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Research Article

Particulate Matter Capture of Plants used as Shelterbelts **Around a Poultry Farm**

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Abstract

Plant species comprised of American holly (Ilex opaca), Arborvitae (Thuja plicata x standishii), Arizona cypress (Cupressus arizonica), Eastern red cedar (Juniperus virginiana) and Yaupon (Ilex vomitoria) established as shelterbelts around a poultry farm were studied on their efficiency on particulate matter capture and effects on plant's stomatal characteristics. Particulate matter adsorbed on leaf surfaces were quantified through gravimetric analysis and were found to be significantly different among the five species at particulate matter sizes of PM₂₀ (p=0.0001) and PM, (p=0.0002). Using stomatal imprints and leaf discs, particulate matter trapped into intercellular parts (e.g. leaf cuticle) of the leaves were inspected and categorized into four particle sizes. Only particulate matter size division of $PM_{<2.5}$ (p=0.0211) and $PM_{>5.<10}$ (p=0.0173) were found to be significantly different upon deposition on plants. Stomatal characteristics of Arizona cypress plants were significantly (p<0.0001) affected by particulate matter deposition compared to other plants, which can further inhibit gas exchange essential physiological function of plants. Stomatal length (p=0.3969) and stomatal pore surface (p=0.1187) were not observed to be significantly different among all species, therefore adverse effects from direct exposure to dust from poultry emission cannot be adequately assessed.

Keywords: Particulate Matter Loading, Intercellular Deposition, Gravimetric Analysis, Stomata.

Introduction

Plants play an important role in combating air pollution and working as a 'natural pollutant sink' [1] especially for the exposed surface of trees (e.g. bark, leaves) as they provide sites for the gravity or wind-blown settlement of particulate matter [2]. Plants reduce air pollution by intercepting suspended particulate matters (SPM) and aerosols and retain them on the leaf surface [1]. Leaves are the primary route of the uptake which is controlled by the stomatal aperture and conductance to gas diffusion [3]. Vegetation constantly exposed to atmospheric pollutants may absorb, accumulate and integrate pollutants impinging on the foliar surfaces [4] but only as a temporary retention site for many atmospheric particles [5].

Particulate matter refers to the mixture of solid and liquid particles suspended in the air which will form an aerosol that vary in sizes, shape, surface area, chemical composition, solubility and origin [6] categorized in ambient air as tri-modal [7] ranging from ultrafine, fine, to coarse particles present in the atmosphere. Coarse particles are derived primarily from suspension or resuspension of dust, soil or other crustal materials from roads, farming, mining, windstorms, volcanoes and other natural processes which may be in the form of sea salts, pollen, mold, spores, and other plant parts. Fine particles are derived

primarily from direct emissions from combustion processes which may consist of transformation products such as sulfate and nitrate particles. Ultrafine particles may also result from vehicle exhaust and atmospheric photochemical reactions that coagulate to form larger complex aggregate or may translocate from the lung to the blood and other parts of the body. On conventional particle deposition theory, larger particle sizes with diameter greater than 10 µm effectively show efficient rates of gravitational settling and deposition onto vegetation [8]. Another study shows efficient vegetation capture of particles larger than 5 µm through interception and impaction while capture of particles 0.1 µm to 5 µm in diameter on vegetation is inefficient. Particles smaller than 0.1 µm effectively transport through the viscous sub-layer by Brownian diffusion providing significant deposition rates [9].

In confinement poultry houses, dust is one of the primary contaminants of concern [10]. Broiler-rearing facilities with over 100,000 birds in South Norfolk, United Kingdom become one significant source of fine particulate matter (PM_{2.5}) resulting in a substantial emissions of 6 tpy in the area [11]. According to Adrizal et al. [10] total suspended particulate matter (TSP) and respirable dust concentrations of 4.4 mg/m³ and 0.24 mg/ m³, respectively, in confinement poultry houses may pose adverse environmental



and health impacts to nearby communities. Establishment of plant shelterbelts along poultry buildings can serve as buffer to lessen the impact of emissions to the neighborhood. Planting trees around livestock buildings helps minimize the dispersion of dust to the environment [12] but emissions from livestock buildings might impact plant shelterbelts survival. In a study by Kulshreshtha et al. [13] particulate deposition on the leaf surface shows cuticle injury and increase in the epidermal cell and stomata size and frequency on the plants as the main receptor is continuously exposed to roadside air.

