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Heavy metal accumulation, chlorophyll content and stomatal density of shade plants in response to air pollution in Banda Aceh, Indonesia

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Abstract. *Zahara M, Arifin VN, Rachman F, Aryani DS. 2025. Heavy metal accumulation, chlorophyll content and stomatal density of shade plants in response to air pollution in Banda Aceh, Indonesia. Biodiversitas 26: 5449-5457.* There is increasing air pollution in Banda Aceh as the number of population and vehicles are growing. This study aimed to investigate the impacts of air pollution on shade plants by measuring heavy metals (i.e. Fe, Cu, Pb) accumulation, chlorophyll content and stomatal density of the leaves. Purposive sampling was conducted to collect leaves of plants exposed to two different levels of pollution, i.e. polluted plants along the busy streets in Jambo Tape Sub-district and less polluted plants (control) in Lamsayeun Village, Ingin Jaya Sub-district. There were five plant species that dominated the surveyed streets, i.e. *Tamarindus indica*, *Polyalthia longifolia*, *Pterocarpus indicus*, *Plumeria acuminata* and *Athrotaxis* sp. Leaf samples of the five dominant species were brought for laboratory analysis to measure heavy metal, chlorophyll content and stomatal density with three replicates taken from different individual plants of each species. Key findings included an increase in stomatal density in the polluted plants compared to the controls with the highest observed was in *P. longifolia* (807.82 mm^{-2}). Chlorophyll a, b, and total chlorophyll levels were generally lower in polluted plants compared to those as control. These findings suggest an adaptation mechanism to pollution. There was an elevated heavy metal level (Fe, Cu, Pb) in foliage, particularly in *Athrotaxis* plant leaves with Fe content reaching 247.2 mg/kg exceeding toxicity thresholds. The study confirmed a significant level of pollution in the area studied. These findings provide critical insights for selecting plant species in urban greening initiatives, with the potential to enhance pollution mitigation and support ecosystem health.

Keywords: Air pollution, bioindicators, chlorophyll content, foliar heavy metals, urban stress tolerance

Abbreviation: AAS: Atomic Absorption Spectroscopy, Chl: Chlorophyll, Cu: Copper, Fe: Iron, H_2O_2: Hydrogen Peroxide, HNO_3: Nitric acid, MDS: Microwave Digestion System, Pb: Lead, SNI: Indonesian National Standard

INTRODUCTION

Urban population is increasing due to the migration of people from rural areas to cities to look for a better job and improve life quality (Aslam et al. 2025). This condition leads to increasing number of motor vehicles (Nogimori 2020), leading to higher levels of air pollution. It is estimated that around 60% of global carbon emissions come from transportation activities in urban areas (Meutia et al. 2017; Cavicchioli et al. 2019), that consist of various harmful pollutants such as Carbon monoxide (CO), Nitrogen dioxide (NO_2), and fine particulate matter (PM2.5) (Khreis 2020; Greenbaum 2023; Meo et al. 2023).

Banda Aceh, a major city located in the Aceh Province of Indonesia, has experienced rapid urbanization and growth in recent decades (Achmad et al. 2014; Ramadhan and Idami 2020; Amri et al. 2023). In 2023, the population of Banda Aceh was 257,313 people (BPS 2023). The city expansion has led to significant changes in various aspects of urban life, including a growing number of motor vehicles, reaching approximately 322,410 units in 2023 (BPS 2023). As a result, the rise in vehicular traffic has also caused a significant increase in air pollution levels, primarily due to the emissions released by these vehicles. The air pollution in Banda Aceh can be observed through the Air Quality Index (AQI) website (www.aqi.in). The historical data showed that air quality index in Banda Aceh increased from 35 in 2022 to 46 in 2023, where the higher number index, the worse the air quality is, indicating a trend of increasing in air pollution. One suggested solution to reduce pollution is to implement a strategy that involves planting trees along the roadside, particularly on major roads that are heavily used by motorized vehicles. This particular approach is often known as Green Road Corridors (GRC) (Meutia et al. 2017; Deshmukh et al. 2019).

Beside air pollution, other negative effects of urban activities include the increase in air temperature, higher levels of noise, more dust particles, a rise in harmful substances, a decrease in humidity and the loss of habitat for various species of birds and wildlife due to the loss of vegetation and green spaces (Aktaş and Donmez 2019; Chiguvi and Thithe 2022; Sumasgutner et al. 2023). These harmful consequences have adverse impact on plant health, potentially causing physical damage and changes in plant processes, as well as alterations in the chemical composition of cells, which ultimately affect all parts of the plant, including the root system, stem structure and leaves (Pandit and Sharma 2020).

