

Effect of Air Pollution on Plant Secondary Metabolites in Selected Trees of Delhi

Milinda Lahiri

University of Delhi

Kalyani Krishna

kalyani30jun@gmail.com

University of Delhi

Research Article

Keywords: Air Pollution, Air Quality Index (AQI), Plant Secondary Metabolites (PSMs), Urban Plantation

Posted Date: February 17th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2569925/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Environmental Quality Management on October 24th, 2023. See the published version at <https://doi.org/10.1002/tqem.22130>.

Abstract

Air pollution is emerging as one of the most important stresses, particularly in urban areas, of both developed and developing countries. It severely affects plant growth and development besides being a health hazard to humans and animals. Delhi has the dubious distinction of being one of the most polluted cities in the world. Incidentally, it is also one of the green cities with ongoing programs to increase the already considerable forest cover and biodiversity parks. Plants often act as sinks for airborne pollutants, including particulate matter. Hence it is important to evaluate their efficacy in absorbing pollutants and use the promising species for urban plantation. In the present study, the stress tolerance of four common trees of Delhi, growing in three areas with varying pollution levels, was estimated by measuring Plant Secondary Metabolites (PSMs) and the total chlorophyll content of the leaves.

Psidium guajava showed a significant increase in the contents of phenols, tannins, and alkaloids with increasing Air Quality Index (AQI). Increased amounts of phenols and flavonoids were observed in *Alstonia scholaris*, those of phenols and alkaloids in *Murraya koenigii*, and only of alkaloids in *Moringa oleifera*. There was a decrease in the total chlorophyll content of the leaves of all four species investigated. *P. guajava* and *A. scholaris* can be recommended for plantation along the sides of roads with heavy traffic movement. Since PSMs are seen to accumulate as a defense mechanism against air pollution too, as in various abiotic and biotic stresses, they can be used as biomarkers for stress tolerance in plants. Besides, higher commercial benefits can be accrued from the increased amounts of PSMs from the tree species investigated.

Introduction

Plants, owing to their sessile habit, are especially vulnerable to numerous abiotic and biotic stresses like temperature, salinity, freezing, thermal amplitude, solar radiation, drought, light (quantity and quality), rainfall, macro and microelements, flooding, metals, predators, etc. Among the environmental stresses, air pollution in toto is being increasingly investigated for its deleterious effects and possible adaptive strategies of plants.

Deteriorating air quality, especially in urban areas, is a severe stress faced by plants and animals alike (WHO, 2018). It seriously undermines the well-being of humans as well as that of the environment. Urban air pollutants are mainly particulate matter (PM), nitrogen oxides, sulfur dioxide, ozone, and carbon monoxide (CPCB, 2007). Delhi is one of the cities most affected by air pollution and was ranked 10th among the major cities with poor air quality globally, in the year 2020. Its high Air Quality Index (AQI) is attributed to vehicular exhaust which is the main contributor to pollutants like nitrogen dioxide, sulfur dioxide, and PM_{2.5}, the latter being additionally contributed by construction work (Sindhwani and Goyal, 2014).

Vegetation acts as a sink for air contaminants and is often severely damaged as a result (Ali, 1993). Chlorosis, necrosis, decreased photosynthesis, deblooming, etc. are some of the deleterious effects of air pollution on plants. Exposure to high quantities of PM for prolonged periods can cause morphological and physiological damage to plants (Agbaire and Esiefarienrhe, 2009). PM enters the plant tissue through stomata and causes partial denaturation of chloroplasts, hence decreasing photosynthetic pigment content in leaves (Rao and LeBlanc, 1966). Thus, leaves exposed to air pollution stress show that chloroplasts are the most severely affected organelles (Tripathi and Gautam, 2007). It is also observed that PM often clogs the stomatal pores, lowering carbon dioxide (CO₂) flux and thus reducing total photosynthetic activity (Rai, 2016).

Nitrogen Dioxide (NO₂) metabolizes directly in the leaves and forms organic nitrogenous compounds (Stulen et.al., 1998). At higher concentrations, it causes oxidative damage to biochemical and physiological processes in plants (Lu et.al., 2002). The level of NO₂ is generally 180 ppb in unpolluted areas (Sheng et.al., 2021). An increase in its concentration of more than 500 ppb generally has a negative effect on net photosynthesis (Freer-Smith and Taylor, 1992). Schiffgens-Gruber and Lutz (1992) found that high NO₂ causes swelling in thylakoids and a decrease in the number of grana stacks in chloroplasts. Interestingly, Caporn (1988) found that the negative effects of oxides of nitrogen on photosynthesis are often reversed by the addition of CO₂. Since CO₂ concentrations on the Earth are currently rising, resulting in climate change, the effects of NO_x may not be the cause of alarm yet.

SO₂, being extremely water-soluble, readily dissolves in mesophyll tissues to produce sulfite and bisulfite ions

which photo-oxidize to less toxic – SO₂–S (Asada and Kiso, 1973). This reaction involves the production of free radicals which damage cellular components like chlorophyll pigments (Shimazaki et.al., 1980) and inactivate various enzymes involved in chlorophyll synthesis. It has been reported that SO₂ in high concentrations can cause a reduction in chlorophyll content as it replaces the Mg²⁺ in the chlorophyll molecule with two hydrogen atoms, rendering it incapable of trapping sunlight (Rao and LeBlanc, 1966).

Ozone negatively affects various plant processes like photosynthesis, water use efficiency, dry matter production, and senescence (Krupa, 1997). The decrease in photosynthesis is because of reduced CO₂ flux (Unsworth and Black, 1981). Chernikova et al. (2000) have shown that prolonged exposure to O₃ causes chlorophyll loss, increased membrane permeability, decrease in seed yield, loss of total sulfhydryl groups, reduction of soluble protein content, and increase in guaiacol peroxidase activity, in some soybean cultivars.

