



# Effects of air pollution on physiological traits of *Ligustrum lucidum* Ait. leaves in Luoyang, China

Xiping Zhao · Pingping Guo  · Yongqiang Yang · Haixin Peng

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**Abstract** Leaves of trees experience the maximum brunt of exposure and undergo certain changes in physiological traits responding to air pollution, and then, the specific leaf traits can be the indicators of air pollution in an area. However, due to the diversity of sources, the composition of air pollutants is very complex. This makes it difficult to predict air pollution using physiological differentiation of leaves. The purpose of this investigation was to examine potential of *Ligustrum lucidum* Ait. leaf measurement as a method to predict the air pollutants in Luoyang, China. Leaves of roadside *L. lucidum* were studied from the city center with serious air pollution to relatively unpolluted areas. Leaf size, stomatal traits, and non-structural carbohydrate were measured. The particulate and gaseous pollutants (including sulfur dioxide, nitrogen dioxide, carbon monoxide, and

ozone) were investigated too. The results showed that the leaf area and soluble sugar content decreased, while the aspect ratio of leaves increased in heavily polluted areas. As pollution increased, the stomatal traits in different crown positions were changed differently. No significant correlation was found between ozone content and the measured traits of leaves. The responses found in the physiological differentiation of the leaves reflect acclimation to air pollution. The soluble sugar content of the leaves could be used to indicate the short-term stress of air pollution, the area, and aspect ratio of leaves are indicative of the long-term stress due to air pollution. Therefore, physiological traits of *L. lucidum* leaves appeared to be significant predictive factors for the air pollutants in urban areas.

**Keywords** Air pollution · Leaves · *Ligustrum lucidum* Ait. · Physiological traits

X. Zhao · P. Guo (✉) · Y. Yang  
College of Forestry, Henan University  
of Science and Technology, Luoyang 471003,  
People's Republic of China  
e-mail: guopingping\_1982@126.com

X. Zhao  
e-mail: zhaoxiping1977@126.com

Y. Yang  
e-mail: 1271043872@qq.com

H. Peng  
Department of Biosystems Engineering, College  
of Engineering, Auburn University, Auburn, AL 36849,  
USA  
e-mail: hzp0033@auburn.edu

## Introduction

Rapid economic growth, industrialization, and urbanization have resulted in deterioration of air quality in developing countries like China (Alnawaiseh et al., 2015; Angelevska et al., 2021). Action and improvement needs to be done to counteract this increasingly prevalent health risk, for instance, alternatives to crop residue burning (Kumar & Singh, 2021), green transition from coal to other energy resources

(Neru, 2021), planning road transportation, and shifting from private car to public transportation based on reducing the impact of road transportation on air quality (Abdulkareem et al., 2020; Angelevska et al., 2021). Besides these air pollution mitigation measures, urban greening is an effective and long-term strategy of controlling air pollution in urban areas (Grote et al., 2016; Nowak et al., 2006; Wang, 2014). Plants can clean the air, block the particles in haze, and absorb toxic gases (Pugh et al., 2012). But when the pollutants in the air far exceed the carrying capacity of plants, it impacts plant growth negatively (Ozolincius et al., 2005; Yue et al., 2017). Due to their unique position in plants, the leaves change first under different air pollution stress. Firstly, although leaves have the function of capturing dust, leaves are saturated when the dust particle concentration is too high, affecting the plant respiration (Bae et al., 2009). Secondly, in haze weather, the dust particles in the air cover the sunlight, thus affecting photosynthesis, which is not conducive to the plant growth (Lin et al., 2019). Finally, the toxic substances in the air can impair the growth and development of the leaves (Schiffgens-Gruber & Lütz, 1992; Steubing et al., 1989), and even lead to the plant death in serious pollution (Saltan et al., 2020).

*Ligustrum lucidum* Ait., a native evergreen species of China, is cultivated in many southern and southwestern provinces (Wang, 1961). It is also common in North Korea, India, and Nepal. *L. lucidum* is widely distributed in the landscape because of its dense branches and leaves, strong germination, and low requirements for soil and climate (FRPS, 1992). *Ligustrum lucidum* is an ornamental and roadside tree species, which is highly resistant to sulfur dioxide and other toxic gases, and can also tolerate high levels of dust and smoke pollution (Graziani et al., 2019; Liang et al., 2008; Rossini Oliva & Valdés, 2004).

The strong tolerance of plants to polluted air may be obtained by changing the physiological, ecological, or structural characteristics. For instance, the leaf area became smaller (Khan et al., 1990), the contents of pigment and sugar in leaves of plants decreased (Yousafzai et al., 2018), while ascorbic acid and sulfate increased in the areas with high air pollution (Appalasamy et al., 2017; Ierhievwie et al., 2014; Sharma & Tripathi, 2009). Therefore, leaf-level physiological traits are often used as a valid method for studying air pollution (Terekhina & Ufimtseva,

2020). However, the source of air pollution is diverse, including thermal power plants (Zhu et al., 2016), industrial enterprises (Graziani et al., 2019), emissions of motor vehicles (Alnawaiseh et al., 2015; Dyvák et al., 2018), and, often to a certain extent, crop residue burning (Kumar & Singh, 2021; Li et al., 2021). Due to the diversity of sources, the composition of air pollutants is very complex (Gibergans-Baguena et al., 2020; Zhang et al., 2012). This makes it difficult to predict air pollution using physiological differentiation of leaves. The aim of this work was to assess the impact of air pollutants from the urban environment on the physiological traits of *L. lucidum* leaves, and which traits should be used to assess the quality of the urban environment.

