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Influence of Environmental Pollution on Leaf Properties of Urban Trees in China and USA: A Comparative Study using Stomatal Density

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ABSTRACT

Impacts of air pollution on plant anatomy in urban areas were studied using two different tree species, namely birch tree (Betula papyrifera) and sweetgum tree (Liquidambar styraciflua), at two globally different locations with different air pollution levels. Wuhan City in China is considered as a commercial center for central China while Union City in USA is located close to New York City, one of the mega cities in the world. Stomatal density measurements were used to record the response rate of the two different tree species to different levels of air pollution. We found significantly higher stomatal densities for samples collected in China regardless of the tree species while the air pollution levels in China was worse compared to the ones in USA. Our observation suggests that the studied tree species likely have developed metabolic resilience mechanisms to cope with the chronic high level of air pollution exposure.

Keywords: Air pollution, *Betula papyrifera*, Bioindicators, Leaf anatomy, Stomatal density, Metabolic resilience

INTRODUCTION

Air pollution is a serious threat to an environment in many parts of the world especially in industrialized areas. Studies have shown that emissions from motor vehicles in urban areas account as a major source of pollution impacting the urban forestry and biodiversity WHO, Viskari et al. [1,2] have demonstrated that air pollutants from motor vehicle exhausts significantly impact the metabolism of roadside plants even before any physical changes can be noticed. Other studies further demonstrated that abnormal foliar anatomy morphology modifications chlorophyll content changes and peroxidase activity alterations are strongly correlated to high exposure rate of plants such as *Platanus orientalis* to road traffic emissions in urban locations [3-7]. Interestingly, these trees are widely planted throughout Europe, Asia and USA in parks and along the roads to improve the microclimatic conditions in cities yet they are now endangered due to high exposure to pollution [8-10].

Plant species have been used as bioindicators to assess air quality and degree of air pollution due to the inherent plant metabolic interaction with atmosphere through photosynthetic reactions [11-14]. Some studies used the concentration of settled contaminants from air on surfaces of leaves as an index to identify the degree of air pollution emitted into the environment from transportation, industry and fossil fuels [2,15-17]. Other studies used pioneer plant species in urban areas as the leaves of studied plants capture dust and aerosols in the air. For example Renzhong et al. [18] analyzed heavy metal concentrations on broad bean (*Vicia faba L.*) leaves and identified effects of contaminants on plant physiology. Recent studies have used stomata small pores on the surfaces of leaves and stems which control the exchange of gases between the interior of plant and the atmosphere as an indicator to identify the influence of environmental air pollutions on plants [16,19-24]. Allen identified a substantial stomatal density decrease with an increase of CO_2 concentration [25]. Darrall also found that increase in air pollution cauzed an inhibition of photosynthesis likely due to the low reactivity of stomata [26]. On the contrary Kärenlampi et al. [27] found that increasing ozone exposure resulted in a reduced shoot dry weight, an accelerated autumn yellowing of leaves but an increased stomatal density in birch trees (*Betula pendula*) in Finland. Bell et al. [3] has recorded physiological responses of a wide range of plant species within the urban sites in India to different levels of air pollution and reported that air pollution could negatively influence crop yield. However they did not conduct any molecular level interpretation or correlated their observations to plant metabolic activities so further work was required to account for a more definitive cause of the low plant productivity due to air pollution. Interestingly Polle et al. [13] found that many of the physiological and anatomical features of urban trees (*Platanus orientalis*) were unaffected by the air pollution levels that trees indeed developed resilience toward disturbed environmental changes.

As demonstrated here the impacts of air pollutants on plant physiology and anatomy including stomata functions are too complex to be summarized or generalized concisely due to its complicated response mechanisms to different environmental stimuli. Future research into physiological effects of air pollutants should incorporate an integrated approach in which both key physiological parameters and growth parameters are measured together with estimates of the effective dose of pollutant. In this way, the underlying mechanisms to changes in growth and development will be more fully understood.

In this research we used stomatal density as an indicator for measuring impact of air pollution levels on plant anatomy in the city of Wuhan in China. We also conducted a comparative study by collecting similar tree leave samples in Union, New Jersey and counted the stomatal density. Total of 20 different sample collection sites for both China and USA were selected. Our study will demonstrate the different levels of plant responses in two different global locations to understand the impact of air pollution on plant anatomy.