In the absence of any reliable mechanical and chemical device which can control emissions at the source, plants alone are capable of absorbing the pollutants already released into the atmosphere (Rai, 2016). The importance of plants in the abatement of pollution and improving air quality by scavenging airborne particles and gases has been increasingly recognized in recent years. As plants are variable in their sensitivity and tolerance to air pollution, Air Pollution Tolerance Index (APTI) is recommended as a

measure of this variability (Singh et al., 1991). A gradation of sensitivity and tolerance can be used to determine the Anticipated Performance Index (API) of plant species and hence their suitability for urban plantations (Rai, 2016). It is of vital importance in the landscaping of areas that are susceptible to pollution.

Air pollution drastically alters the physiological processes of plants resulting in changes in carbon allocation and utilization of various resources (Agbaire and Esieffari, 2009). Plant Secondary metabolites (PSMs) have great significance in this regard as their levels often show marked increases under various stresses (Isah, 2019) at the expense of primary metabolism. PSMs are a large repertoire of molecules produced in low concentrations by living plant cells. They do not have a role in primary metabolism but are important in many aspects of plant life, especially in the alleviation of stresses, due to their antioxidant properties. Despite their being energetically expensive, the allocation of resources to produce PSMs is biochemically viable and beneficial (Austen et al., 2019). PSMs are divided into three categories: terpenes, phenolics (tannins and flavonoids included), and nitrogenous compounds (alkaloids and glycosides).

Extensive investigations have been carried out in recent decades on biochemical pathways, transcription factors, and effects of various abiotic and biotic stresses on PSM production, among others (reviewed by Khare, et al., 2020). However, changes in PSM levels in plants exposed to gaseous contaminants in the surrounding air are reported in far fewer studies.

Treatment with cadmium and copper (metals often found in PMs) reduces total phenolics, flavonoids, and saponin content in *Gynura procumbens* due to the inhibition of Phenylalanine Ammonia Lyase (PAL) activity (Ibrahim et al., 2017). Lead (a component of vehicle exhaust), on the other hand, causes an increase in PSM levels in *Phaseolus vulgaris* (Santisree et al., 2020).

Lavola (1998) reported that high SO₂ concentrations cause an increase in the accumulation of precursors of a few phenolic compounds, flavonoids, and condensed tannins in the needles and stem of *Betula*. It is suggested that plants that have sulfur-containing secondary metabolites reportedly utilize atmospheric SO₂ to produce them (Van der Kooij et al., 1997).

In controlled environmental conditions, O₃ and NO₂ cause the accumulation of iso-flavonoids in soybean, albeit a little lower with NO₂ (Keen and Taylor, 1975).

Some lichens (MacGillivray and Helleur, 2001) and stems of higher plants (Lavola et al., 1994) tend to accumulate phenolics with an increase in O₃ concentration. Studies also show that plants with low ascorbic acid content are more prone to the deleterious effects of O₃ (Conklin et al., 1996), while those with higher ascorbic acid content show considerable O₃ tolerance (Krupa et al., 2001). Incidentally, the exogenous application of ascorbic acid to leaves results in higher levels of phenols, flavonoids, and tannins in water-stressed common bean (Gaafar et al., 2020).

O₃ has no direct role in plant metabolism and plants tolerate it at low levels. Appropriate concentrations of NO₂ and SO₂ have useful metabolic roles in plants. High concentrations of gases in the atmosphere are harmful (Singh and Verma, 2007) and increase the pollution load.

NO at low concentration is actually a signaling molecule in plants and has diverse metabolic functions. It moderates osmolyte accumulation and metabolic reprogramming and increases plant adaptation to stress by inducing PSM synthesis. It is a positive or negative regulator of stress depending on local concentration (Santisree et al., 2020). CO in proper concentration helps in enhancing abiotic stress resistance through cross-talk with other signaling molecules, including NO (Wang and Liao, 2016).

Since the role of secondary metabolites in the adaptation of plants to air pollution has not been well documented, this study attempts to correlate the changes in the quantity of secondary metabolites in some selected tree species with ambient air pollution levels and to suggest the possible use of the tolerant species for urban ecosystem restoration, by using PSM levels as biomarkers for air pollution tolerance. Passive bio-monitoring was used to assess the effects of ambient pollution. The effects of individual components of air were not evaluated. Phytochemical analysis for various secondary metabolites and chlorophylls was carried out from leaves collected from trees growing in three areas with different pollution levels.

When pollutants are given singly or in combinations, using fumigation chambers under controlled conditions, it is found that the damage is synergistic if more than one pollutant was present in the air (Singh and Verma, 2007). Studies on the effects of stresses on plant growth and development are often carried out by maintaining the plants under natural or simulated conditions of stress. It is recommended that studies on defense compounds produced by plants in response to climatic stress and their bioactivity should be conducted using plant samples from natural habitats that are already challenged by various climatic stresses (Yeshe et al., 2022).

Materials And Methods

Area of Study: The city of Delhi lies in latitudes 28°24'17" and 28°53'00"N, and longitudes 77°45'30" and 77°21'30"E. The capital made by the Britishers in the colonial rule initially, for two lakh people, now harbors more than 3.5 crore people. With the ever-increasing population and industrialization, air pollution has increased tremendously. 72% of air pollution in Delhi is caused by vehicular emissions (Sindhwani and Goyal, 2014), while other sources include domestic waste, industrial emissions, and dust from construction sites (CPCB, 2007).