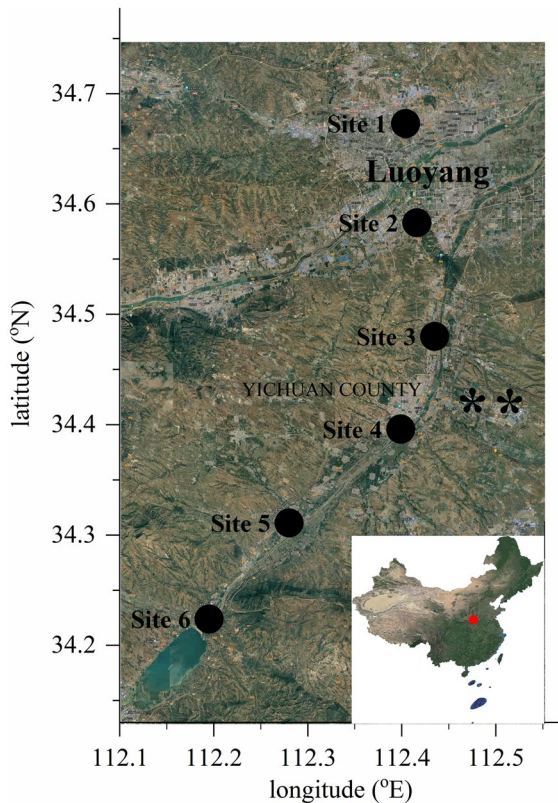
## Materials and methods

### Study area

This study was carried out in Luoyang, a tourist city in the center area of China. Luoyang is famous for its 5000 years of civilization, 4000 years of city building, and 1500 years of capital building. Unfortunately, the air quality in Luoyang is deteriorated in recent years. According to the data released by Luoyang Environmental Protection Department,  $PM_{2.5}$  (i.e., fine particles with aerodynamic diameter  $\leq 2.5 \mu m$ ), one of the most important parameters reflecting the air pollution level, reached through  $879 \mu g/m^3$  on January 4, 2016, which was due to be the large-scale dust storm that day and local industrial pollutants (Tian et al., 2018). Luoyang used to be one of the most important old industrial bases in China. The industrial production brought enormous economic benefits, but also produced a lot of pollutants (Wang et al., 2010). Additionally, the air quality in Luoyang continue to deteriorate due to the urbanization spread, in particular the rapid increase of motor vehicles, which also led to a rise in exhaust emissions (Zhang et al., 2012).

### Sampling sites

Sampling was conducted along Wangcheng Avenue in Luoyang, China. Wangcheng Avenue is a road with a high level of urbanization and traffic. The roadside trees are dominated by *L. lucidum*. Six sampling sites were selected along the road (Fig. 1). South of the



**Fig. 1** Map of the People’s Republic of China, showing the location of Luoyang city (solid red circles), sampling sites (solid black circles), and coal power plants (asterisks)

intersection of Wangcheng Avenue and Zhongzhou Road (Site 1) is situated in the city center with a high density (>2600 pcu/h) and low speed (10–15 km/h) of traffic. North of the intersection of Wangcheng Avenue and Yiluo Road (Site 2) is situated in the suburbs with about 1100 pcu/h traffic density and 40 km/h speed of vehicles. North of Yichuan County (Site 3) is situated in a newly developed area of the County with high traffic density (about 900 pcu/h) and 60 km/h speed of vehicles. South of Yichuan County (Site 4) is closer to two coal power plants in the east of Yichuan County with about 350 pcu/h traffic density and 70 km/h speed of vehicles. Mingguao Town (Site 5) and Tianhu Town (Site 6) were chosen as controls and are about 50 km and 60 km away from the city center respectively, making them the least polluted sites in a sparsely populated area with low traffic density (about 180 pcu/h) and 80 km/h speed of vehicles, absence of industry around farmland.

### Air quality monitoring

The air quality in the six sampling sites was investigated using micro air quality monitor (OSEN-AQMS, Osen Purification Technology Co., Ltd., Shenzhen, China) for a period of one month (May 2017). Data were collected at a height of 2 m and in 3-h intervals between 06:00 to 20:00 h (local time) for each day. The monitored particulate and gaseous pollutants included particles with aerodynamic diameter  $\leq 2.5 \mu\text{m}$  ( $\text{PM}_{2.5}$ ), particles with aerodynamic diameter  $\leq 10 \mu\text{m}$  ( $\text{PM}_{10}$ ), sulfur dioxide ( $\text{SO}_2$ ), nitrogen dioxide ( $\text{NO}_2$ ), carbon monoxide (CO), and ozone ( $\text{O}_3$ ). The mass concentration value of each pollutant is then converted into the air quality index (AQI) values using the Chinese Standard HJ 633–2012 (China SAotPsRO, 2012), which equals the American standard (US-EPA, 2003). The highest AQI value for the individual pollutants is the AQI value for that day in the study area.

### Leaf size measurements

Sampling was conducted on May 31, 2017. At each sampling site, three healthy trees with similar size were chosen (Table 1). With the help of a curved arm aerial vehicle, the fresh leaves of the current year’s branches were collected carefully from the upper, middle, and lower crown facing the street side. The leaves were sealed in plastic bags and were stored in a refrigerator (0–4 °C) until transporting them back to the laboratory. Ten leaves were sampled from each crown position (i.e., 30 leaves per tree). Nine of the leaves were used for stomatal imprints, the others for leaf size and non-structural carbohydrate.

The leaf area, length, and width of leaves were measured with a image computer analysis system (TDY5.2, Tianyu Science and Technology Co., Ltd., Beijing, China). The aspect ratio of leaves was calculated as the ratio between leaf length and width (Fascella et al., 2014).