MATERIALS AND METHODS

On June 25th, 2017, 20 different sample sites in Central China Normal University (CCNU) in Wuhan, China were selected. CCNU is located in a central part in the city of Wuhan in China. Wuhan is a capital of Central China's Hubei province and is known as a commercial center of central China. CCNU is one of the largest universities in the city with lots of exposure to motor vehicle traffics as many typical city universities often experience. In each of sample site locations at CCNU campus, both birch tree (*Betula papyrifera*) and sweetgum tree (*Liquidambar styraciflua*) were chosen and 5 leaves from each tree species were collected. We also collected GPS locations on August 20th 2017, we mapped out environmentally similar locations at Kean University in Union, NJ (USA). Kean University is located in urban location in the City of Union in USA. Union is approximately 8 miles away from Manhattan in New York city which is one of the global mega cities. Kean University experiences similar traffic levels and exposures to motor vehicle exhausts level as CCNU. Selection of same tree species and same sampling strategies were applied for Kean University sampling sites in order to conduct a comparative analysis between the two locations. See Figure 1 for sample site comparisons.

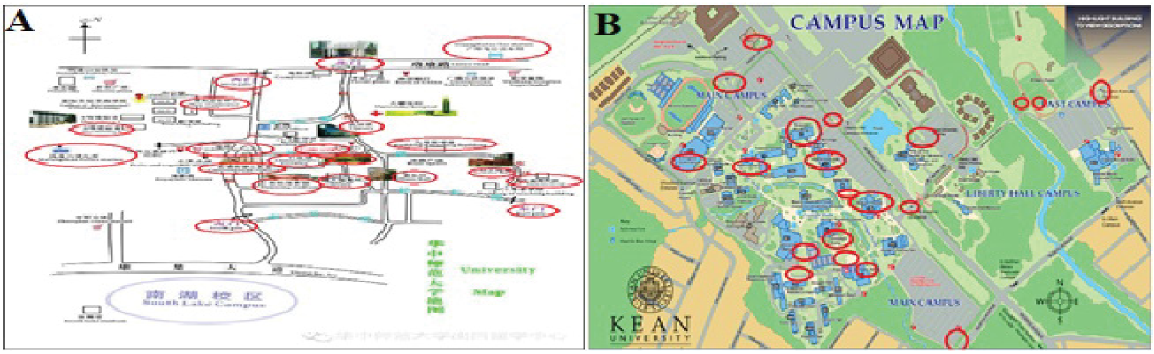

Figure 1: Campus map of (A) China Central Normal University (CCNU) in Wuhan, China, and (B) Kean University in Union, USA. Red circles indicate relative locations where tree leaf samples were collected. See Table 1 for complete details for each sampling site locations and descriptions.

Once all leaves were collected from both CCNU and Kean University each leaf sample was coated with a solution of clear nitrocellulose dissolved in butyl acetate on the posterior portion of the leaf. A section of 1 cm by 1 cm of treated leaf sample was then selected and affixed on an adhesive layering. The sectioned layering with plant leaf was examined under richter optica binocular microscope to count for stomatal density. See Figure 2 for slides prepared for stomatal count analysis.

Air quality data of the sampling date for both CCNU and Kean University were collected using the national air quality index website. Air quality data for particulate matter and chemical compositional concentrations were gathered for the month samples were collected for each site.

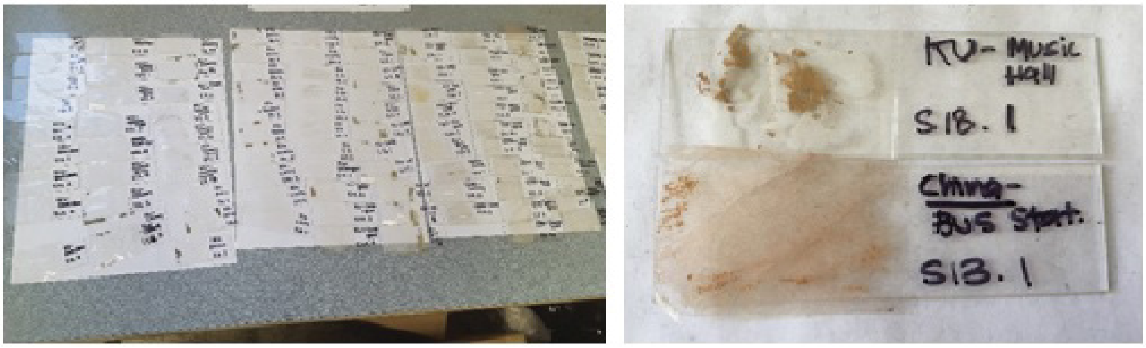

Figure 2: Slides of tree leaf samples prepared for microscopic analysis.