Pollutants can easily enter the plant leaves through stomata and dissolve in surface moisture, forming acids that harm leaf tissues. These chemical compounds trigger oxidative stress, leading to chlorophyll degradation, reduced photosynthesis, and changes in stomatal density (Taiz et al. 2023). In areas with low light intensity, plants thrive by developing larger leaves with larger stomata, producing fewer and thinner leaves with larger intercellular spaces. On the other hand, plants in high-light intensity areas have smaller leaves with increased thickness and smaller but more abundant stomata (Yahia et al. 2019; Sakoda et al. 2020). The environment in which plants grow greatly affects their morphological, physiological and anatomical characteristics (Taiz et al. 2023).

There are several studies which looking at the effect of air pollution on plants. Air pollution adversely affects plants, with SO_2 reducing growth and chlorophyll in *Calendula officinalis* in India (Gupta et al. 2022), and NO_x and SO_2 lowering biomass in bioindicator plant species in Brazil (Kateivas et al. 2022). Despite global studies on pollution-plant interactions, limited data exist on tropical shade plants' physiological adaptations to vehicular emissions, particularly in Southeast Asian cities like Banda Aceh. Therefore, it is essential to conduct detailed studies that focus on the morphology, anatomy (especially stomata), and physiology of shade plants located along Banda Aceh's main roads. The primary goals of this study are to (i) quantify heavy metal uptake in roadside plants, (ii) assess pollution-induced changes in chlorophyll and stomatal density, and (iii) evaluate species-specific tolerance for urban greening. This study provides evidence-based recommendations for selecting tolerant roadside plants to support sustainable urban management in Banda Aceh.

MATERIALS AND METHODS

Study site and period

This study was carried out in Banda Aceh City from March to May 2023. The collection of plant materials exposed to air pollution took place in Jambo Tape Sub-district, from T. Hasan Dek to Teuku Moh Daud Beureueh streets with length of approximately 2 km, specifically along the roadsides (Figure 1). For comparison, we also collected plant materials less exposed to air pollution treated as control which were taken from a village with relatively few vehicles, located in Lamsayeun village, Ingin Jaya Sub-district. This area experienced good air quality in 2023, with an Air Quality Index (AQI) ranging from 26 to 29, indicating clean and pollution-free air.

Sampling procedure

This study used the explorative survey method with purposive sampling (Daud et al. 2019; Intan et al. 2023). Identification of shade plants growing alongside the surveyed streets was carried out which resulted in five species of dominant plants being selected for observation. Leaf samples from three plants (i.e. replicates) of each selected species were collected either by hand or using a pole, for both control and sample plants. The leave samples selected were those determined to be most directly exposed to vehicles, assuming these would be the most polluted. The identification and enumeration of the shade plants in the designated research area were manually executed using a hand tally counter. While the air pollution information was observed through the Air Quality (AQI) website (www.aqi.in). Plant sampling complied with local regulations and institutional guidelines.

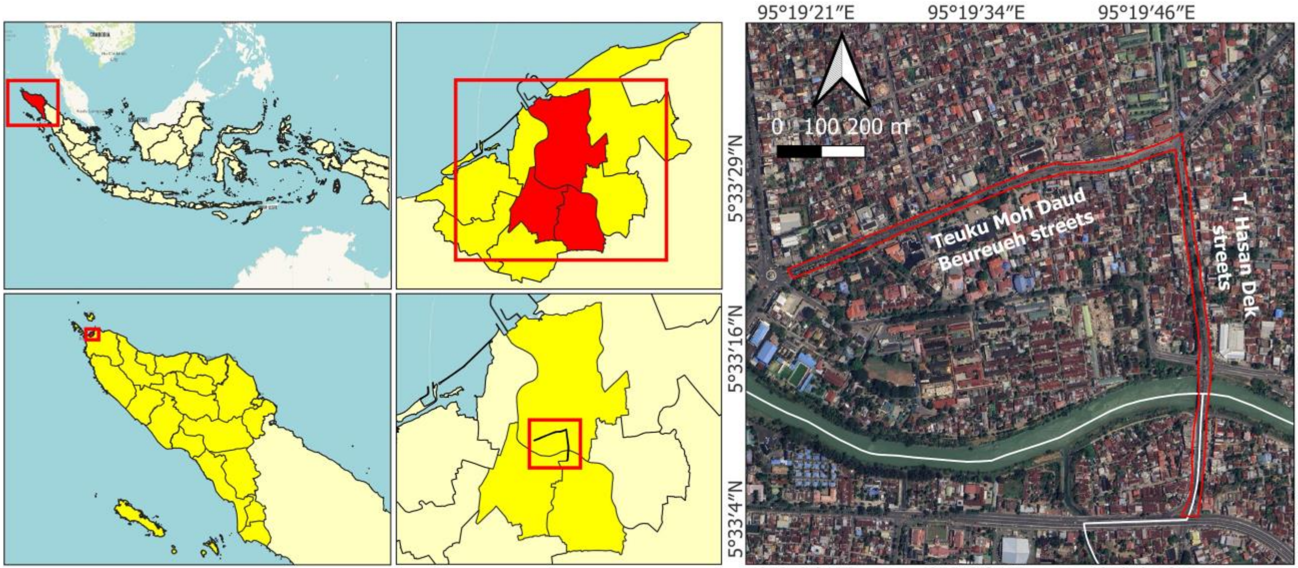

Figure 1. Map of study area along T. Hasan Dek and Teuku Moh Daud Beureueh streets, Banda Aceh City, Aceh Province, Indonesia