In the present study, three sites with varying pollution levels were identified, i.e. Pocket-4, Mayur Vihar, Phase-1 (High Pollution or **HP** site), Gurgaon Sector 49 (Moderate Pollution or **MP** site), and Sri Venkateswara College, Dhaula Kuan (Low Pollution or **LP** site). The location of these sites and their AQI are shown in Figure 1 and Table 1, respectively. Four tree species growing in these areas, namely *Psidium*

guajava, *Murraya koenigii*, *Alstonia scholaris*, and *Moringa oleifera* were chosen for the investigation of total phenols, total flavonoids, total tannins, total alkaloids, and chlorophyll content.

Table 1: AQI of the sample sites for the year 2020. Data represents the concentration of pollutants expressed as $\mu\text{g}/\text{m}^3$

Category	Prescribed Standard	LP	MP	HP
AQI	100	42	214	411
PM _{2.5}	60	23	214	411
PM ₁₀	100	42	89	325
NO ₂	80	10	28	50
SO ₂	80	18	20	49
O ₃	180	13	28	50
CO	4	40	53	136

Source: Delhi Pollution Control Committee (DPCC), Central Pollution Control Board (CPCB)

Sampling: Mature leaves from trees of *Psidium guajava*, *Murraya koenigii*, *Alstonia scholaris*, and *Moringa oleifera* from all three locations were collected in February 2021. It was ensured that all the trees selected had more or less similar conditions of light, water availability, and soil. Fresh leaves (harvested and stored at 4°C) were thoroughly washed with distilled water, blotted dry, and processed for analysis within two hours of collection for total phenols, total flavonoids, and total alkaloids or oven-dried overnight at 80°C for total tannins and chlorophyll estimation. All samples were processed in both biological and technical triplicates.

Quantitative Phytochemical Analysis

1. **TOTAL PHENOLIC COMPOUNDS:** Aqueous extract of 100 mg of leaf samples was made in 100 mL of double-distilled water (DDW). 1 mL of this solution was taken in a test tube along with 0.5 mL 2N of Folin-Ciocalteu reagent and 1.5 mL 20% of Na₂CO₃ solution and finally, the volume was made up to 8 mL with DDW, followed by vigorous shaking. The solutions were allowed to incubate in dark at room temperature for two hours followed by taking the absorbance at 765 nm. The data were used to estimate the total phenolic content by comparing it to a standard curve obtained from various dilutions of Gallic Acid. (Hagerman et al., 2000)
2. **TOTAL FLAVONOIDS:** Methanolic extract of the leaf samples (10 mg/mL) was made and 100 μL was taken in a test tube. 100 μL of 20% aluminium trichloride in methanol and a drop of acetic acid were added and the volume was made up to 5 mL with methanol. The samples were incubated at room temperature for forty minutes. The absorption was taken at 415 nm. Blank samples were made

from 100 µL of sample extract and a drop of acetic acid, diluted to 5 mL. (Kumaran and Karunakaran, 2006)

3. **TOTAL ALKALOIDS:** 5g of leaf samples were homogenized and incubated for four hours at room temperature in 200 mL of 10% acetic acid in ethanol. The solution was filtered, and the extract was concentrated in a water bath to one-fourth of the original volume. Concentrated ammonium hydroxide solution was added dropwise, and the solution was filtered to obtain the precipitate. After washing thrice with dilute ammonium hydroxide solution, the alkaloid residue was air-dried and weighed. (Harborne, 1973)
4. **TOTAL TANNINS:** 500 mg of homogenized samples were suspended in 50 mL of DDW in conical flasks. The samples were shaken at room temperature at medium speed for an hour in a mechanical shaker. The solution was then filtered and made up to 50 mL. To 5 mL of this filtrate, 2 mL of 0.1 N HCl and 0.008 M potassium ferrocyanide were added. The absorbance was measured at 605 nm within 10 minutes of addition. The total tannins were calculated as per the formula given by Van Buren and Robinson (1969).
5. **DETERMINATION OF TOTAL CHLOROPHYLL:** 0.5 g of leaf samples were ground in 10 mL of 80% acetone. The solution was filtered, and absorbance was measured at 662 and 644 nm. The values were used to calculate the Chlorophyll a and b content using the following equations (Asimovic et.al., 2016):

$$\text{Chlorophyll a (mg/mL)} = 9784 \times A_{662\text{nm}} - 990 \times A_{644\text{nm}}$$

$$\text{Chlorophyll b (mg/mL)} = 21426 \times A_{644\text{nm}} - 4650 \times A_{662\text{nm}}$$

Statistical Analysis: Data are expressed as mean $\pm$ standard error of the mean (SEM). Data were analyzed by ANOVA at the significant value of $p < 0.05$.

Results

In this study, quantitative phytochemical analysis of leaves of *Psidium guajava*, *Murraya koenigii*, *Alstonia scholaris*, and *Moringa oleifera* were conducted for total Phenols, Flavonoids, Alkaloids, and Tannins. In addition, the estimation of Chlorophyll a and b quantification was also carried out.

Total Phenols: The levels of total phenols estimated were higher in *P. guajava*, *M. koenigii*, and *A. scholaris* from polluted environments when compared to those at lower pollution levels. The results are shown in figure 2. However, the estimated total phenol content of leaves of *M. oleifera* from the HP site was less than that from the samples collected from less polluted sites.

Total Flavonoids: The levels of total Flavonoids estimated were lower in *P. guajava*, *M. koenigii*, and *M. oleifera* from heavily polluted environments when compared to those from lower pollution levels. The results are shown in figure 3. However, the estimated total flavonoid content of *A. scholaris* from the HP site was more than that of the samples collected from less polluted sites.