RESULTS AND DISCUSSION

Tables 1 and 2 shows sampling locations and physical descriptions for each sampling sites for China and USA. We collected an equal number of tree leaf samples for both locations. We also ensured that an equal number of leaf samples for sweetgum trees and birch trees were collected for both locations to ensure a homogeneous data distribution.

Table 1: Sample Site Locations and Descriptions-Wuhan, China

ID	Sample Species	Location	GPS Location	Physical Descriptions
S01	Sweet gum	Science building	N30 31.209 E114 21.591	Side of the bldg.., vegetation on the ground around it. Thorns next to pathway, kind of busy side street with cars and bikes
S02	Cherry birch	North gate	N30 31.538 E114 21.338	Off the road on a ledge, hill, very buggy but quiet of traffic since higher off the ground. though the road was very busy
S03	Sweet gum	Book store	N30 30.962 E114 21.406	Very local area, lots of foot traffic, and car traffic. about 20 feet away from the side road a lot of food places around this area
S04	Sweet gum	Gym	N30 30.994 E114 21.482	Less foot action, quiet area a lot more kids in this area, same type of trees close to one another
S05	Sweet gum	Admin. Bldg	N30 31.227 E114 21.481	Across the street, inside a park, very busy area, road way is very active with cars bikes and people
S06	Sweet gum	Library	N30 31.306 E114 21.301	Shaded pathway, pretty busy with foot traffic, no cars rally quiet next to class building
S07	Sweet gum	Tennis courts	N30 30.953 E114 21.523	Students walked by, but didn't really come close to the courts and the tree specifically. The pathway is brick and barely used
S08	Yellow birch	Police	N30 31.245 E114 21.590	Right next to it, kind of a shaded region. Pretty busy right next to the road, busy with foot and car traffic
S09	Yellow birch	Dorms	N30 30.918 E114 21.351	Lots of foot traffic, not a lot of cars and bikes. Very quiet, hilly compared to other locations. intersection of the road way
S10	Cherry birch	Cafe	N30 30.972 E114 21.435	Across the street, a lot of foot traffic, not so much car traffic, same vegetation as store, closer a forest area
S11	Cherry birch	East gate	N30 31.055 E114 21.731	Right next to a main street, about 10 m from the road. Lots of traffic taking place by cars/ motorbikes along with foot traffic.
S12	Yellow birch	Forest	N30 30.998 E114 21.443	Surrounding area is very busy, pathways through the site, noticed a lot of people walking through. Also very buggy
S13	Sweet gum	Soccer field	N30 30.922 E114 21.491	Next to road, cars parked next to the tree, other vegetation, across the street is building for classroom, some motorbike traffic, lots of walking around
S14	Sweet gum	South gate	N30 33.115 E112 54.160	Traffic is very busy from all areas in every road way. Close to a lot of food shops next to life science building
S15	Sweet gum	Conference hall	N30 31.224 E114 21.662	Cars coming in and out and a lot of walking. Next to major road that goes through campus.
S16	Gray birch	West gate	N30 31.471 E114 21.137	Right next to road, very busy area with car motorbikes and people. Tree is on the other side of the concrete wall, is hanging of the road
S17	Gray birch	Theatre	N30 31.188 E114 21.512	Hidden path. Surrounding areas very busy since building and parking are right next to the area. A water gate is near
S18	Sweet gum	Music hall	N30 31.196 E114 21.525	A lot of traffic, similar to theatre, depending on hour of day, by garbage and electrical boxes in the ground
S19	Sweet gum	Teaching building	N30 31.344 E114 21.234	Shaded area, lots of bamboo, concrete walkway with a parking lot close to the building. not much foot traffic
S20	Gray birch	Bus stop	N30 31.570 E114 21.321	Off the main road, cars are around very little, along with people. very busy

As noted in Tables 1 and 2, sampling site selections and collections were planned carefully to confirm that the similar motor vehicle and foot traffics were observed in both locations in order to minimize any human variations or local variations on the environmental conditions. We also conducted a Pearson correlation statistical analysis and found that motor vehicle traffic was positively correlated with human foot traffics at significance level of 0.01, further confirming our selection of sampling sites represented the local environments well.