Heavy metals absorption analysis

Sample preparation

This study used 2 g of the plant sample, which was placed into a microwave digestion vessel with the addition of a mixture of 6 mL nitric acid (HNO_3) and 1 mL Hydrogen Peroxide (H_2O_2) prepared in a fume hood. The Microwave Digestion System (MDS) creates conditions with elevated pressure and temperature, resulting in rapid and complete digestion. These digested samples were placed into a 50 mL volumetric flask and diluted to the mark. The resulting solution was then analyzed using Atomic Absorption Spectroscopy (AAS) (Kucak and Blanusa 1998).

Atomic Absorption Spectroscopy (AAS)

A calibration blank and working solutions for Fe at 248.3 nm, Cu at 324.7 nm and Pb at 283.3 nm were prepared for accurate measurement. The aspirator tube was rinsed with a diluent solution prior to analysis. A high correlation coefficient ($r \geq 0.995$) was expected for the calibration curve, indicating linearity and suitability for quantitative analysis. The metal concentration of samples was calculated by interpolating their absorbance on the calibration curve (Suhaimi 2017). The analysis was performed using a PerkinElmer® Instrument, and the calibration was carried out using the manufacturer's standard. The method followed was based on MDS and the Indonesian National Standard SNI 6989-84:2019. Fe, Cu, and Pb were prioritized due to their prevalence in vehicular emissions and documented phytotoxicity (Briffa et al. 2020). The experiments were conducted in the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Aceh, Indonesia.

Chlorophyll content analysis

Chlorophyll concentration was measured using a spectrophotometer at wavelengths of 663 nm and 645 nm. The total chlorophyll content was calculated using Arnon's (1949) equations to account for proportions of chlorophyll a, to chlorophyll b. These equations are critical for quantifying the chlorophyll content accurately, which is essential for various biological and agricultural studies.

The chlorophyll content was estimated using Arnon's (1949) equations as follow:

Chlorophyll a (µg/mL) = 12.7 (A663)-2.69 (A645)

Chlorophyll b (µg/mL) = 22.9 (A645)-4.68 (A663)

Mg total chlorophyll tissue = 20.2(A645) + 8.02(A663) × V/100 × W

Where :

A	: Absorbance at a specific wavelength
V	: Final volume of chlorophyll extract
W	: Weight of fresh tissue extracted

Stomatal density analysis

The third leaf from the top was used for the calculation of stomatal density, three times from three different plants of each species. The selected plant leaves were those most exposed to vehicular emissions, and it is presumed that they were subjected to air pollution. To observe stomatal type, stomatal number, the stomatal density, and epidermal cells under the microscope the replica method was employed to prepare the slide for more detailed observation. The stomatal density (10× magnification) was calculated using the following equation by Salisbury (1927):

$$Stomatal\ density = \frac{number\ of\ stomata}{field\ of\ view\ under\ the\ microscope}$$

The number of stomata was also screened with 10× magnification, while the field of view was calculated with the same formula as with circle area formulas, where the field of view under microscope = $\pi \times r^2$. r = 0.25 mm^2 for 10× magnification (Lestari 2006; Zahara 2019). The analysis was conducted in the Biology Laboratory, Department of Biology, Faculty of Islamic Studies, Universitas Muhammadiyah Aceh, Banda Aceh, Indonesia.

Statistical analysis

The significant differences in stomatal density and chlorophyll content between polluted and control plants were analyzed using independent-samples *t*-test for each species. Normality and homogeneity of variance were checked and met the assumptions for *t*-test. Data are presented as mean±Standard Deviation (SD). Statistically significant was considered at $p<0.05$. All analyses were performed using SPSS. A one-way ANOVA was conducted for each metal to test for significant differences among the plant species. Following ANOVA, a Tukey HSD post-hoc test was applied to identify which species differed significantly from each other.

RESULTS AND DISCUSSION

Species identification

Within the study area, a wide variety of plants were identified, and these plants exhibit a diverse range of species from different families (Table 1). Among the plants primarily cultivated as shades along the road, there were 173 individuals of *Tamarindus indica*, 133 individuals of *Athrotaxis* sp., 90 individuals of *Pterocarpus indicus*, 24 individuals of *Polyalthia longifolia* and 23 individuals of *Plumeria* sp. These five plants, which were the most planted, functioned as representatives for the other shade plants for the assessment of chlorophyll content, stomatal characteristics, and observations of heavy metals.