Total Tannins: The levels of total tannins estimated were lower in *M. koenigii*, *A. scholaris*, and *M. oleifera* from more polluted environments when compared to those at lower pollution levels. The results are shown in figure 4. However, the estimated total phenol content of *P. guajava* from the HP site was more than that of the samples collected from less polluted sites.

Total Alkaloids: The levels of total alkaloids estimated were higher in *P. guajava*, *M. koenigii*, and *M. oleifera* from polluted environments when compared to those from less polluted sites. The results are shown in figure 5. However, the estimated total alkaloid content of *A. scholaris* showed an opposite trend.

Total Chlorophyll: The levels of chlorophyll a and b were reduced in all four trees with increasing air pollution levels in varying degrees. The results are shown in figures 6 and 7. The amount of Chlorophyll-a estimated was less than that of chlorophyll b.

Among all four tree species selected, *P. guajava* had the highest amount of total phenols, flavonoids, and tannins, while *A. scholaris* had the highest amount of alkaloids. The highest Chlorophyll content was observed in *M. koenigii*. Additionally, the alkaloid content of *M. oleifera* was the lowest.

Upon perusal of the results, it is seen that:

- Total phenol content increased in *P. guajava*, *M. koenigii*, and *A. scholaris* with an increase in air pollution.
- Total flavonoid content, on the other hand, decreased in *P. guajava*, *M. koenigii*, and *M. oleifera* with increasing air pollution.
- *M. koenigii*, *A. scholaris*, and *M. oleifera* showed a decrease in total tannins with an increase in air pollution.
- Total alkaloid content, however, increased in *P. guajava*, *M. koenigii*, and *M. oleifera* with increasing air pollution.
- Chlorophyll a and b content in all trees selected decreased with an increase in AQI values

Discussion

Air in India's urban areas is heavily polluted. 10 of our cities are on the list of 15 most polluted cities in the world (World Air Quality Report, IQAir, 2022). Plants have been widely recognized as effective means of entrapping airborne pollutants. Thus, vegetation is an indicator of air pollution and is used to monitor air quality. Trees have tougher physiognomy and survive better in urban areas compared to lower plants (Rai, 2016). Hence it is imperative to understand how they react to pollutants in the atmosphere and develop adaptive strategies. In the present study, four common trees of Delhi were chosen to evaluate their tolerance to air pollution and consequently their suitability for plantation in the NCR region.

APTI is the most common indicator to measure stress tolerance and is calculated using four criteria namely, relative water content, ascorbic acid content, total protein, and pH of leaf extract (Singh et al., 1991). The suitability of the tree species to be used for urban greening is established by considering their APTI values along with other characteristics like habit, canopy structure, type of plant, laminar structure, and economic value (Rai and Panda, 2014). Higher APTI values indicate higher tolerance of the tree species to airborne pollutants. Trees have been recommended for plantation in big and small cities in India and abroad, based on APTI values (see review by Das et al., 2018). Identification of suitable plants as biomonitors and bioindicators has also been done in many areas of India and the world (reviewed by Rai, 2016).

It is interesting to note that the same species is suitable for plantation in one area while it may not be recommended for another area. *P. guajava* is suggested for green belts in Varanasi due to its high APTI value which does not differ much in control and polluted areas (Singh et al., 1991), while Agbaire and Esiefarienrhe (2009) found the tree unsuitable for planting near a gas plant in Nigeria, based on the considerable change in its APTI value in polluted areas compared to unpolluted conditions. It seems that interpretation of the evaluation criteria is important in recommending trees for planting. Some other reasons for the differential behavior of plants that affect their APTI scores are geographic location, environmental, and edaphic factors (Raza et al., 1985). Similarly, though trees with acicular leaves are more efficient in capturing PM_{2.5} (Chen et al., 2017) and have more APTI (Bui, H-T, 2021), trees with broad leaves only can be evaluated in plains due to the scarcity of the former. Also, the needle-like leaves of conifers differ in their ability to entrap PM_{2.5} and PM₁₀ and are also damaged by excessive capture of pollution after a short time (Chen et al., 2017), making it difficult to judge their suitability.

Among the APTI criteria, chlorophyll and ascorbic acid are the most significant and determining factors on which tolerance depends (Rai and Panda, 2014). Reduction in the amounts of chloroplast pigments is due to their extreme sensitivity to even small alterations resulting in several irreversible photochemical reactions like oxidation and reduction (Puckett et al., 1973), chloroplast damage (Pandey et al., 1991), and inhibition of chlorophyll biosynthesis (Ali, 1993). There is an overall degradation of chlorophylls, carotenoids, and ascorbic acid due to pollution (Chauhan and Joshi, 2008). Though a few studies have reported an increase in chlorophyll content under stress (Agbaire and Esiefarienrhe, 2009, see Rai, 2016), loss of chlorophyll seems to be a more likely effect of stress, especially in plants of the same age (Das et al., 2018). As high values of AQI are the immediate cause of chlorophyll degradation, chlorophyll estimation is an important tool for analyzing the effect of air pollution on plants (Rai, 2016). The results from the current study show that an increase in gases in the atmosphere of Delhi lowers the concentrations of chlorophylls in the leaves of all the species investigated.