Gostin [14] found out that plants growing on industrial areas and near the major roads were observed to have significant decrease in the size of the stomata and increase on stomatal density of the leaves. Dust deposition on leaf cuticle due to particulate penetration into the epicuticular wax may reduce light incidence and reduce net photosynthesis [1]. In metropolitan areas, smoke particles that settle out of the air accumulate as film on plant surfaces, cutting down the amount of light the plants receive. Foliage with sticky or hairy surfaces suffers the most [15]. Rice plants (Oryza sativa), situated one kilometer away from a cement factory, were shown to receive high dust loads and observed to have lower biomass by 44% to 60% [16]. Plant's capacity to hold dust or particulate matter (PM) can be speciesdependent. Trees take up more pollutants, including PM, than shorter vegetation [1]. Malone [17] reported reduction of dust by 50 to 53% at a distance of 14.6 meters downwind of a roaster house beyond three rows of trees of cypress and red cedar but does not specifically describe PM fractions trapped by different plant species.

The overall goal of this study was to examine the effectiveness of several species of trees on intercepting PM from poultry buildings. The potential of foliage of different species to trap particulates was evaluated to determine the effectiveness of plants in controlling air pollutant dispersion. Furthermore, stomatal features were assessed to determine the possible deposition of PM on the leaf surface and into the leaf cuticle.

Materials and Methods Field Site and Species Selection

Plants used as shelterbelt were planted around the vicinity of the Broiler Research Center (BRC) located at Stephen F. Austin State University's (SFASU) Walter C. Todd Agricultural Research Center in Nacogdoches, Texas. The shelterbelt was established in the zone that was directly opposite the exhaust fans of the four poultry buildings at the BRC. Five species of plants, which were a mixture of shrubs and conifers and moderately to fast growing trees, were used as test species for determining the particulate capture. The plant species were American holly (Ilex opaca), Arborvitae (Thuja plicata x standishii), Arizona cypress (Cupressus arizonica), Eastern red cedar (Juniperus virginiana) and Yaupon (Ilex vomitoria). These plant species are commonly found in East Texas.

Laboratory Measurement

Twenty (20) leaf/fascicles samples consisted of the most matured leaves were selected and taken at random points from low to mid-crown and more exposed branches of each plant species. The collection of leaf samples was done about a year after the shelterbelt has been exposed to emissions from the poultry buildings. The collected fresh plant foliage was brought to the Environmental Assessment Laboratory at SFASU for particulate matter loading, particle counting and stomatal characteristics analyses (Figure 1).

Particulate Matter Loading

Particulate matter (PM) loading was analyzed through particle gravimetric analysis. A 0.02% solution of heptamethyltrisiloxane (>98.0%, Tokyo Chemical Industry, Portland, OR) was used as a surfactant [10] to completely remove the particulate matter from each filter. Foliage samples were transferred to a flask after rinsing the collection bottles with distilled water which was also added to the flask. Distilled water (125 mL) was added to each flask containing 95 µL of heptamethyltrisiloxane to prepare 0.02% surfactant solution. Flasks were stoppered and placed in the refrigerator and soak for 24 hours. After the 24-hour soaking, flasks were placed on a reciprocating shaker (Eberbach Corp, Arbor, MI) at 200 rpm and kept in operation for 30 minutes. Leaf samples were sprayed vigorously on all sides and removed from the flask allowing the distilled water to be collected in the flask. Solution was then successively filtered using three different pore sizes of filter papers suited for gravimetric analysis. The collected solution was subjected to gravimetric analysis to determine the weight of the PM using 55-mm diameter hardened-ash less filter paper with three different pore diameters to separate particulate matter sizes of $PM_{2.5}$ (Whatman 42), PM_{8} (Whatman 40) and $PM_{>20}$ (Whatman 541). A digital microbalance (AB104-S Line Balance, Mettler Toledo LLC, Columbus, OH) was used to measure the initial and final weight of the filter paper after overnight drying of filter paper in the desiccator (Dry Keeper, C-Type, Frederick, MD).