Tamarindus indica, belonging to the Fabaceae family, displays a wide range of uses. The presence of tamarind trees not only provides a natural habitat for avian species but also serve as a source of tamarind fruits, which have been utilized both in their raw form and as well as being processed into tangy treats and drinks (Bayoï and Bianra 2023). Moreover, these tall trees have a wide canopy, creating a calming and moderate atmosphere that brings a cooling effect to the surroundings (Sarwadi et al. 2019).

Table 1. Shade plant species along T. Hasan Dek and Teuku Moh. Daud Beureueh streets, Banda Aceh, Indonesia

Scientific name	Local name	Family	Number
Tamarindus indica	*Asam Jawa*	Fabaceae	173
Athrotaxis sp.	*Cemara*	Cupressaceae	133
Pterocarpus indicus	*Angsana*	Fabaceae	90
Polyalthia longifolia	*Glodokan*	Annonaceae	24
Plumeria acuminata	*Kamboja*	Apocynaceae	23
Roystonea regia	*Palem raja*	Arecaceae	20
Ficus benjamina	*Beringin*	Moraceae	9
Swietenia macrophylla	*Mahoni*	Meliaceae	8
Samanea saman	*Trembesi*	Fabaceae	8
Handroanthus chrysotrichus	*Tabebuya*	Handroanthus	5
Terminalia mantaly	*Ketapang*	Combretaceae	4
Bougainvillea sp.	*Bunga kertas*	Nyctaginaceae	3
Azadirachta indica	*Mimba*	Meliaceae	2
Ficus benjamina	*Beringin putih*	Moraceae	2
Muntingia calabura	*Kersen*	Muntingiaceae	1
Mimusops elengi	*Tanjung*	Sapotaceae	1
Spathodea campanulata	*Tulip Afrika*	Bignoniaceae	1

Athrotaxis sp. a type of evergreen native to Tasmania, has the remarkable ability to thrive in low sunlight, making it ideal for planting alongside shaded roads. Additionally, *Athrotaxis* sp. can reproduce vegetatively through submerged stems and root suckers, sometimes forming clonal patches. This capacity for spreading and covering large areas helps expand shaded regions and enhances roadside environments, making it an excellent choice for roadside plantings (Cullen and Kirkpatrick 1998).

Pterocarpus indicus, indigenous to tropical and temperate Asia, is found in various regions in the world (Lee et al. 2023). This plant, belonging to the Leguminosae family, is cultivated along highways, pathways, parks, and in villages for ornamental and shading purposes. Their popularity for planting along roadsides can be attributed to an exceptional adaptability and rapid growth in moist conditions. Originating from Southeast Asia, this tree is often selected for urban planting due to its visual appeal and shade-providing capabilities. The distinctive characteristics of *P. indicus,* include its wide canopy and expansive branches, making it an outstanding provider of shade (Samsuri et al. in 2023).

Street trees provide numerous benefits to pedestrians, particularly on small-scale meteorological level, depending on the specific type of tree, amount of canopy coverage, and characteristics of the street canyon. The microclimate advantages that come from having tree canopy coverage can be influenced by various attributes or characteristics of the trees (de Abreu-Harbich et al. 2015; Sanusi et al. 2016; Sharmin et al. 2023). Different species of trees can cause different changes to the microclimate and utilize various shading strategies due to differences in leaf size, orientation, transpiration rates, and overall canopy structure (Sanusi et al. 2016).

Heavy metals absorption

Air pollution is one of the major negative outcomes associated with increasing urbanization (Ukaogo et al. 2020; Shimod et al. 2022). The research conducted by Briffa et al. (2020) and Cui et al. (2024) clearly showed that heavy metals in the atmosphere act as harmful pollutants, posing significant risks to the environment and human well-being. Among the various heavy metals considered pollutants, Cadmium (Cd), Zinc (Zn), Mercury (Hg), Arsenic (As), Silver (Ag), Chromium (Cr), Copper (Cu), Iron (Fe), and Lead (Pb) are particularly noteworthy for the risks they pose to human and environmental health. For this study, we focused exclusively on measuring the adsorption of three metals: Copper (Cu), Iron (Fe), and Lead (Pb).

Plants can absorb and accumulate pollutants through three different processes. Firstly, stomatal uptake occurs when gaseous pollutants enter the leaf through stomata, thus disrupting photosynthesis and causing oxidative stress. Surface adsorption involves the deposition of particulate matter (PM) and heavy metals on leaf surfaces, where some particles stick to the cuticle while others may be washed away by rain. Finally, root absorption happens when pollutants or heavy metals from contaminated soil and water are taken up by roots and transported through the xylem, accumulating in different plant tissues (Page and Feller 2015). Research has shown that vegetables and fruit plants can accumulate heavy metals because of their specific structures for uptake (Edelstein and Ben-Hur 2018).