### Stomatal trait measurements

Stomata in lower epidermis were selected to measure the traits in this study. Two imprints closed to both sides of the main leaf veins from the lower epidermis

**Table 1** Basic condition of sampling trees (mean  $\pm$  Std)

Sites	Tree diameter at breast height (cm)	Tree height (m)	Under branch height (m)	Crown width (m)
1	26.2 $\pm$ 5.1	10.8 $\pm$ 1.8	2.6 $\pm$ 0.3	5.4 $\pm$ 0.1
2	24.3 $\pm$ 4.6	9.8 $\pm$ 1.6	2.4 $\pm$ 0.2	5.1 $\pm$ 0.1
3	24.0 $\pm$ 4.3	10.1 $\pm$ 1.8	2.3 $\pm$ 0.2	5.4 $\pm$ 0.3
4	24.8 $\pm$ 4.6	9.9 $\pm$ 1.5	2.4 $\pm$ 0.2	5.4 $\pm$ 0.2
5	23.2 $\pm$ 3.6	9.6 $\pm$ 1.4	2.5 $\pm$ 0.2	5.4 $\pm$ 0.2
6	24.5 $\pm$ 4.4	10.1 $\pm$ 1.6	2.4 $\pm$ 0.2	5.3 $\pm$ 0.1

were taken using the clear nail polish method (Hilu & Randall, 1984). An average of 30 stomata was measured per imprint. Using the image computer analysis system (TDY5.2, Tianyu Science and Technology Co., Ltd., Beijing, China), the following stomatal traits were measured: length and width of the guard cells, length and width of stomata, and stoma density.

#### Non-structural carbohydrate measurements

The leaf area measured leaves were dried in an oven at 105 °C for 30 min, 70 °C for 2–3 days, and then were ground to pass through 80-mesh. The content of soluble sugar and starch was determined by the modified phenol sulfuric acid method (Buysse & Merckx, 1993). The powdered samples were extracted with 80% v/v ethanol. The extracted solution was used for determining the soluble sugar content with a spectrophotometer (722S, Jinghua Instrument Co., Shanghai, China) at a wavelength of 485 nm. The residues were hydrolyzed in 39.2 mol/l perchloric acid solution to measure the starch content identically. Total non-structure carbohydrate (TNC) was the sum of soluble sugar and starch content (Zhang et al., 2013).

#### Statistical analysis

Differences in leaf traits among the sampling sites were evaluated by analyses of variance (ANOVA), with the significance assessed at the alpha value  $< 0.05$ . Tests of the normality and homogeneity of variances were performed using the Shapiro-Wilk and Levene tests. The Pearson's correlation among leaf traits and air pollutants including CO, NO<sub>2</sub>, SO<sub>2</sub>, O<sub>3</sub>, PM<sub>2.5</sub>, and PM<sub>10</sub> were determined (Yousafzai et al., 2018). All analyses were

performed using the IBM SPSS Statistics software (Version 24.0, International Business Machines Corporation, Armonk, New York, United States).

## Results and discussion

### Air quality

Table 2 showed the mean values for each pollutant measured. The highest AQI was observed in the city center (Site 1), whereas the lowest AQI was found at a location far away from the city center (Site 6). From Site 1 to Site 6, the AQI tended to decrease. Site 4 showed the second highest AQI after Site 1. Results from ANOVA analysis showed that there was a significant difference for AQI between each site ( $p < 0.05$ ) except Site 2 and Site 3. From the city center to the outside, PM<sub>2.5</sub>, PM<sub>10</sub>, and CO showed similar patterns of change to the AQI; the sites with the high traffic density had the high concentration value of pollutants, which confirmed that the vehicular traffic was an active source of pollution (Dyvak et al., 2018). The maximum values of NO<sub>2</sub> and SO<sub>2</sub> were found at Site 4 which confirmed that air quality of the site was affected by the additional complex mixture of pollutants from the two nearby coal power plants (Shon et al., 2019). Site 4, which was located 5 km southwest of the two power stations, had relatively higher SO<sub>2</sub> and NO<sub>2</sub> levels compared to the other Sites and attributed this to strong NE winds (Chen, 1998; Liu et al., 2018). The amount of O<sub>3</sub> at all sites varied from 87 to 167  $\mu\text{g}/\text{m}^3$ , as shown in Table 2, is higher than the estimated background ozone level of 70  $\mu\text{g}/\text{m}^3$  by World Health Organization (WHO, 2006). O<sub>3</sub> was found in high concentrations  $> 100 \text{ mg}/\text{kg}$  (air quality guideline) at the sites except Site 2 and Site 6, which might be harmful to public health. The causes of O<sub>3</sub>

**Table 2** Air quality of sampling sites

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
AQI	121 ± 0.58a	86.5 ± 0.87c	82 ± 0.58c	100.5 ± 4.91b	64 ± 0.58d	50 ± 5.20e
PM2.5 (µg/m <sup>3</sup> )	91 ± 0.29a	63.5 ± 0.87b	47 ± 8.02c	72.5 ± 5.48b	31.5 ± 2.02d	31 ± 2.31d
PM10 (µg/m <sup>3</sup> )	178 ± 0.58a	115 ± 2.31c	113 ± 0.59c	149 ± 9.24b	78 ± 1.16d	54 ± 7.51e
SO <sub>2</sub> (µg/m <sup>3</sup> )	32 ± 4.04b	29.5 ± 3.75bc	34.5 ± 0.87ab	43 ± 0.58a	22 ± 1.73 cd	20 ± 3.46d
NO <sub>2</sub> (µg/m <sup>3</sup> )	30 ± 3.18bc	35 ± 2.31b	32.5 ± 0.29b	42.5 ± 3.75a	24 ± 0.29c	23 ± 2.31c
CO (µg/m <sup>3</sup> )	1.85 ± 0.03a	1.5 ± 0.06b	1.15 ± 0.09c	1.55 ± 0.09b	0.9 ± 0.06c	1.1 ± 1.73c
O <sub>3</sub> (µg/m <sup>3</sup> )	167 ± 7.51a	87 ± 5.77c	108 ± 20.79bc	131 ± 16.74ab	137 ± 1.73ab	88 ± 12.12c