Mean leaf surface area (cm²) was derived from five scanning operations using a leaf area meter (Model CI-202, CID Bio-Science Inc., Camas, WA). Particulate loading was the difference between the weights of the filter before and after filtration divided by the leaf surface area as shown in Equation 1.

$$W = \frac{(W_2 - W_1)}{A}$$
 (Equation 1)

Where:

 $W = dust loading (g/cm^2)$

 $W_{\gamma} = \text{final weight (g)}$

 $W_1^2 = initial weight (g)$

 $A = \text{total leaf area, cm}^2$



Figure 1: Laboratory procedures for the determination of particulate matter loading, particle counting, and stomatal characteristics analyses.

Particulate Matter Deposition

Leaf discs of 6 millimeter (mm) diameter were cut from each leaf samples using a sharp device (Single Hole Punch). Leaf discs was then transferred onto a microscope glass slide and carefully placed onto the base of acetone vaporizer (Perm-O-Fix, Atlanta Lab Systems, LLC) and subjected to a discharge



of 1 drop of acetone. The glass slide was warmed up for the acetone to dry up before Triacetin (EMSL Analytical Inc.,) was added. A drop of Triacetin was added to discs instead of performing leaf staining to avoid confusion on detection of particles deposition on the leaf surface. The filter clearing method was used to detect deposition of particles on internal parts of the leaf surface. Leaf particles deposition on the outer leaf surface was of secondary importance on this part of the analysis. Leaf discs were then set for 24 hours to facilitate leaf clearing prior to particle counting. A camera (MA 1000, Amscope, Irvine, CA) mounted to a compound microscope (T690-C, Amscope, Irvine, CA) was used to investigate the number of particles deposited on a given leaf surface area at 10 x 10 magnification.

Stomatal Imprints

Five leaf discs were cut and prepared from each plant species except for conifers where leaf fascicle was obtained from each needle for stomatal imprints. A base or top coat polish (Nail Treatment, Beauty 21 Cosmetics, Ontario, CA) was used as adhesive and solution to adhere to leaf surface for a maximum of 30 minutes to completely cover the entire area. After thorough drying, the hardened solution in a form of a film was carefully peeled and transferred to an adhesive tape with the abaxial surface facing the sticky side of the tape prior to mounting on a microscope glass slide cover. The imprints were subjected to observations using the compound microscope at 40 x 10 for stomatal density (SD). The stomatal pore surface (SPS) (µm2) was measured using the length (L) and width (W) values, assuming an elliptical shape of the pore [18]. The number of stomata was counted in a given microscopic field area of the leaf to calculate stomatal density.

Statistical Analysis

All data were analyzed using the SAS statistical software (Version 9.2 (32) Cary, NC). Significant difference on particulate matter loading (g/cm²), dust deposition on leaf cellular surfaces and stomatal characteristics were determined through one-way analysis of variance. Test of least significant difference was performed when the F-test was significant (P < 0.05). Data was pooled after a significant ANOVA with Tukey HSD procedure to compare difference on means.

Results and Discussion

Dust loading varied significantly among each species (Table 1). At PM₂₀ sizes, a significant statistical difference (p<0.0001) existed. Highest particulate matter loading was observed in Yaupon and Arborvitae plants (0.00025 g/cm²) followed by Eastern red cedar (0.00012 g/cm²), Arizona cypress (0.00002 g/cm²), and American holly (0.00001 g/cm²). At PM_o sizes, no significant difference on particulate matter was observed (p=0.0516). Yet on PM_{2.5} sizes, highest dust loading (0.00036 g/ cm²) was also observed in Arborvitae plants but fewer particles retained inside the leaf cuticle (Figure 2) and was significantly different (p=0.0002) from the other four plant species. Total