Table 2 shows the specific metallic elements found in the leaves of five shade-plants along the surveyed roads in Banda Aceh City. Significant differences ($p<0.05$) were observed in the concentrations of Fe, Cu, and Pb among the studied plant species. *Athrotaxis* sp. exhibited the highest Fe content (247.21 µg/g), followed by *P. acuminata* (176.70 µg/g) and *P. longifolia* (158.70 µg/g), whereas *T. indica* (118.31 µg/g) and *P. indicus* (124.70 µg/g) showed the lowest levels. Cu concentrations were greatest in *T. indica*, *P. longifolia*, and *P. indicus* (5.16-5.25 µg/g), with no significant differences among them, while *Athrotaxis* sp. (3.96 µg/g) and *P. acuminata* (3.41 µg/g) contained lower levels. Pb content was highest in *P. acuminata* (4.43 µg/g) and lowest in *P. indicus* (3.07 µg/g), with *T. indica*, *P. longifolia*, and *Athrotaxis* sp. exhibiting intermediate concentrations.

The presence of heavy metals in the leaves of shade plants is a great concern due to the potential health risks they present and their potential impact on the plants' growth. Heavy metals such as arsenic, cadmium, lead (Pb), and mercury can be absorbed by plants through their roots and leaves, thus exposing humans to these metals and negatively affecting the plants' metabolism and structure (Edelstein and Ben-Hur 2018; Sulaiman and Hamzah 2018; Angulo-Bejarano et al. 2021). Table 2 shows that all the shade plants had elevated levels of Fe, Cu, and Pb content in their leaves. The shade plant, *Athrotaxis* sp., specifically showed the highest Fe content at 247.20±0.30 mg/kg (which exceeded the threshold of 100 mg/kg in plants) (Harish et al. 2023). The iron concentration in plant leaves, expressed on a dry weight basis, ranged from approximately 50-150 mg/kg (Amorós et al. 2018). Fe and Cu are micronutrients that play a vital role in plant metabolism. However, these micronutrients can become toxic when it presents beyond the optimal levels (Tripathi et al. 2015).

Table 2. The average of heavy metal absorption in the leaves of shade plants along the surveyed streets in Banda Aceh, Indonesia

Shade plant species	**Heavy metals absorption in leaves (mg/kg)**		
	Iron (Fe)	**Copper (Cu)**	**Lead (Pb)**
Tamarindus indica	118.30±0.19 d	5.20±0.13 a	3.44±0.19 b
Polyalthia longifolia	158.70±1.12 c	5.24±0.15 a	3.26±0.25 d
Pterocarpus indicus	124.70±0.18 d	5.16±0.02 a	3.07±0.19 c
Plumeria acuminata	176.70±0.14 b	3.40±0.19 c	4.43±0.04 a
Athrotaxis sp.	247.20±0.30 a	3.96±0.08 b	3.54±0.12 b

Note: Data are means±standard error of 3 replicates. Means with the same letter are not significantly different, while means with different letters are significantly different at $p<0.05$

The presence of Fe toxicity in plants is characterized by the development of yellow, orange, or reddish-brown foliage, a result of the oxidation of beta-carotene by iron, and the subsequent decrease in beta-carotene levels (Stutz et al. 2015; Hamzah et al. 2024). The concentration of Copper (Cu) in plant tissue shows a similar trend. Table 2 provides evidence that all Cu uptake in shade plants was very high. Cu is a vital metal for plants and plays a crucial role in various physiological functions such as photosynthesis and respiration. The available Cu can have significant effects on important processes in plant metabolism. However, human activities often contribute to the contamination of the environment, leading to the release of substantial amounts of Cu. When Cu is present in excess, it can have toxic properties that are harmful to plant life (Yruela 2009).

The result showed significant amount of lead absorption by the leaves of plants located in the shade (Table 2). The harmful effects of lead poisoning in plants are diverse and include hindering the normal growth and development of plants, causing chlorosis, blackening of the root system, suppressing photosynthesis, disrupting membrane structure, affecting enzyme activity, disturbing water balance, and interfering with mineral nutrition. Moreover, lead (Pb) poisoning can also result in cellular death. Furthermore, the buildup of lead (Pb) can impede seed germination, slow down seedling growth and reduce the absorption and transportation of essential nutrients (Collin et al. 2022; Gupta et al. 2024). Additionally, lead (Pb) can disrupt plant metabolic processes, impede the development of spindles and cell walls, and hinder root growth (Collin et al. 2022). The extent of Pb toxicity depends on both the duration of exposure and the concentration of lead present (Nas and Ali 2018; Zulfiqar et al. 2019; Aslam et al. 2021).