Means ± SE with different letters within a row were significant at  $p \leq 0.05$ . PM2.5 was the fine particles with aerodynamic diameter  $\leq 2.5 \mu\text{m}$ . PM10 was the fine particles with aerodynamic diameter  $\leq 10 \mu\text{m}$ . AQI was the air quality index. SO<sub>2</sub>, NO<sub>2</sub>, CO, and O<sub>3</sub> were sulfur dioxide, nitrogen dioxide, carbon monoxide, and ozone, respectively

formation are complex. For instance, the amount of O<sub>3</sub> may occasionally exceeded the guideline value due to natural causes (Kim et al., 2021). However, Site 1 showed high O<sub>3</sub> concentrations, up to 167 µg/m<sup>3</sup> which exceeded the Interim target-1 (167 µg/m<sup>3</sup>) level established by WHO (2006). Such high concentrations of O<sub>3</sub> would result in middle level ambient pollution in the city center, which is believed to be influenced by traffic exhaust, energy consumption, and industrial emission changes besides meteorological factors. This is confirmed by the analysis of O<sub>3</sub> sources in many cities (Unger et al., 2020; Yan et al., 2021).

Leaf size

The leaf area of *L. lucidum* at the Site 1 and 2 with serious pollution was smaller than those at sites with higher air quality (Table 3). Our study was consistent with the observations of previous studies; i.e., air pollution led to the reduction of leaf area (Cotrozzi, 2019;

Khan et al., 1990; Pandey, 2005). Although the leaf area did not show a significant difference among the sites (Table 3), there was a significant negative correlation with PM<sub>2.5</sub>, PM<sub>10</sub>, CO, and NO<sub>2</sub> relating to the crown positions (Table 4). Maher et al. (2008) confirmed that air pollutants increased with height in the crown by detecting the concentrations of heavy metals in the leaves. The uneven distribution of air pollutants in the crown may affect the growth and development of leaves. However, the variation of leaf area in crown may also be due to the long-term evolution of plants (Nikolov et al., 2019). Thereafter, to understand the relationship between leaf area and air pollutants, there are many challenges ahead. The variation of leaf area at Site 4 indicated negative responses in leaf size exposed to coal-fired power plant emission consistent with the studies by Jones et al. (1987), suggesting the leaf area are indicators of potential in monitoring air pollution. A controlled experiment made by Trlica et al. (1985) indicated that leaves possibly were injured by coal-fired power plant emission, and

**Table 3** Leaf size of *Ligustrum lucidum* Ait

Crown layer	Leaf size	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Whole	Leaf area (cm)	27.39 ± 1.03a	26.92 ± 1.12a	29.92 ± 1.21a	27.07 ± 1.30a	29.64 ± 0.99a	30.38 ± 1.14a
	Aspect ratio	1.99 ± 0.04a	2.02 ± 0.04a	1.93 ± 0.03ab	1.93 ± 0.32ab	1.82 ± 0.32bc	1.82 ± 0.43c
Upper	Leaf area (cm)	28.28 ± 2.57a	28.25 ± 1.36a	28.23 ± 1.69a	27.91 ± 2.04a	29.78 ± 1.85a	31.38 ± 1.80a
	Aspect ratio	2.00 ± 0.42a	1.89 ± 0.27ab	1.88 ± 0.29ab	1.71 ± 0.25b	1.83 ± 0.57ab	1.86 ± 0.28ab
Middle	Leaf area (cm)	24.36 ± 2.06a	24.37 ± 2.32a	29.67 ± 2.51a	26.49 ± 1.54a	28.76 ± 1.50a	29.73 ± 2.07a
	Aspect ratio	2.02 ± 0.37a	1.90 ± 0.30a	1.93 ± 0.37a	1.85 ± 0.33a	1.89 ± 0.38a	1.99 ± 0.32a
Lower	Leaf area (cm)	28.68 ± 1.14a	28.42 ± 1.91a	27.52 ± 2.05a	26.68 ± 2.64a	30.37 ± 1.84a	29.96 ± 2.16a
	Aspect ratio	2.05 ± 0.34ab	1.99 ± 0.38ab	1.98 ± 1.98ab	1.90 ± 0.29bc	1.74 ± 0.32c	2.13 ± 0.31a

Means ± SE with different letters within a row were significant at  $p \leq 0.05$

**Table 4** Correlation between air quality parameters and leaf size of *Ligustrum lucidum* Ait

Crown layer	Leaf size	AQI	PM2.5 ( $\mu\text{g}/\text{m}^3$ )	PM10 ( $\mu\text{g}/\text{m}^3$ )	SO <sub>2</sub> ( $\mu\text{g}/\text{m}^3$ )	NO <sub>2</sub> ( $\mu\text{g}/\text{m}^3$ )	CO ( $\mu\text{g}/\text{m}^3$ )	O <sub>3</sub> ( $\mu\text{g}/\text{m}^3$ )
Whole	Leaf area (cm)	-0.835*	-0.872*	-0.827*	-0.719	-0.821*	-0.841*	-0.278
	Aspect ratio	0.783	0.809	0.758	0.566	0.637	0.801	0.115
Upper	Leaf area (cm)	-0.826*	-0.743	-0.834*	-0.853*	-0.835*	-0.625	-0.353
	Aspect ratio	0.402	0.496	0.388	0.457	0.602	0.572	0.418
Middle	Leaf area (cm)	-0.662	-0.768	-0.628	-0.251	-0.398	-0.801	-0.333
	Aspect ratio	0.645	0.758	0.608	0.328	0.463	0.728	0.013
Lower	Leaf area (cm)	-0.610	-0.578	-0.650	-0.965**	-0.931**	-0.522	-0.053
	Aspect ratio	0.909*	0.847*	0.890*	0.597	0.560	0.752	0.526

