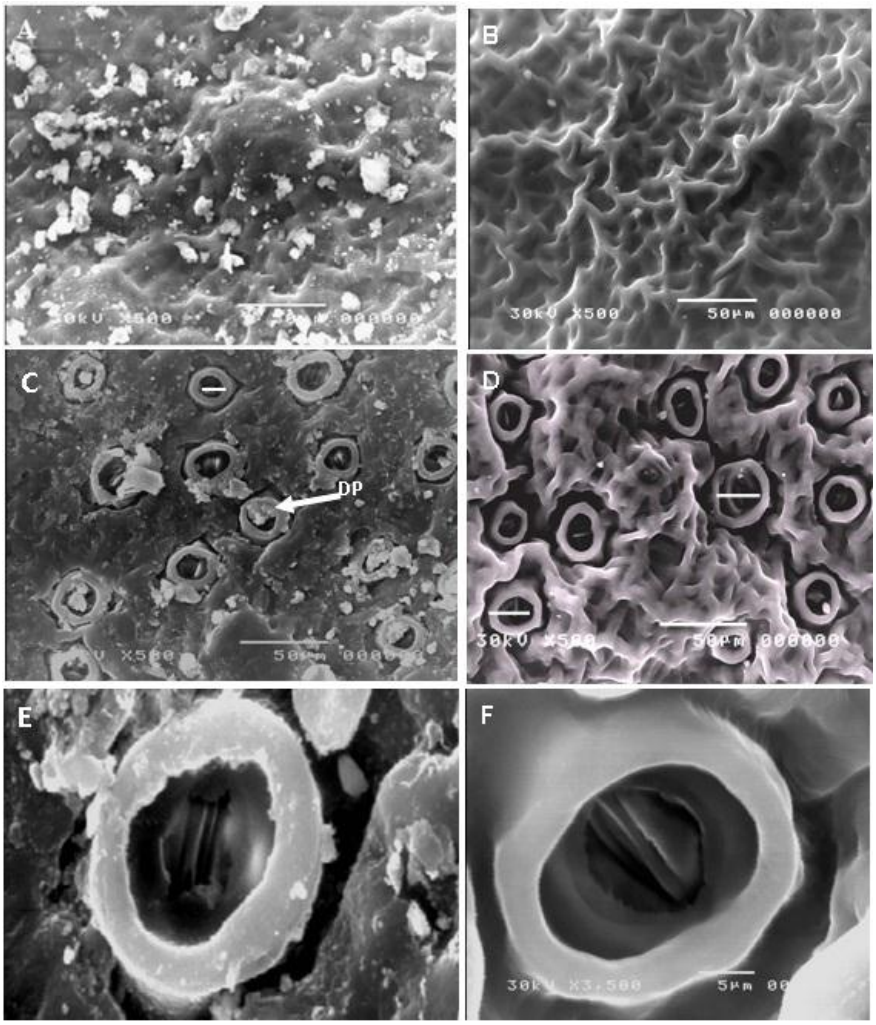

Plate 1. Scanning electron micrographs of particles deposited on adaxial leaf surface of *Ficus nitida* under dust pollution stress (A) and control conditions (B); the abaxial leaf surface of polluted leaf (C); the abaxial leaf surface of control leaf sample (D), scale bar =50µm; damage stomata structure on abaxial leaf surface under dust pollution stress (E) and healthy stomata structure of control leaf samples (F), scale bar=5 µm and (3,500X).