Chlorophyll content

Primary productivity in plants is strongly regulated by the photosynthetic rate of leaves and the availability of essential resources, including water, nitrogen, and suitable temperature conditions (Jiang et al. 2017; Nowicka et al. 2018; Plett et al. 2020). The reduction in chlorophyll levels due to stress is directly related to a plant's physiological response to stressors. This phenomenon provides valuable insights into how vegetation reacts to changes in the environment (Kumar et al. 2006; Arellano et al. 2017).

The study findings show that the plants exposed to pollution experienced a significant decline in their chlorophyll concentrations (Chl a, Chl b, and Total Chl) levels compared to the unpolluted plants, as shown in Figure 2. Furthermore, the analysis of *T. indica* revealed a decrease of 13% in Chl a, a 15% decrease in Chl b, and a reduction in total Chl. When examining *P. longifolia*, it was discovered that there was 11.5% decrease in Chl a, a 13% decrease in Chl b, and a 10% reduction in total Chl. Similarly, for *P. indicus*, the study showed a decrease of 11% in Chl a, a 10% decrease in Chl b, and a 14% reduction in total Chl. Additionally, the examination of the leaves of *P. acuminata* and *Athrotaxis* sp indicated a significant amount of dust on both the upper and lower surfaces, which directly impacted the chlorophyll content, as shows in Figure 3, specifically, *P. acuminata* demonstrated a reduction of up to 28% in Chl a, an 18% decrease in Chl b, and a 25% decrease in total Chl. Similarly, *Athrotaxis* sp. exhibited a reduction of 27% in Chl a, a 30% decrease in Chl b, and a 25% decrease in total Chl.

Using independent samples t-tests, the analysis showed that CHL a and b responded differently to pollution. Significant reductions in CHL a were observed in *T. indica*, *P. indicus*, *P. acuminata*, and *Athrotaxis* sp., while *P. longifolia* showed no significant change. CHL b, however, decreased significantly in all five species, with the strongest effects in *P. acuminata* and *Athrotaxis* sp. Total CHL generally tended to be lower under polluted conditions, with a significant decrease only in *P. acuminata*, indicating its higher sensitivity. Overall, control plants consistently had higher CHL a, b, and total CHL, confirming that air pollution negatively affects photosynthetic capacity, with species-specific tolerance differences.

Previous research has confirmed that air pollution leads to a significant decrease in chlorophyll levels in shade plants, due to the absorption of heavy metals from the air. Pimple (2017) observed that exposure to air pollutants, especially those emitted by factories and cars, led to a decline in carotenoids, chlorophyll a, and chlorophyll b in various plant species. Air pollution affected the photosynthetic pigments and chlorophyll a/b content ratio on hardwood trees and shrubs (Ghafari et al. 2021). Additionally, the decrease in chlorophyll content was also observed in plant species such as *A. indica*, *Conocarpus erectus*, *Guiacum officinale*, and *Eucalyptus* sp., when planted in areas with heavy traffic, as noted by Iqbal et al. (2015). The decrease in photosynthesis and chlorophyll content can be attributed to the deposition of dust particles or the formation of crust on the leaf surface, as emphasized by Lin et al. (2021).