\*Correlation was significant at the 0.05 level (2-tailed). \*\*Correlation was significant at the 0.01 level (2-tailed)

the leaf injury increased near the plant. Many studies have demonstrated that the leaf area can be used as a measure of the severity of air pollution. Deng et al. (1981), for instance, showed that leaf area of *Robinia pseudoacacia* could be used as bio-indicators for SO<sub>2</sub> pollution in Shenyang, China. However, some studies have shown that the leaf area increases due to air pollution. For instance, In the works of Chen et al. (2015), leaf area was considerably larger in a site with high concentration PM 2.5 than in the control site. The adaptive change of leaf area caused by air pollution has complex species dependence and is also related to the stress mechanism of one or more air quality parameters (Wuytack et al., 2011). Clearly, the effect of air pollution on leaf area is related to the type of pollutants..

Interestingly, the aspect ratio of leaves in the areas with serious pollution is larger than that at the Site 5 and Site 6 with better air quality (Table 3). These indicated the leaves became narrow due to air pollution. Large leaves are more likely to retain dust (Chaturvedi et al., 2013). Narrowing leaves in a polluted site could help reduce the risk of being covered by dust (Leonard et al., 2016). Results from correlation analysis showed that the aspect ratio of leaves in the lower crown had a significant positive correlation with AQI, PM2.5, and PM10 of sites ( $p=0.05$ ), suggesting the aspect ratio of leaves in the lower crown could be an indicator for particulate matters rather than for other air pollutants.

#### Stomatal traits

Stomatal traits responding to air pollutants of the sampling sites varied among the crown positions

(Tables 5 and 6). Despite differences in stomatal traits of leaves in whole crown or in middle crown, the traits did not reveal a regular pattern of change among sites (Table 5) and did not show significant correlation with air quality parameters, except for guard cell width in upper crown (Table 6). However, the size (length and width) and density of stomata in the upper crown at the polluted sites were much smaller than the Site 5 and Site 6 with better air quality. The reduction in size and density of stomata could be considered as a favorable adaptation as it might be important for preventing the capture of pollutants (Hoshika et al., 2015). However, the first emergency response of stomata to the stress of air pollutants may be the closure of some stomata, which in turn reduces the stomatal conductance of leaves. Consequently, this study demonstrates the inability of using these *L. lucidum* stomatal traits (except guard cell width in upper crown) to monitor air pollutants concentration.

The size of guard cell was larger in leaves from the upper crown at the polluted sites than in those of leaves at the Sites 5 and 6 with better air quality, except for guard cell length at site 3 (Table 5). Furthermore, the guard cell length from the upper crown showed a significant positive correlation with AQI, PM10, PM<sub>2.5</sub>, and CO content of the sites (Table 6). The guard cells are primarily and directly affected by the stream of the pollutants entering the stomata, as the stomata are open (Christodoulakis, 1993). Ozone, for example, acts directly on guard cells by altering the activity of ion channels in the guard cell plasma membrane (Torsethaugen et al., 1999). Thus, increasing the size of guard cells was good for combating the air pollution (Iqbal et al., 2010a).

**Table 5** Stomatal traits of *Ligustrum lucidum* Ait. leaves

Crown layer	Stomatal traits	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Whole	Stomatal length (µm)	16.69 ± 0.20c	17.72 ± 0.20b	15.62 ± 0.19d	18.47 ± 0.19a	18.51 ± 0.20a	16.89 ± 0.23c
	Stomatal width (µm)	7.95 ± 0.12b	8.40 ± 0.12a	7.88 ± 0.09b	8.51 ± 0.10a	8.64 ± 0.11a	7.47 ± 0.11c
	Stomatal density (N/mm <sup>2</sup> )	160.89 ± 4.66b	148.87 ± 3.75b	197.94 ± 8.47a	197.12 ± 8.22a	202.54 ± 10.44a	158.85 ± 4.65b
	Guard cell length (µm)	22.92 ± 0.19c	23.20 ± 0.21bc	20.78 ± 0.21d	23.93 ± 0.23a	23.63 ± 0.24ab	23.81 ± 0.09ab
	Guard cell width (µm)	3.42 ± 0.06b	3.05 ± 0.05c	3.04 ± 0.04c	3.10 ± 0.05c	2.75 ± 0.04d	3.67 ± 0.06a
Upper	Stomatal length (µm)	17.0 ± 0.36b	17.31 ± 0.42b	15.57 ± 0.39c	18.87 ± 0.32a	17.67 ± 0.27b	18.94 ± 0.39a
	Stomatal width (µm)	7.81 ± 0.22c	8.42 ± 0.18ab	7.96 ± 0.15bc	8.53 ± 0.15a	8.32 ± 0.16abc	8.61 ± 0.16a
	Stomatal density (N/mm <sup>2</sup> )	155.23 ± 4.81b	161.64 ± 6.28b	191.0 ± 10.86ab	175.28 ± 15.37b	222.75 ± 16.56a	177.62 ± 10.25b
	Guard cell length (µm)	23.55 ± 0.32ab	22.40 ± 0.36c	21.32 ± 0.46d	24.66 ± 0.36a	22.59 ± 0.30bc	24.04 ± 0.47a
	Guard cell width (µm)	3.57 ± 0.11a	2.98 ± 0.08b	3.14 ± 0.07b	3.14 ± 0.09b	2.71 ± 0.06c	2.73 ± 0.08c
Middle	Stomatal length (µm)	16.61 ± 0.26c	18.6 ± 0.28b	15.52 ± 0.34d	17.9 ± 0.27b	19.53 ± 0.32a	17.94 ± 0.34b
	Stomatal width (µm)	7.99 ± 0.17 cd	8.64 ± 0.19ab	7.77 ± 0.16d	8.37 ± 0.17bc	9.05 ± 0.18a	8.20 ± 0.21bcd
	Stomatal density (N/mm <sup>2</sup> )	163.55 ± 8.13bc	142.16 ± 5.28c	216.92 ± 16.81a	207.09 ± 13.90a	192.09 ± 11.98ab	154.17 ± 9.12c
	Guard cell length (µm)	22.54 ± 0.27c	24.18 ± 0.34ab	20.36 ± 0.33d	23.38 ± 0.38bc	24.87 ± 0.44a	23.88 ± 0.47ab