Ascorbic acid levels are high under conditions of stress as they favor stress tolerance due to the active defense mechanism of plants while low levels indicate that it is consumed in the course of removal of cytotoxic free radicals (Vazquez-Leon et al., 2017). In *M. oleifera* leaves, ascorbic acid and PSMs were influenced by atmospheric humidity and radiation. Interestingly, while ascorbic acid content decreased with tree age, PSM levels remained unchanged (Vazquez-Leon et al., 2017). It was reported recently that

foliar spray with ascorbic acid actually causes an increase in the levels of PSMs in common bean plants under stress (Gaafar et al., 2020), thus making the antioxidant system of the plants more effective. Stress plays a crucial role in the activation of genes and signal transduction systems in the production of PSMs elevating their levels as a defense mechanism (Isah, 2019). Antioxidant protection against stress requires high amounts of carotenoids, ascorbic acid, alpha-tocopherol, glutathione, phenolics, and flavonoids that increase in quantity under stressful conditions, thus inhibiting the formation of reactive oxygen species and suppressing them (Krishnaveni, 2013). Since there are nearly 100,000 PSMs (Li et al., 2020), they provide a varied choice for accumulation under different stresses. Hence, the increase in their level has been used as a biomarker in the present investigation. Biochemical parameters are recommended as early indicators of pollution before visible symptoms set in (Tripathi and Gautam, 2007).

In the present study, *P. guajava* showed the highest level of increase as well as the highest values of three of the metabolites investigated, namely, phenols, tannins, and flavonoids, and hence appears to be most tolerant to ambient pollution. *A. scholaris* showed increased levels of phenols and flavonoids, apart from having the highest amount of alkaloids of the four species studied. This might confer higher resistance to pollution over *M. konegii*, which also showed an increase in phenols and alkaloids but the overall content and degree of increase were much lower. *M. oleifera* had appreciable levels of flavonoids and tannins, but their levels declined along with those of phenols. Though there was a small elevation in alkaloids over the initially low amount, the overall status of PSMs in relation to pollution makes *M. oleifera* a sensitive species.

Thus, *P. guajava* and *A. scholaris* appear to be suitable for planting in the sprawling urban conurbation of Delhi. Their habit, canopy, laminar structure, and economic value make them highly desirable for heavily populated and traffic-dense zones. *M. konegii* is suitable for residential areas and *M. oleifera* can be used as an indicator.

Since pollutants are in combination, the individual PSMs are seen to decrease or increase in relative abundance in a non-uniform and unpredictable manner in this study, as was also shown by Berini et al. (2018), in different environmental conditions (temperature, moisture, and light) in five woody species. According to Austen et al. (2019), change in net amounts of PSMs is specific to the kind of stress and in *Salix*, the flavonoid pathway is suppressed while the shikimate pathway is promoted, during stress. Nevertheless, elevation in PSM levels for free radical scavenging is inevitable in various stresses, even if short-lived (see review by Isah, 2019). It, therefore, appears that the influence of stress and defense responses associated with PSM production is multifaceted and it is worthwhile to identify species-specific and pollutant-specific markers. PSM pathways and their regulation are highly susceptible to environmental variation because the expression of genes involved in PSM synthesis is altered by stresses (Li et al., 2020). PSMs can, therefore, be sensitive and accurate markers. In the present study, one or more PSMs have shown marked changes in their quantities in four tree species in response to air pollution. They can be used as biochemical markers to quickly assess the pollution type and load and to employ possible mitigation strategies. Mukherjee et al. (2019) have recommended phenols, among others, as

markers for pollution tolerance in *Mangifera*. The strategy can be extended to other vegetation of Delhi to keep track of and control changing composition of the atmosphere.

An interesting outcome of detecting enhanced levels of PSMs in the trees used for this study is based on their economic value. Plants are living chemical factories for the biosynthesis of a large variety of PSMs (Li et al., 2020). They are important in a variety of industries like food, pharmaceuticals, agrochemicals, and cosmetics. 25% of drugs used in the world are plant-based (Isah, 2019). Understandably attempts to increase their contents to add nutraceutical quality to plants are being done by techniques ranging from agricultural practices (Luciano et al., 2017) to the analysis of gene expression (Sharma, 2018).

Khare et al. (2020), have summarized the information on the enhanced levels of active compounds in medicinal plants under various abiotic and biotic stresses. In the context of the present study, Negi (2018), has reported a significant increase in the medicinal compounds in *Portulaca* plants in response to pollutants from traffic. All four tree species used in this study have proven medicinal value, along with the food (*P. guajava*) and culinary (*M. konegii* and *M. oleifera*) uses. While *P. guajava* and *A. scholaris* are recommended primarily with the aim of planting in green belts for mitigating pollution, PSMs in larger amounts can be profitably extracted from them and also from the other two species to an extent.

In fact, the possibility of deliberate application of drought stress during the cultivation of spice and medicinal plants to enhance the content of secondary metabolites by carefully balancing the pros and cons with appropriate agricultural practices and the use of phytohormones to overcome the severe repercussions of stress on plant metabolism was suggested by Al-Gabbiesh et al. (2015). But here is a serious concern that even though PSMs increase in amount on gram/unit biomass basis under various stresses, there is a decline in total biomass as primary metabolism is adversely affected (Selmar and Kleinwachter, 2013), thereby offsetting the increase in PSMs. Very few studies have quantified the loss of biomass and the concomitant increase in PSMs on a whole-plant basis. But due to stress-induced over-reduction, highly reduced compounds (PSMs) are formed in the plant which might lead to a net increase in their quantity. In any case, since the species used in the present study are trees, the accompanying loss of biomass, if any, due to the enhanced levels of PSMs may not be a serious concern. On the other hand, higher concentrations of PSMs per unit weight of leaves may actually make the extraction process economical.