surface area of conifer foliage was higher than deciduous plants [2], therefore has more potential to adsorb dust on leaf surface which was consistently observed in Arborvitae from PM_{2.5} to PM_{20} than other conifers used in the study. Adrizal et al. [10] identified conifers (e.g. Norway spruce) to significantly hold more mass of PM10 due to needle arrangement. Khan & Abbasi [19] reported that smaller leaves are efficient particle collectors than larger leaves supporting the higher amount of dust (e.g. PM₈, PM₂₀) that accumulated on Yaupon plants.

Table 1: Mean values of particulate matter loading of three particles sizes on five plant species quantified using gravimetric analysis. Different superscripts indicate significant differences among the means at the 0.05 level.

Plant	Particulate Matter Loading (g/cm²)			
species	PM _{2.5}	PM_8	PM_{20}	
American holly	0.00009 ^b	0.00005 ^b	0.00001 ^b	
Arborvitae	0.00036a	0.00026a	0.00025a	
Arizona cypress	0.00017 ^b	0.00012ab	0.00002 ^b	
Eastern red cedar	0.00007 ^b	0.00007 ^{ab}	0.00012ab	
Yaupon	0.00006 ^b	0.00014ab	0.00025a	

Table 2 shows particulate matter deposition through particle counting using a microscope at 1000x magnification which yielded a significant statistical results among all five species at particulate sizes of PM_{<2.5} (p=0.0211) and PM_{>5,<10} (p=0.0173). No significant statistical difference was observed in PM_{>2.5,5} (p=0.4710) and $PM_{>10}$ (p=0.1307) sizes. At $< PM_{10}$ size division, the number of particles counted in Arizona cypress (Figure 3a) (160.0 PM<10/mm²) was significantly higher compared to Eastern red cedar (95.3 PM<10/mm²) and American holly $(64.3 \text{ PM}_{<10}/\text{mm}^2)$, Arborvitae $(34.7 \text{ PM}_{<10}/\text{mm}^2)$ and Yaupon (23.1 PM_{<10}/mm²). Arizona cypress, American holly and Eastern red cedar trapped more particles from PM_{<5} to PM_{<10} than other plant species. This result was supported by the findings of Joshi & Bora [20] in which they reported that identifying leaf characteristics, particularly the waxy coating and rough surface, result in more dust accumulation on leaf surfaces. At PM_{<2.5} size fraction, Yaupon plants (176.0 PM_{<2.5}/mm²) accumulated more particulate matter (Figure 4a) followed by American holly (51.8 PM_{<2.5}/mm²), Eastern red cedar (45.4 PM_{<2.5}/mm²), Arizona cypress (32.5 PM_{2.5}/mm²) and Arborvitae (14.5 PM_{2.5}/ mm²), respectively. Large-leaved species are less effective barriers against finer dust which can travel greater distances [21]. As opposed to prior findings, this can explain Yaupon plants quantified with most number of particles of sizes greater than PM_{2.5} but less than PM₅.

Table 2: Mean values of four particles sizes of particulate matter (+ s.d.) quantified through microscope optical counting.



Different superscripts indicate significant differences among the means at the 0.05 level.

Plant species	Number of Particles in a leaf area (particles/mm²)				
	PM _{<2.5}	PM _{<5}	PM _{<10}	PM _{<10}	
American holly	51.8 ± 54.5 ab	127.0 ± 118.0 a	64.3 ± 78.8 ab	7.7 ± 8.7 a	
Arborvitae	14.5 ± 18.9 b	29.9 ± 7.2 a	34.7 ± 29.3 ^b	1.9 ± 2.6 a	
Arizona cypress	32.5 ± 36.8 b	156 ± 129.0 a	160.0 ± 99.8 a	19.2 ± 32.1 a	
Eastern red cedar	$45.4 \pm 98.8~^{\mathrm{ab}}$	41.6 ± 42.4 a	95.3 ± 53.0 ab	18.5 ± 25.3 a	
Yaupon	176.0 ± 110.3 a	149.0 ± 114.0 a	23.1 ± 23.8 b	3.8 ± 6.3 a	