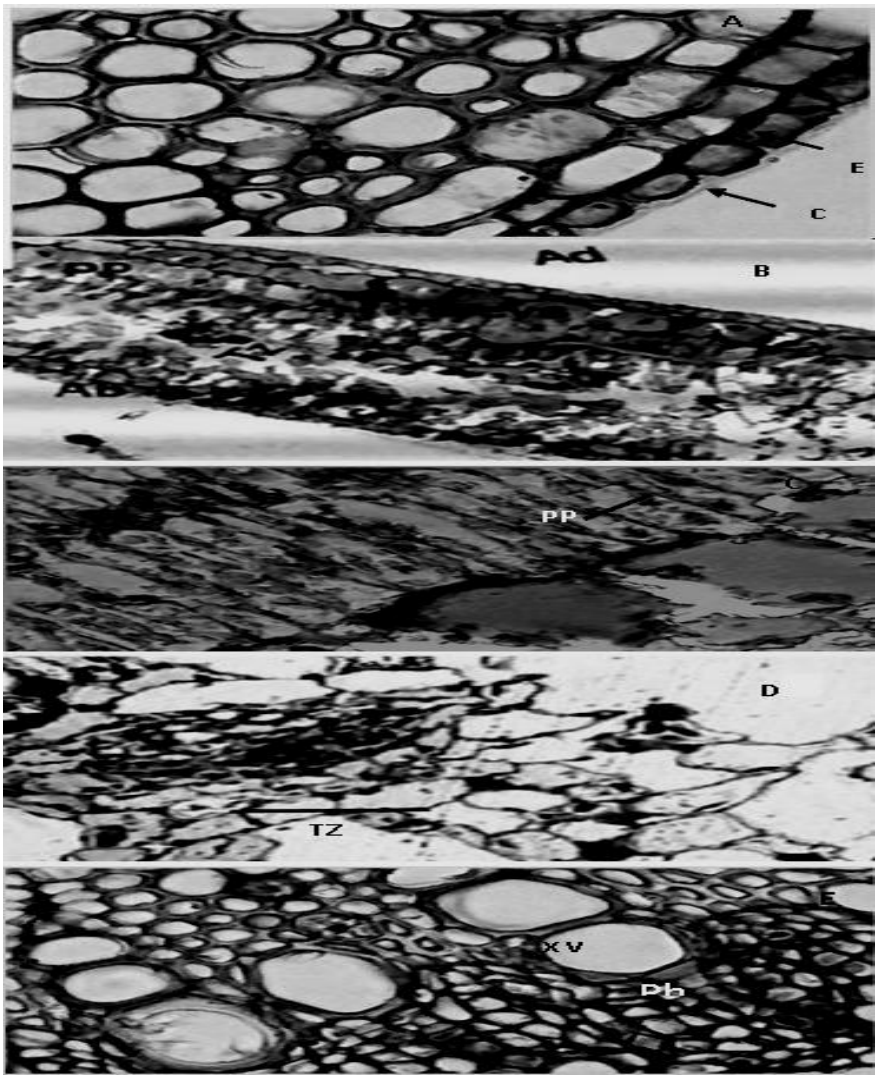

Plate. 2. Cross semi thin section of :

(A): leaf collected from control site showing E: adaxial epidermis with C: very thin cuticle (100x).

(B): Ad: adaxial epidermis, Cys: cystolith, PP: palisade parenchyma, TZ: transition zone, S: stomata, Ab: abaxial epidermis (50x);

(C): palisade parenchyma in double layers with regular shape and many plastids (100x);

(D): regular transition zone (TZ) with small intercellular spaces (100x).

(E): xylem vessels (XV) with small thickness; and healthy shape of phloem cells (Ph) (X400).

Destructive shape of palisade parenchyma cells with a limited number of plastids is showing in Plate (3C). Moreover, spongy parenchyma appeared to have irregular shape, wide intercellular spaces, small tonoplst size and reduced size of chloroplasts (Plate. 3D). Also, the thickness of palisade parenchyma and spongy parenchyma cell wall increased. The transition zone cells were more or less completely damaged and vacuoles occupied most of the cell volume (Plate. 3E), which finally appeared empty from organelles. It is observed that, the alteration degree is related to particulate (dust) mass; consequently the recorded differences in the alteration among sites represent evidence about the load of pollution at the study sites. This is of prime importance when the results of this study considered for biomnitoring perspectives.