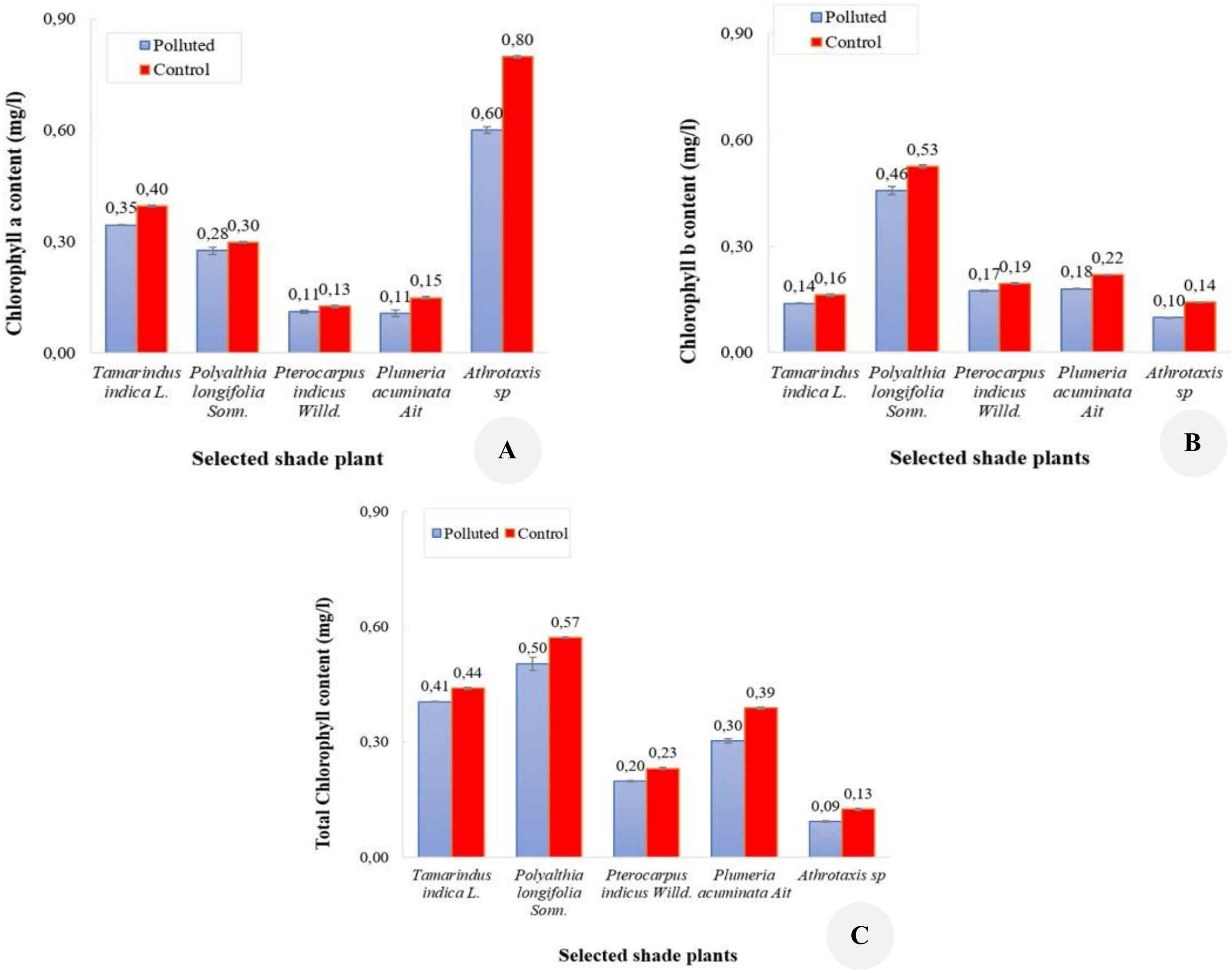

Figure 2. Average chlorophyll content of the leaves of plants exposed to different pollution levels in Banda Aceh City: A. Chlorophyll a, B. Chlorophyll b, and C. Total chlorophyll

Figure 3. The thickness of the dust on the leaf surface: A. *Plumeria acuminata*, and B. *Athrotaxis* sp.

Several other researchers have reported similar findings regarding the impact of air pollution on chlorophyll content concentration, as indicated by studies conducted by Dwivedi et al. (2020) and Roziaty et al. (2023). The standard ratio for the chlorophyll content is defined as follows; a high ratio is >1, a medium ratio is equal to 1, and a low ratio is <1 (Stansfield 1991). The study conducted by Giri et al. (2013) indicates that trees growing in industrial areas have a lower amount of chlorophyll. This decrease in chlorophyll content is primarily caused by the breakdown of chlorophyll into phaeophytin due to the loss of magnesium ions. The quantity of chlorophyll in these trees may vary over time, depending on factors such as pollution levels, weather conditions, and other factors that can affect chlorophyll content.

The heavy metals associated with air pollution can accelerate chlorophyll degradation by disrupting its biosynthesis and enhancing chlorophyllase activity, also inducing Reactive Oxygen Species (ROS) that damage the chloroplast and oxidize the chlorophyll, leading to reduced photosynthetic efficiency (Mehmood et at. 2004; Rao et al. 2025). A reduction in photosynthetic pigments is also found in some hardwood plants that are growing in polluted areas compared to non-polluted areas (Giri et al. 2013). Chlorophyll degradation (up to 28%) aligns with ROS-induced chloroplast damage (Rao et al. 2025), exacerbated by Cu/Pb/Fe toxicity.

Table 2. Stomatal density of the leaves of plants exposed to different pollution levels in Banda Aceh, Indonesia

Shade plants	**The average of stomatal density (mm^{-2})**			
	Adaxial		**Abaxial**	
	Polluted	**Control**	**Polluted**	**Control**
Tamarindus indica	290.81±0.03 a	180.26±0.01 b	601.85±0.32 a	298.53±0.05 b
Polyalthia longifolia	0±0.00 NS	0±0.00 NS	807.82±0.02 a	615.42±0.02 b
Pterocarpus indicus	34.013±0.09 a	13.605±0.15 b	625.85±0.03 a	326.53±0.03 b
Plumeria acuminata	0±0.00 NS	0±0.00 NS	224.48±0.04 a	150.122±0.01 b
Athrotaxis sp.	0±0.00 NS	0±0.00 NS	598.63±0.02 a	472.78±0.01 b