Table 2: Sample Site Locations and Descriptions–Union, USA

ID	Sample Species	Location	GPS Location	Physical Descriptions
S01	Sweet gum	Science building	N40 40.841 W74 14.069	Not a lot of foot traffic. Decent amount of cars, tree located about 15 feet from the front of the building
S02	Sweet gum	North gate	N40 40.904 W74 14.210	Lots of car traffic, on the main road and entrance into the university. Decent amount of foot traffic as well, close to the train station
S03	Sweet gum	Book store	N40 40.943 W74 14.192	Right next to walkway and parking lot. Pretty busy with car and foot traffic.
S04	Sweet gum	Gym	N40 40.822 W74 14.230	Not a lot of foot traffic but during the semester there is a significant increase in traffic. About 10 feet from the road
S05	Gray birch	Admin. Bldg	N40 40.792 W74 14.194	Foot traffic medium, not a lot of car traffic due to the visitor's lot being in the front of the building.
S06	Cherry birch	Library	N40 40.697 W74 14.041	Right in front, next to walkway. Not a lot of foot traffic, but busy area during the semester
S07	Sweet gum	Tennis courts	N40 40.646 W74 14.023	Very shaded region, not a lot of traffic. Right next to the fence of the courts
S08	Sweet gum	Police Stn.	N40 40.625 W74 13.989	Shaded region, off the walkway not a lot of traffic. Mostly just the golf carts and benches are at this location
S09	Yellow birch	Dorms	N40 40.601 W74 14.082	Move in day, lots of foot traffic, next to walkway and street. Not much motor vehicle traffic.
S10	Sweet gum	Cafe	N40 40.645 W74 14.119	Not a lot traffic by any means. Right outside the cafeteria, 10 ft from walkway
S11	Gray birch	East gate	N40 40.895 W74 13.452	Right next to the fences, main road and lots of car traffic, very residential area, close to another electrical box.
S12	Cherry birch	Forest	N40 40.882 W74 13.627	Not so close to the road or the river. No foot traffic, area covered with invasive species such as Japanese knotweed.
S13	Cherry birch	Soccer field	N40 40.872 W74 13.601	Close to the road, decent amount of car traffic, next to the fence and goals.
S14	Cherry birch	South gate	N40 40.504 W74 13.738	Right next to the parking lot, a very busy road. Lots of traffic and foot traffic.
S15	Yellow birch	Stem building	N40 40.822 W74 13.820	Off the path, not a lot of foot traffic. closer ot ursino, there is an electrical box is next to the tree
S16	Sweet gum	West gate	N40 40.695 W74 13.854	Right next to road and very close to the main road (Morris Ave). Lots of traffic.
S17	Yellow birch	Wilkins theatre	N40 40.706 W74 13.896	A lot of foot traffic. In the front of the building. close to one of the main entrances into campus for cars as well as people
S18	Sweet gum	Music hall	N40 40.691 W74 13.928	Foot traffic not much, but this is another main foot entrance into campus
S19	Sweet gum	Class Rm	N40 40.782 W74 14.039	In front of the building, off the walkway, shaded area with not a lot of foot traffic no car traffic
S20	Yellow birch	Bus stop	N40 40.835 W74 14.042	Next to the parking lot, lots of foot traffic, car and trolley traffic at a steady rate

As shown in Figure 3, The average value of stomatal density measured from all tree leaf samples were significantly higher for the samples collected in China. The average stomatal density for tree leaf samples collected in China was 41.9 ± 3.7 whereas the ones for USA was 22.4 ± 7.8. We further analyzed the data by separating the information based on different tree species. Figure 4 shows the stomatal density of tree leafs depending on the tree species. We still found that the stomatal densities for tree leaf samples collected in China, regardless of the tree species, were higher.

Air quality information during the sampling date in China or USA was gathered from Air Quality Index Service website (https://aqicn.org/). Table 3 shows a comprehensive measurement data to assess air quality for both cities, Wuhan in China and Union in USA. It is clear that the air quality of the Union City in USA was significantly better in terms of all aspects of measurements (e.g., PM2.5, O_3, NO_2, and CO, etc.) compared to the Wuhan City in China.