The vascular elements (XV and Ph) of the polluted samples (Table 2, Plate. 3F) appeared with relatively thick cells walls and a slightly alteration in phloem cells. A clearly increase in the thickness of the lignified secondary wall reinforcement in the xylem vessels was observed, resulting in a reduction in the vascular elements size and change the phloem appearance; the walls turned sinuous and thick. In addition, the contents of some of the phloem cells became denser. The estimated data (Table, 2) revealed that the maximum diameter for either MX or PX vessels was recorded at control site with values 26 and 16 µm, respectively. The values of MX and PX for the samples of polluted sites were 16.66 and 10 µm, respectively. The results of xylem vessels wall thickness supported the above results, where it found that samples collected from control site had the lowest thickness values (2.66 and 2.2 µm) of MX and PX vessels wall respectively, while the highest thickness values 8.66 and 5.66 µm were recorded for the samples of polluted sites, for both MX and PX, respectively. However, the negative impact of dust pollutants was quantitatively highlighted through the occurred increase in the thickness of the wall of both proto- and metaxylem vessels. This may be attributed to the fact that under pollution stress the deposition of secondary products on these walls will be increased. Another evident for this impact will be taken from the diameter of the xylem vessels, where the diameter of both types was exhibited the same trend of decreasing, and had a noticeable deviation from those of control.

TABLE 2. Diameter and thickness of xylem vessels wall of leaf samples of *F. nitida* tree collected from different sites. All values (Mean ± SD) expressed in mm, n= 3).

SITE	MX vessel diameter	PX vessel diameter	MX vessel wall thickness	PX vessel wall thickness
control	26 ± 2	16 ± 2	2.666 ± 0.577	2.2 ± 0.124
Polluted	16.66± 1.154	10 ± 2	8.66 ± 1.154	5.66 ± 0.235
Significance levels	$P<0.01$	$P<0.05$	$P<0.05$	$P<0.05$