We think that trees with tolerance to pollution and possessing high quantities of PSMs can be planted by determining the target pollutant and target species using the elevated one or more PSMs as biomarkers to utilize the existing pollution to an advantage. Though selection of trees should be guarded by target pollution types (Chen et al, 2017), much more research is necessary as several factors influence a stress, making it a complex phenomenon requiring comprehensive elucidation

There is also the problem of accumulation of heavy metals from polluted air in economically important plants and the resultant harm to the health of humans who consume them (Radwan et al., 2018). The use of NEEs (non-essential elements) to increase Reactive Oxygen Species (ROS) and elicitors, leading to increased PSM levels has been tried in agriculture by Luciano et al. (2017), who recommended that the

elements should not be in high concentrations and should not go to the edible parts in order to prevent damage to human health. By keeping the PM in Delhi's air in limit and sequestering the heavy metals away from the leaves of the trees, it may be possible to take advantage of the increase in the levels of PSMs in the species used in this study.

Since natural products and medicinal plants are bound by legislation and societal regulations, wild plants and those with increased concentrations of PSMs (due to stress) can be used for drug discovery and the production of new compounds using tissue culture under a conditioned environment (Yeshe et al., 2022). Studies on the physiological and metabolic status of plants under stress also provide the rationale for application in metabolic engineering in the production of high-value and novel PSMs using sequencing techniques and other molecular approaches (Isah, 2019).

While it is clear that selecting trees using various parameters for greening urban spaces to combat anthropogenic sources of pollution has gained great importance in recent years, it is also necessary to select trees not only for traffic-dense, highly polluted areas but also for various other regions of metros, to have a discernible impact. In fact, many factors other than tree species selection should also be considered, viz. street design, wind velocity and direction, buildings, traffic movement (one-way or two-way), and spacing between trees, in conjunction with economic, social, and political policies strongly linked to nature (Chaudhuri and Kumar, 2022). Since most initiatives on amelioration of urban pollution have failed to deliver the desired outcomes so far, the pollution-related increased production of valuable PSMs may prove to be a temporary succor. In fact, plants that are being adversely affected by climate change may even be a rich source of discovery of novel drugs and bioactive compounds (Yeshe et al., 2022).

Conclusions

In the present study, upon evaluation of the secondary metabolite status of four trees under the influence of air pollution, it was observed that there were significant changes in the PSM content with respect to air quality. Hence PSMs were used as biomarkers for pollution tolerance based on which *P. guajava* and *A. scholaris* were recommended for planting in traffic and other emission-rich areas. *M. oleifera* can be used as an indicator of air pollution. The increase in PSMs under pollution stress can be gainfully utilized by extracting higher quantities of these chemicals from stressed trees. Moderate loss in biomass may not be a concern in the tree species investigated. The assimilation of heavy metals by experimental trees investigated needs to be assessed. Other economically important trees should be considered for studies on pollution tolerance. Selection of trees for targeted plantation for pollution type vis-a-vis kind of PSMs accumulated is desirable. Plantations should be done for different requirements in different areas of the cities.

Declarations

Funding: Not Applicable

Conflicts of Interest: The authors have no conflicts of interest.

Availability of Data and Material: All the data provided in this study has been generated by the authors unless mentioned otherwise.

Code Availability: Not Applicable

ACKNOWLEDGMENTS

The authors thank Prof. C. Sheela Reddy, Principal, Sri Venkateswara College for her support and the lab staff, Department of Botany, Sri Venkateswara College for technical assistance. Thanks are also due to Dr. Yogendra Kumar Gautam for helping with the statistical analysis.

AUTHOR CONTRIBUTIONS: Conceptualization and Methodology: M.L. and K.K.; Performance, Data Analysis: M.L.; Writing - original draft preparation: M.L.; Writing - Sections of the manuscript: M.L. and K.K.; Writing - review, editing, and approval of final version: K.K. Supervision: K.K.; Review of Literature: M.L. and K.K. All authors have read and agreed to the final version of the manuscript.

References

1. Agbaire, P. O., and Esiefarienrhe, E. (2009). Air Pollution Tolerance Indices (APTI) of some plants around Otorogun Gas Plant in Delta State, Nigeria. *Journal of Applied Sciences and Environmental Management*, 13. doi: 10.4314/jasem.v13i1.55251
2. Al-Gabbiesh, A., Kleinwächter, M., and Selmar, D. (2015). Influencing the contents of secondary metabolites in spice and medicinal plants by deliberately applying drought stress during their cultivation. *Jordan Journal of Biological Sciences*, 147.
3. Ali, E. A. (1993). Damage to plants due to industrial pollution and their use as bioindicators in Egypt. *Environmental Pollution*, 81. doi: 10.1016/0269-7491(93)90207-5
4. Asada, K., and Kiso, K. (1973). Initiation of aerobic oxidation of sulfite by illuminated spinach chloroplasts. *European Journal of Biochemistry*, 33. doi: 10.1111/j.1432-1033.1973.tb02677.x
5. Asimovic, Z., Cengic, L., Hodzic, J., and Murtic, S. (2016). Spectrophotometric determination of total Chlorophyll content in fresh vegetables. *Works of the Faculty of Agriculture and Food Sciences*, University of Sanjevo, Vol. LXII, No. 66/1.
6. Austen, N., Walker, H. J., Lake, J. A., Phoenix, G. K., and Cameron, D. D. (2019). The regulation of plant secondary metabolism in response to abiotic stress: interactions between heat shock and elevated CO₂. *Frontiers in Plant Science*, 10. Doi: 10.3389/fpls.2019.01463
7. Berini, J. L., Brockman, S. A., Hegeman, A. D., Reich, P. B., Muthukrishnan, R., Montgomery, R. A., and Forester, J. D. (2018). Combinations of abiotic factors differentially alter the production of plant secondary metabolites in five woody plant species in the boreal-temperate transition zone. *Frontiers in plant science*, 9. doi: 10.3389/fpls.2018.01257.