**Table 5** (continued)

Crown layer	Stomatal traits	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Lower	Guard cell width ( $\mu\text{m}$ )	3.37 $\pm$ 0.08a	3.19 $\pm$ 0.10ab	2.98 $\pm$ 0.07bc	3.06 $\pm$ 0.08b	2.77 $\pm$ 0.08c	3.35 $\pm$ 0.11a
	Stomatal length ( $\mu\text{m}$ )	16.28 $\pm$ 0.5 cd	17.57 $\pm$ 0.28bc	15.77 $\pm$ 0.28d	17.96 $\pm$ 0.40ab	19.27 $\pm$ 0.62a	15.86 $\pm$ 0.32d
	Stomatal width ( $\mu\text{m}$ )	8.10 $\pm$ 0.29b	8.19 $\pm$ 0.21ab	7.95 $\pm$ 0.15b	8.68 $\pm$ 0.23ab	8.89 $\pm$ 0.28a	6.84 $\pm$ 0.14c
	Stomatal density (N/ $\text{mm}^2$ )	165.37 $\pm$ 12.71bc	140.56 $\pm$ 4.92c	181.80 $\pm$ 11.06b	216.6 $\pm$ 16.15a	153.02 $\pm$ 8.75bc	153.14 $\pm$ 5.86bc
	Guard cell length ( $\mu\text{m}$ )	22.78 $\pm$ 0.47b	23.27 $\pm$ 0.32b	20.88 $\pm$ 0.3cb	23.58 $\pm$ 0.46ab	24.59 $\pm$ 0.60a	23.71 $\pm$ 0.23ab
	Guard cell width ( $\mu\text{m}$ )	3.28 $\pm$ 0.09b	3.01 $\pm$ 0.08bc	3.03 $\pm$ 0.07bc	3.09 $\pm$ 0.08bc	2.90 $\pm$ 0.13c	4.09 $\pm$ 0.08a

Means  $\pm$  SE with different letters within a row were significant at  $p \leq 0.05$

Following air quality of sampling sites, the stomatal traits of leaves in lower crown revealed an opposite pattern of change compared with that in upper crown (Table 5). For example, there was a marked increase in guard cell size of leaves in upper crown, whereas a slight decrease was observed in lower crown of *L. lucidum* with the increase of pollutant concentrations in sampling sites. This study suggested that the response of leaf stomata to air pollutants may also depend on the height and crown structure of trees. Under the influence of tree height, crown structure, and wind speed, the distribution of air pollutants in the canopy was heterogeneous (Albaugh et al., 1992). Wang (2014) found the vertical distribution of atmospheric particle concentration was related to tree height in the main roadside. However, it was difficult to accurately measure the distribution of pollutants due to the complexity in the array of leaves and branches and the complex process of air flow within a crown (Bache, 1979; Lin & Khlystov, 2012). Detailed estimates of the effects of crown position on pollutants collection by leaves still require a lot of work to be done.

#### Non-structural carbohydrates

The soluble sugar content in leaves at the polluted sites was generally lower than in those of leaves at the site with better air quality, except for the soluble sugar content at Site 3. The lowest soluble sugar content in leaves was in the Site 1 (Table 7). This was in agreement with Liang et al. (2008). Their study revealed a loss of soluble sugar in all tested species including *L. lucidum* at polluted sites. Inhibitory action of pollutants on photosynthesis in leaves was believed to reduce availability of soluble sugar (Iqbal et al., 2010b; Ierhievwie et al., 2014). The inhibitory effect included blocking the light by suspended particulate matter in the air and stomatal occlusion due to the deposition on leaf surface (Bae et al., 2009). This was revealed in this study by the negative correlation between the soluble sugar content in leaves and particle content in air (Table 8). Some studies have observed that a decreased soluble sugar was linked to various toxic gases (He, 2002; Ito et al., 1985). However, most of these studies were carried out using high pollutant concentrations comparing