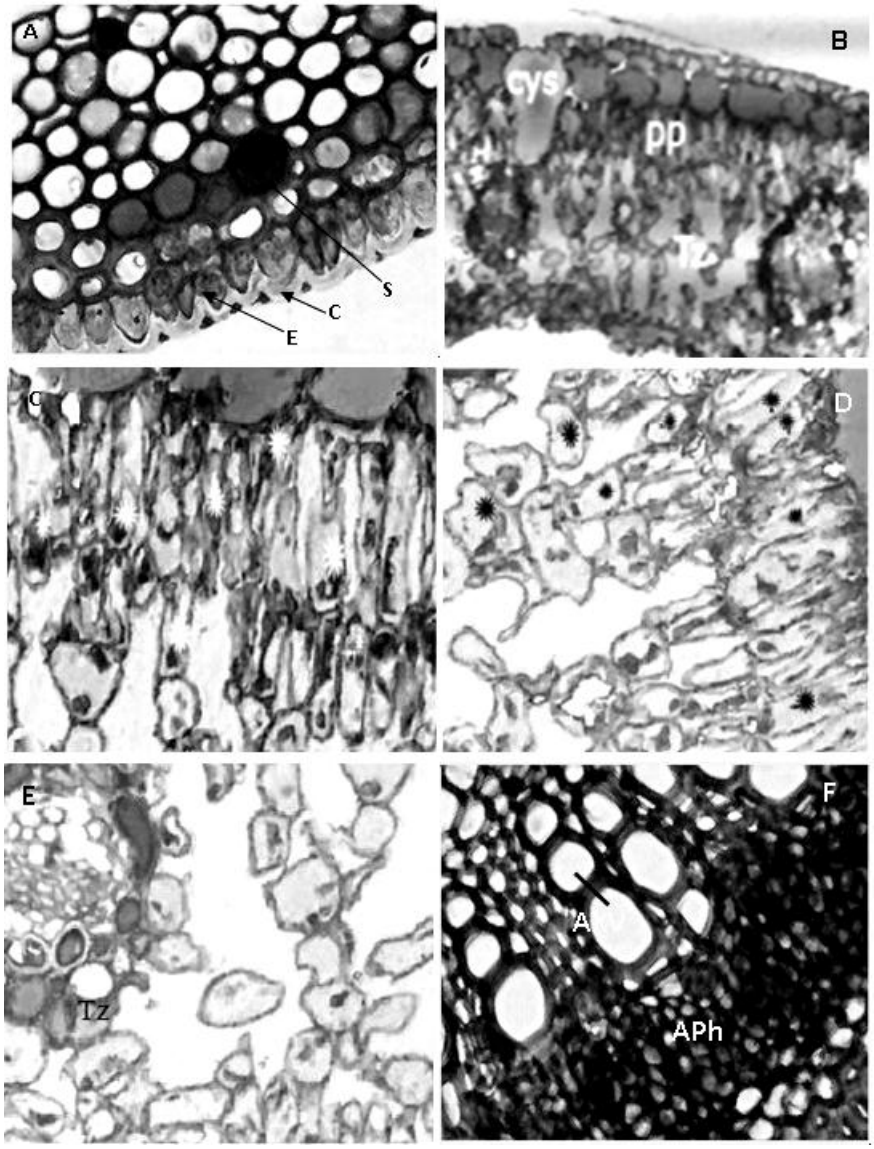

Plate 3. Cross semi thin section of leaf collected from polluted site showing (A): thicker cuticle layer (C), collapsed epidermis (E), phenolic substances (S) (X100); (B) relatively large cystolith (Cys) and less regular transition zone (Tz); (C): irregular shape of palisade parenchyma with limited number of plastids (*); (D): abnormal and destructive shape of palisade parenchyma cells and vacuoles occupied most of the cell volume; (E): irregular transition zone; (F): altered xylem vessel (AXV), altered phloem cells (APh); (X=400).

Conclusion

The current research represents one of a series of research carried out to study the impact of airborne dust particles to the anti-oxidants, and protein patterns in *Ficus nitida* tree leaves, in order to assess the possibility of using these features in the bio-monitoring of air pollution. Here, too, the changes in the morpho-anatomical features reflect again the effects resulting from the stress caused by this type of pollutants, where the severity of impact on the some morphology, anatomy, and ultra structure features of tree leaf was observed to have relation with the captured particles mass. Fortunately, although the tree leaves showed clear alterations, it continues to grow until maturity (flowering stage). Based on the above, the current research nominates the morpho-anatomical modifications as promising measures to estimate the air quality of the urban habitat.

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الرصد الحيوى لملوثات جزيئات الغبار المتطايرفى الهواء بواسطة الاستجابات المورفو- تشريحية لاوراق اشجار المناطق الحضرية النامية فى المناخ الجاف

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تضمنت الدراسة مقارنة مورفو- تشريحية لاوراق اشجار نبات الفيكس النامية تحت تأثير ملوثات جزئيات الغبار مع مثيلاتها النامية فى مناطق الضابطة، الخالية من التلوث. من الناحية الموفولوجية، اظهرت عينات الاوراق الملوثة انخفاضا معنويا فى كل من الحجم، المساحة النوعية و كذلك كثافة الثغور عن مثيلاتها بعينات الضابطة، كما لوحظ ظهور اعراض مرض الاخضرار/الاصفرار و ايضا بقع سوداء على سطحها العلوى. تضمنت الخصائص التشريحية لعينات الاوراق الملوثة ظهور الادمة السميكة، البشرة المنهارة، تضخم الحوصلة الحجرية ، الترسبيات الفينولية السوداء داخل النسيج الاسفنجى، زيادة نسبية فى سمك جدر الاوعية الخشبية و تخسرات طفيفة فى خلايا اللحاء. لقد اختلفت درجة هذه التغيرات وفقا للاختلافات فى كتلة الغبار المتجمع على اسطح الاوراق، الامر الذى يمكن استخدامه فى عمليات الرصد الحيوى لتلوث الهواء.