There was a significant statistical difference in stomatal density among five plants (Table 3). Yaupon's frequency of stomata (Figure 4b) was significantly higher compared to the four other plants (p=0.0001). The number of closed and open stomata in Arizona cypress (Figure 3b) was also statistically different from the species (p<0.0001). All stomata were observed closed upon inspection in the microscope. Stomata that were obstructed and covered with particulate matter were also identified as closed stomata. In Sinha & Singh [1], dust deposited on the leaf surface clogged stomata which may inhibit physiological functions of plants. Stomatal length (p=0.3969) and stomatal pore surface (p=0.1187) showed no significant statistical differences (Table 4).

Table 3: Stomatal characteristics (stomatal density (SD), closed stomata, open stomata) of five plant species as inspected through microscope optical counting. Different superscripts indicate significant differences among the means at the 0.05 level.

Plant species	Closed stomata	Particulate Matter Size			
		%	Open stomata	%	Total stomata
American holly	0.80 ^b	3.98	16.6 b	96.02	17.40 ь
Arborvitae	2.60 в	18.64	10.4 ^b	81.36	15.00 ь
Arizona cypress	12.20 a	100	0 a	100	12.20 ь
Eastern red cedar	0.80 ь	6.88	10.40 b	93.12	11.20 ь
Yaupon	5.80 b	18.71	22.60 ь	81.29	28.40 a

Table 4: Stomatal features (stomatal length (SL), stomatal pore surface (SPS)] of five plant species as inspected through microscope optical counting. Different superscripts indicate significant differences among the means at the 0.05 level.

Plant species	Stomatal features		
	Stomatal length	Stomatal Pore surface	
American holly	0.005 a	0.000016 a	
Arborvitae	0.019 a	0.000007 a	
Arizona cypress	0.009 a	0.000000 a	
Eastern red cedar	0.006 a	0.000017 a	
Yaupon	0.003 a	0.000003 a	

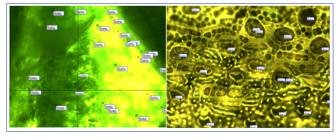


Figure 2: Dust deposited on intercellular surface of Arborviate upon microscope inspection at 10x magnification and stomatal characteristics at 40x magnification

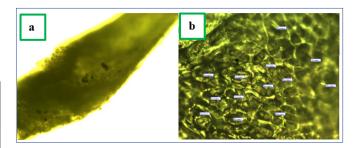


Figure 3: a) Dust deposited on Arizona cypress plants upon microscope inspection at 10x magnification and b) stomatal characteristics at 40x magnification.

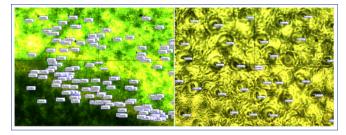


Figure 4: a) Dust deposited on Yaupon plants upon microscope inspection at 10x magnification and b) stomatal characteristics at 40x magnification.

Conclusions

This study compared the effectiveness of five different plant species in capturing particulate matter emissions from poultry buildings by measuring the particulate matter loading and stomatal characteristics. The following conclusions were drawn from this study.

- 1. The gravimetric analysis used to determine the particulate matter loading can be used in future studies on assessing the effectiveness of shelterbelts around animal facilities in trapping and controlling dust dispersion.
- Yaupon plants effectively trapped particulate matter larger than 20 µm, while Arborvitae was effective for both fine particulate matter ($PM_{2.5}$) and those larger than 20 μ m.
- Optical counting showed that Yaupon had the highest loading of particles up to 5 µm in diameter. Arizona cypress deposited significantly more particles up to 10 µm in diameter resulting in more blocked stomata.
- Stomatal length and stomatal pore surface did not significantly differ among the five plant species, which indicates that these stomatal characteristics were not adversely affected by direct exposure to dust from poultry emissions.

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Conflict of Interest

The authors have no conflict of financial interest.

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