8. Bui, H. T., Odsuren, U., Kwon, K. J., Kim, S. Y., Yang, J. C., Jeong, N. R., and Park, B. J. (2021). Assessment of air pollution tolerance and particulate matter accumulation of 11 woody plant species. *Atmosphere*, 12. doi :10.3390/atmos12081067
9. Caporn, S. J. (1989). The effects of oxides of nitrogen and carbon dioxide enrichment on photosynthesis and growth of lettuce (*Lactuca sativa* L.). *New phytologist*, 111. doi: 10.1111/j.1469-8137.1989.tb00710.x
10. Central Pollution Control Board (CPCB). (2007). Report: The Air quality trends and action plan for control of air pollution from seventeen cities, CPCB – NAAQM Series (NAAQMS/29/2006-2007). New Delhi, India
11. Chaudhuri, S., and Kumar, A. (2022). Urban greenery for air pollution control: a meta-analysis of current practice, progress, and challenges. *Environmental Monitoring and Assessment*, 194. doi: 10.1007/s10661-022-09808-w
12. Chauhan, A., and Joshi, P. C. (2008). Effect of ambient air pollution on photosynthetic pigments on some selected trees in an urban area. *Ecology Environment and conservations*, 14.
13. Chen, L., Liu, C., Zhang, L., Zou, R., and Zhang, Z. (2017). Variation in tree species ability to capture and retain airborne fine particulate matter (PM_{2.5}). *Scientific reports*, 7. doi: 10.1038/s41598-017-03360-1
14. Chernikova, T., Robinson, J. M., Lee, E. H., and Mulchi, C. L. (2000). Ozone tolerance and antioxidant enzyme activity in soybean cultivars. *Photosynthesis Research*, 64. doi: 10.1023/A:1026500911237
15. Conklin, P. L., Williams, E. H., and Last, R. L. (1996). Environmental stress sensitivity of an ascorbic acid-deficient *Arabidopsis* mutant. *Proceedings of the National Academy of Sciences*, 93. doi: 10.1073/pnas.93.18.9970
16. Das, M., Das, M., and Mukherjee, A. (2018). Air pollution tolerance index (APTI) used for assessing air quality to alleviate climate change: A review. *Research Journal Of Pharmaceutical Biological And Chemical Sciences*, 9.
17. Freer-Smith, P.H., Taylor, G. (1992). Comparative evaluation of the effects of gaseous pollutants, acidic deposition, and mineral deficiencies on gas exchange of trees. *Agriculture, Ecosystems and Environment*. 42. doi: 10.1016/0167-8809(92)90007-X
18. Gaafar, A. A., Ali, S. I., El-Shawadfy, M. A., Salama, Z. A., Şekara, A., Ulrichs, C., and Abdelhamid, M. T. (2020). Ascorbic acid induces the increase of secondary metabolites, antioxidant activity, growth, and productivity of the common bean under water stress conditions. *Plants*, 9. doi: 10.3390/plants9050627.
19. Hagerman, A., Muller, I., and Makkar, H. (2000). Quantification of Tannins in tree foliage. *A laboratory manual*, Vienna: FAO/IAEA, Pp. 4-7.
20. Harborne, J.B. (1973). *Phytochemical methods: A guide to modern techniques and plant analysis* (Third Edition). Chapman A and Hall, London.
21. https://app.cpcbcr.com/AQI_India/
22. <https://www.iqair.com/in-en/world-air-quality-ranking>

23. Ibrahim, M. H., Chee Kong, Y., and Mohd Zain, N. A. (2017). Effect of cadmium and copper exposure on growth, secondary metabolites and antioxidant activity in the medicinal plant Sambung Nyawa (*Gynura procumbens* (Lour.) Merr). *Molecules*, 22. doi: 10.3390/molecules22101623
24. Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biological research*, 52. doi: 10.1186/s40659-019-0246-3.
25. Keen, N. T., and Taylor, O. C. (1975). Ozone injury in soybeans. *Plant Physiology*, 55.
26. Khare, S., Singh, N. B., Singh, A., Hussain, I., Niharika, K. M., Yadav, V., Bano, C., Yadav, R.K, and Amist, N. (2020). Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints. *Journal of Plant Biology*, 63. doi:10.1007/s12374-020-09245-7
27. Krishnaveni, M. (2013). Air pollution tolerance index and antioxidant activity of *Parthenium hysterophorus*. *Journal of Pharmacy Research*, 7. doi: 10.1016/j.jopr.2013.03.025
28. Krupa, S. V. (1997). *Air pollution, people, and plants: an introduction* (No. 04; QH545. A3, K7.). St. Paul, Minnesota, USA: APS Press.
29. Krupa, S., Mcgrath, M. T., Andersen, C. P., Booker, F. L., Burkey, K. O., Chappelka, A. H., Chevone, B. I., Pell, E. J., and Zilinskas, B. A. (2001). Ambient ozone and plant health. *Plant Disease*, 85.
30. Kumaran, A. and Karunakaran, R. J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chemistry*, 97. doi: 10.1016/j.foodchem.2005.03.032
31. Lavola, A. (1998). Soluble carbohydrates and secondary phytochemicals in *Betula* as affected by SO₂ pollution. *Water, Air, and Soil Pollution*, 107. doi: 10.1023/A:1004981625721
32. Lavola, A., Julkunen-Tiitto, R., and Pääkkönen, E. (1994). Does ozone stress change the primary or secondary metabolites of birch (*Betula pendula* Roth.)? *New Phytologist*, 126. doi: 10.1111/j.1469-8137.1994.tb02959.x
33. Li, Y., Kong, D., Fu, Y., Sussman, M. R., and Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*, 148. doi: 10.1016/j.plaphy.2020.01.006
34. Lu, M., Li, Y. J., and Lu, J. P. (2002). The study of greening trees on the atmospheric pollutant absorption ability. *Urban Environ. Urban Ecol*, 15.
35. Luciano, Á. J., Irineo, T. P., Virginia, O. V. R., Feregrino-Pérez, A. A., Hernández, A. C., and Gerardo, G. G. R. (2017). Integrating Plant Nutrients and Elicitors for Production of Secondary Metabolites, Sustainable Crop Production and Human Health: A Review. *International Journal of Agriculture and Biology*, 19. doi: 10.17957/IJAB/15.0297
36. MacGillivray, T., and Helleur, R. (2001). Analysis of lichens under environmental stress using TMAH thermochemolysis–gas chromatography. *Journal of Analytical and Applied Pyrolysis*, 58.
37. Mukherjee, S., Chakraborty, A., Mondal, S., Saha, S., Haque, A., and Paul, S. (2019). Assessment of common plant parameters as biomarkers of air pollution. *Environmental monitoring and assessment*, 191. doi: 10.1007/s10661-019-7540-y