**Table 6** Correlation between parameters of air quality and stomatal traits *Ligustrum lucidum* Ait

Crown layer	Stomatal traits	AQI	PM2.5 (µg/m3)	PM10 (µg/m3)	SO2 (µg/m3)	NO2 (µg/m3)	CO (µg/m3)	O3 (µg/m3)
Whole	Stomatal length (µm)	-0.066	-0.028	-0.061	0.012	0.204	-0.060	0.137
	Stomatal width (µm)	0.229	0.150	0.232	0.307	0.441	0.015	0.281
	Stomatal density (N/mm <sup>2</sup> )	-0.089	-0.282	-0.039	0.310	0.159	-0.460	0.551
	Guard cell length (µm)	-0.154	-0.012	-0.157	-0.229	-0.058	0.066	0.061
	Guard cell width (µm)	-0.012	0.149	-0.017	-0.190	-0.250	0.322	-0.125
Upper	Stomatal length (µm)	-0.280	-0.128	-0.262	-0.142	-0.009	-0.023	-0.111
	Stomatal width (µm)	-0.600	-0.464	-0.583	-0.211	-0.023	-0.356	-0.603
	Stomatal density (N/mm <sup>2</sup> )	-0.600	-0.750	-0.581	-0.363	-0.414	-0.768	-0.016
	Guard cell length (µm)	0.145	0.282	0.168	0.158	0.183	0.364	0.231
	Guard cell width (µm)	0.941**	0.910*	0.939**	0.636	0.473	0.860*	0.626
Middle	Stomatal length (µm)	-0.370	-0.305	-0.378	-0.336	-0.109	-0.292	-0.085
	Stomatal width (µm)	-0.341	-0.331	-0.360	-0.375	-0.158	-0.362	-0.039
	Stomatal density (N/mm <sup>2</sup> )	0.080	-0.110	0.136	0.519	0.325	-0.279	0.287
	Guard cell length (µm)	-0.345	-0.236	-0.368	-0.481	-0.249	-0.177	-0.084
	Guard cell width (µm)	0.281	0.461	0.258	-0.036	-0.026	0.630	-0.076
Lower	Stomatal length (µm)	0.080	0.110	0.136	0.519	0.325	0.279	0.581
	Stomatal width (µm)	0.409	0.276	0.418	0.430	0.455	0.096	0.528
	Stomatal density (N/mm <sup>2</sup> )	0.407	0.339	0.471	0.838*	0.700	0.249	0.321
	Guard cell length (µm)	-0.312	-0.225	-0.323	-0.425	-0.259	-0.188	-0.087
	Guard cell width (µm)	-0.416	-0.259	-0.415	-0.456	-0.481	-0.078	-0.326

\*Correlation was significant at the 0.05 level (2-tailed). \*\*Correlation was significant at the 0.01 level (2-tailed)

to that in real atmosphere. We reported here that the soluble sugar in leaves showed no significant correlation with SO<sub>2</sub>, NO<sub>2</sub>, and O<sub>3</sub> concentration at the sites. The soluble sugar in leaves showed a significant negative correlation with CO concentration at the sites. However, the significant correlation was insufficient to draw conclusions on the effect of CO on soluble

sugar. The leaves could not only absorb CO from the atmosphere (Bidwell & Fraser, 1972), but also produce CO themselves (Fischer & Ttge, 1978). Thus, we think the decreased soluble sugar may be an indicator for early damage of *L. lucidum* leaves, which, among other stress factors, reacted sensitively to the increasing load of particulate matter pollutants.

**Table 7** Non-structural carbohydrate contents of *Ligustrum lucidum* Ait. leaves

Crown layer	Leaf size	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Whole	TNC (%)	6.12±0.70b	13.02±2.25a	12.92±1.97a	18.05±1.33a	12.36±2.44a	13.90±1.23a
	Sugar (%)	0.94±0.17b	1.61±0.37ab	2.33±0.53a	1.60±0.43ab	2.61±0.15a	2.62±0.09a
	Starch (%)	5.18±0.53c	11.41±1.95b	10.59±1.74b	16.46±1.10a	9.96±2.31bc	11.28±1.20b
Upper	TNC (%)	7.94±0.09b	17.15±0.33 ab	8.93±3.50 ab	16.0±1.64 ab	18.14±3.51a	13.31±2.30ab
	Sugar (%)	1.39±0.05a	2.09±0.51a	2.49±1.36a	1.67±0.87a	2.89±0.23a	2.76±0.30a
	Starch (%)	6.55±0.47b	15.06±0.84a	6.44±2.36b	14.41±0.79a	15.25±3.29a	10.55±2.00ab
Middle	TNC (%)	6.22±0.10a	13.15±5.94a	16.26±0.70a	17.68±3.09a	7.91±0.07a	17.03±0.07a
	Sugar (%)	0.95±0.02a	1.84±0.93a	2.32±0.23a	1.23±0.30a	2.20±0.02a	2.58±0.02a
	Starch (%)	5.27±0.07a	11.31±5.18a	13.94±0.47a	16.46±2.79a	5.71±0.05a	14.46±0.05a
Lower	TNC (%)	4.19±0.49d	10.14±1.39c	15.56±1.40b	21.38±1.63a	8.15±0.48 cd	11.36±0.91c
	Sugar (%)	0.48±0.02b	1.06±0.21ab	2.09±0.47ab	1.85±1.16ab	2.58±0.02a	2.53±0.02a
	Starch (%)	3.71±0.47d	9.08±1.18c	13.48±0.93b	19.53±0.47a	5.57±0.47d	8.83±0.93c

Means ± SE with different letters within a row were significant at  $p \leq 0.05$ . TNC was the sum of soluble sugar and starch content