Note: Data are means±standard error of 3 replicates. Different letters indicate significant differences at $p<0.05$ (independent t-test). NS: Not Significant

Stomatal density

The results revealed that polluted plants exhibited higher stomatal density compared to the control (Table 2). Stomatal density on the adaxial leaf surface varied significantly between polluted and control plants for *T. indica* and *P. indicus*. The mean stomatal density of *T. indica* in polluted conditions was 290.82±0.04 mm^2, compared to 180.26±0.02 mm^2 in control plants ($p<0.001$). Similarly*, P. indicus* showed 34.02±0.09 mm^2 in polluted plants and 13.61±0.16 mm^2 in control plants ($p<0.01$). Other species (*P. longifolia, P. acuminata*, and *Athrotaxis* sp.) had zero stomatal density in both conditions, showing no significant differences (ns). While on the abaxial (lower) leaf surface varied significantly between polluted and control plants for all five species. *T. indica* showed a mean stomatal density of 601.75±0.30 mm^2 in polluted plants compared to 298.54±0.05 mm^2 in control plants ($p<0.001$). *P. longifolia* exhibited 807.83±0.02 mm^2 in polluted plants and 615.43±0.03 mm^2 in control plants ($p<0.001$). Similarly, *P. indicus, P. acuminata*, and *Athrotaxis* sp. all had significantly higher stomatal densities in polluted plants than in control plants ($p<0.05$ for each).

This increase in stomata is likely a protective measure to reduce transpiration and facilitate the absorption of heavy metals. Some plant species, such as *P. longifolia*, *P. acuminata*, and *Athrotaxis* sp., are classified as hypostomatic plants, having stomata exclusively on the lower (abaxial) surface of the leaves, as described by Richardson et al. (2017). This can be attributed to the impact of air pollution, which not only increases the stomatal density in the leaf but also affects the overall leaf structure. When plants are exposed to pollution and dust, they respond by increasing their stomatal density as a protective mechanism against excessive transpiration. This finding is consistent with a study conducted by Bhattacharjee et al. (2018), who provided an explanation for this plant response, that the significant increase in stomatal density in polluted plants in Assam, India, functions as a defense mechanism against cement particulates. Based on Qur'ania et al. (2024), stomatal density was classified as low (<300/mm^2), medium density (300-500/mm^2) and high density (>500/mm^2).

The density of stomata and the ratio of palisade parenchyma to spongy parenchyma in black locust (*Robinia pseudoacacia*) increased due to air pollution, as reported by Rashidi et al. (2012). According to Harrison et al. (2019), stomata play a crucial role in regulating the exchange of gases in and out of leaves. Lin et al. (2022) explain that studying stomata provides valuable insights into plant-environment interactions, including the impact of air pollution. Plants exhibit the ability to adjust their stomatal characteristics over different time scales. For example, Haworth et al. (2021) confirmed that they can regulate stomatal properties during leaf formation and in the short-term optimizing carbon dioxide and water vapor exchange.

Stomatal closure is induced by direct interaction of toxic metal pollution with the guard cells. It is a protective response that helps plants survive harsh conditions, such as drought or exposure to heavy metals. During metal stress, plants produce more Abscisic Acid (ABA) and give a signal to guard cells to close the stomata. This condition reduces water loss and limits the entry of harmful substances; however, closing the stomata also reduces CO$_2$ uptake, leading to lower photosynthesis and plant growth (Rucińska-Sobkowiak 2016; Taiz et al. 2023). An increase in stomatal density was evident in plants exposed to pollution, indicating an adaptive response to environmental stress. This research underscores the resilience of plants in urban environments despite challenges such as heavy metal pollution and reduced chlorophyll content. According to the results obtained, it is essential to observe and select the most suitable plants for cultivation alongside roads, as this allows for consideration of the potential effects on metal cycling and ecological balance.

In conclusion, *T. indica* exhibits the highest tolerance to air pollution. The polluted *T. indica* showed only a slight reduction in Chl levels, indicating sustained photosynthetic capacity despite pollutant exposure. It also showed high stomatal density on both leaf sides, indicating a strong mechanism to maintain gas exchange and transpiration under stress conditions. The presence of metals also remained within a moderate range compared to other species, reducing the risk of physiological damage. These characteristics suggest that *T. indica* is physiologically and anatomically more resilient to urban air pollution, making it a suitable candidate for urban roadside greening initiatives aimed at improving air quality and ecosystem health. The planting of this plant should be accompanied by regular monitoring of metal accumulation. Considering that the present study relies on single-season observations, future investigations should adopt multi-temporal sampling to capture potential annual variability and strengthen the generalizability of these findings. Implementing these recommendations can contribute to safer and more sustainable urban green spaces.

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