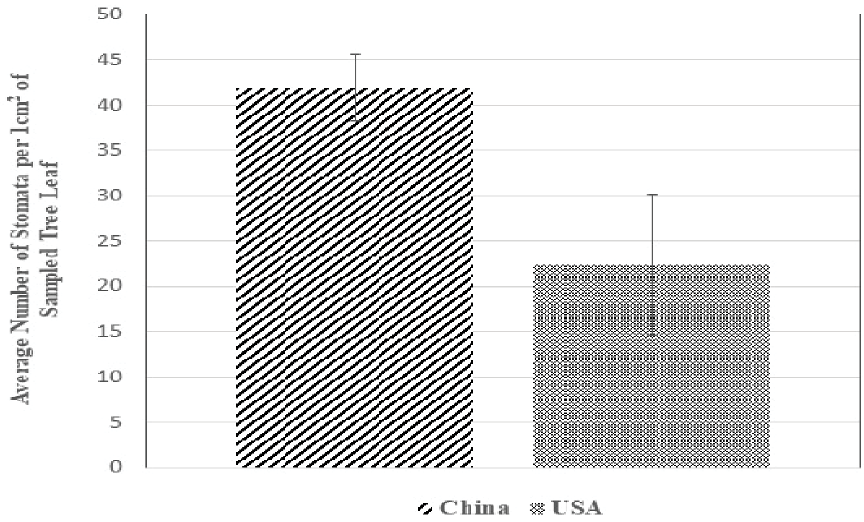

Figure 3: Comparison of stomatal density for all tree leaf samples collected in China and USA.

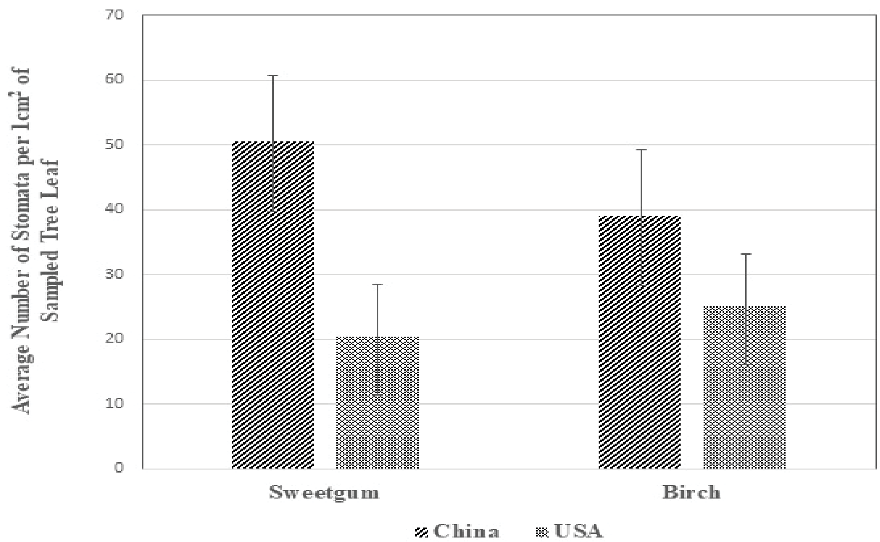

Figure 4: Comparison of stomatal density for sweetgum tree leaf samples and birch tree leaf samples collected in China and USA.

Table 3: Air quality data for Wuhan, China and Union, NJ during the month of june and august 2017 collected from air quality index service.

	Wuhan	Union
*$PM_{2.5}$	162	10
**PM_{10}	77	—
ǂO_3	1	—
ǂNO_2	67	—
ǂSO_2	3	—
ǂCO	30	—

*Particles with a diameter less than 2.5 micrometers (μm). Units in μg/m³, **Particles with a diameter between 2.5 and 10 micrometers (μm). Units in μg/m³, ǂ Units in ppm (mg/L), Note: No data entry indicates non-detectable concentration.

CONCLUSION

In this research, we investigated the impacts air pollution on tree leaf anatomy in two globally different locations. We collected samples from urban areas in China and USA. Stomatal density was measured and used to represent the effects of air pollution on plants. Interestingly we observed higher stomatal density in tree leaf samples collected from China regardless of the tree species while the air quality in China was worse compared to the city in USA. Our study suggests either that: (1) Stomatal density is not a good way to measure impacts of air pollution on plant; or (2) The studied tree species in China have developed higher resilience and resistance to air pollution due to chronic and high concentration exposure to air pollutants. Previous studies suggested that urban conditions and higher air pollution affected structural and physical leaf properties, yet the impacts of air pollution on internal functional anatomy were still in debate. It should be noted that we also cannot rule out the possibility that the geographical and geophysical conditions such as different latitudes lighting time, genetic background of plants, may impact the observed differences in the stomatal densities between the two locations. However, this field study involves two global locations that are both considered to be an urban collage campus environment. Furthermore it is practically impossible to create or choose two global field stations that share the exact identical physical conditions. To determine the extent of air pollution effects on trees and other plants further controlled studies should be conducted measuring a wide range of both structural and physical properties of the plants both in laboratory and field. In the future, we plan to carry experiments with more tree species with different global locations with different levels of air pollution to further identify the relationship between the air pollution and plant responses.

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