Our results showed the starch content was much higher in lower crown layer at Site 4 than that at other sites (Table 7). An unusual increase of starch content at polluted Site 4 could be due to much higher  $\text{SO}_2$  and  $\text{NO}_2$  in the site which closer the two coal power plants.  $\text{NO}_2$  and  $\text{SO}_2$  are two types of major pollutants from coal power plants (Sharma & Tripathi, 2009; Zhu et al., 2016). Steubing et al. (1989) found that carbohydrate metabolism in *Allium ursinum* leaves was altered by fumigation with moderate doses of  $\text{SO}_2$ ,  $\text{NO}_2$ , and  $\text{O}_3$ , leading to starch accumulation in the leaves. Schiffgens-Gruber and Lütz (1992) also

reported an increase in the starch contents in spruce leaves after treatment with gas mixtures of  $\text{O}_3$ ,  $\text{NO}_2$ , and  $\text{SO}_2$ . However, according to Bückner and Ballach (1992), air pollution reduced the starch contents in the leaves of trees. These findings of the effects of  $\text{NO}_2$  and  $\text{SO}_2$  on starch content in leaves are inconsistent, therefore implying the complexity of starch responding to air pollution. The Pearson's correlation analysis in our study showed the starch content in leaves did not show significant correlation with any air quality parameters of the sites (Table 8). Starch was one of the main substances stored in plants for

**Table 8** Correlation between parameters of air pollutants and non-structural carbohydrate contents (TNC) of *Ligustrum lucidum* Ait. leaves

Crown layer	TNC (%)	AQI	PM2.5 ( $\mu\text{g}/\text{m}^3$ )	PM10 ( $\mu\text{g}/\text{m}^3$ )	$\text{SO}_2$ ( $\mu\text{g}/\text{m}^3$ )	$\text{NO}_2$ ( $\mu\text{g}/\text{m}^3$ )	CO ( $\mu\text{g}/\text{m}^3$ )	$\text{O}_3$ ( $\mu\text{g}/\text{m}^3$ )
Whole	Sugar	-0.944**	-0.990**	-0.927**	-0.586	-0.579	-0.981**	-0.522
	Starch	-0.236	-0.198	-0.186	0.423	0.564	-0.189	-0.448
	TNC	-0.389	-0.361	-0.339	0.295	0.429	-0.351	-0.514
Upper	Sugar	-0.947**	-0.990**	-0.947**	-0.717	-0.681	-0.977**	-0.528
	Starch	-0.302	-0.247	-0.303	0.097	0.176	-0.247	-0.253
	TNC	-0.423	-0.376	-0.423	0.193	0.073	-0.374	-0.317
Middle	Sugar	-0.936**	-0.947**	-0.936**	-0.656	-0.614	-0.894*	-0.713
	Starch	-0.232	-0.167	-0.184	0.402	0.460	-0.095	-0.618
	TNC	-0.346	-0.284	-0.299	0.300	0.361	-0.209	-0.686
Lower	Sugar	-0.856*	-0.907*	-0.821*	-0.389	-0.402	-0.919**	-0.389
	Starch	0.060	0.049	0.121	0.720	0.742	0.021	-0.268
	TNC	-0.062	-0.080	0.001	0.634	0.653	-0.108	-0.310

TNC was the sum of soluble sugar and starch content. \*Correlation was significant at the 0.05 level (2-tailed). \*\*Correlation was significant at the 0.01 level (2-tailed)

long term (Zeeman et al., 2010). In the process of plant growth, the conversion between starch and soluble sugar is always going on. Whereas soluble sugar was generally reduced, both decreased and increased starch may be found in leaves (Bücker & Ballach, 1992; Fialho & Bücker, 1996). Also, those of starch in the leaves displayed unstable seasonal variations, compared with the soluble sugar content (Liu et al., 2019). In conclusion, the starch content in leaves of *L. lucidum* is not a good predictor of the air pollutants in Luoyang urban areas.

The content of TNC in leaves of *L. lucidum* at Site 1 was low, especially in the lower crown layer, which was significantly lower than that at the other sites (Table 7). Air pollution reduced the content of TNC, which is consistent with the research results of Cao et al. (2017) on *Machilus ichangensis* and *Taxus wallichiana*. The difference of TNC response to air pollutants between crown layers was related to the daylighting of leaves. According to previous studies (Legner et al., 2014; Li et al., 2020, Masarovicová & štefančík, 1990), crown structure can influence the growth of trees by influencing the proportion of sun and shade leaves. There was no significant correlation between TNC content and air quality parameters (Table 8), which corresponded to starch content. Bücker and Ballach (1992) also found that the response pattern of TNC stored in trees to pollution stress is the same as that of starch. This may be due to the dominance of starch in TNC (83% in this study).

Non structural carbohydrate stored in trees, like starch, has been demonstrated to confer resilience to environmental stress in the short term (Dong et al., 2018; Thalmann & Santelia, 2017). However, the observed changes in non structural carbohydrates in leaves during pollution stress result from multiple activities: flowering (Iqbal et al., 2010b), fruiting (Mesaa et al., 2016), and seasonal growth (Ramírez-Briones et al., 2017). Only one data collection was conducted in this study, which makes the knowledge still fragmentary and hinders our full understanding of the relationship between non structural carbohydrates and air pollutants. To evaluate how leaves respond to interacting factors, a more clearly view of the non structural carbohydrate change during air pollution stress will require more repetitive experiments conducted under different growth conditions.

## Conclusions

Air pollution has affected growth of leaves in the *L. lucidum*. Leaf area and soluble sugar content decreased in trees in polluted area, while aspect ratio of leaves increased. The changes in the stomatal traits with the increase of environmental pollution depend on the sampling position within crown. It is concluded that the area, aspect ratio, and soluble sugar content of leaves and guard cell width of stomas can be used as indicators of air pollution in Luoyang city, but the sampling position in the crown might need to be considered. It is also suggested that such changes in the *L. lucidum* leaves of polluted areas could be of great significance in determining the level of air pollution and the types of air pollutants.

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**Data availability** The datasets supporting the conclusions of this article are included within the article.

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