38. Negi, S. (2018). Quantitative phytochemical analysis of *Portulaca oleracea* Linn. growing in an unpolluted and polluted area. *The Pharma Innovation*, 7.
39. Pandey, D.D., Sinha, C.S., and Tiwari, M.G. (1991). Impact of coal dust pollution on biomass, chlorophyll, and grain characteristics of rice. *Journal of Biology*, 3 (Online).
40. Puckett, K. J., Nieboer, E., Flora, W. P., and Richardson, D. H. S. (1973). Sulphur dioxide: its effect on photo-synthetic ¹⁴C fixation in lichens and suggested mechanisms of phytotoxicity. *New Phytologist*, 72. doi: 10.1111/j.1469-8137.1973.tb02019.x
41. Radwan, A.M., Reyad, N. F., Donia, A. E. R. M. and Ganaie, M.A. (2018). Comparative studies on the effect of environmental pollution on secondary metabolite contents and genotoxicity of two plants in the Asir area, Saudi Arabia. *Tropical Journal of Pharmaceutical Research*, 17. doi: 10.4314/tjpr.v17i8.19.
42. Rai, P. K. (2016). Impacts of particulate matter pollution on plants: Implications for environmental biomonitoring. *Ecotoxicology and environmental safety*, 129. doi: 10.1016/j.ecoenv.2016.03.012
43. Rai, P. K., and Panda, L. L. (2014). Dust capturing potential and air pollution tolerance index (APTI) of some road side tree vegetation in Aizawl, Mizoram, India: an Indo-Burma hot spot region. *Air Quality, Atmosphere and Health*, 7. doi: 10.1007/s11869-013-0217-8
44. Rao, D.N., and LeBlanc, F. (1996). Effects of SO₂ on the lichen algae with special reference to chlorophyll. *Bryologist*, 69.
45. Raza, S. N., Vijaykumari, N., and Murthy, M. S. (1985). Air pollution tolerance index of certain plants of Hyderabad. In Symp Biomonitoring State of Environment, New Delhi: *Indian National Science Academy* (pp. 243-245).
46. Santisree, P., Sanivarapu, H., Gundavarapu, S., Sharma, K. K., and Bhatnagar-Mathur, P. (2020). Nitric oxide as a signal in inducing secondary metabolites during plant stress. *Co-evolution of secondary metabolites*, 593-621. doi: 10.1007/978-3-319-96397-6_61.
47. Schiffgens-Gruber, A., and Lütz, C. (1992). Ultrastructure of mesophyll cell chloroplasts of spruce needles exposed to O₃, SO₂, and NO₂ alone and in combination. *Environmental and experimental botany*, 32. doi: 10.1016/0098-8472(92)90007-0
48. Selmar, D., and Kleinwächter, M. (2013). Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation of natural products. *Plant and Cell Physiology*, 54. doi: 10.1093/pcp/pct054
49. Sharma, A. (2018). Gene expression analysis in medicinal plants under abiotic stress conditions. In Plant metabolites and regulation under environmental stress (pp. 407-414). *Academic Press*.
50. Sheng, Q., Liu, C., Song, M., Xu, J., and Zhu, Z. (2021). Comprehensive Transcriptome Analysis of Rare *Carpinus putoensis* Plants under NO₂ stress. *Genes*, 12. doi: 10.3390/genes12050754
51. Shimazaki, K. I., Sakaki, T., Kondo, N., and Sugahara, K. (1980). Active oxygen participation in chlorophyll destruction and lipid peroxidation in SO₂-fumigated leaves of spinach. *Plant and Cell Physiology*, 21.

52. Sindhvani, R., and Goyal, P. (2014). Assessment of traffic-generated gaseous and particulate matter emissions and trends over Delhi (2000–2010). *Atmospheric pollution research*, 5. doi: 10.5094/APR.2014.051
53. Singh, S. K., Rao, D. N., Agrawal, M., Pandey, J., and Narayan, D. (1991). Air pollution tolerance index of plants. *Journal of Environmental Management*, 32.
54. Singh, S. N., and Verma, A. (2007). Phytoremediation of air pollutants: a review. *Environmental bioremediation technologies*. doi: 10.1007/978-3-540-34793-4_13
55. Stulen, I., Perez-Soba, M., De Kok, L. J., and Van der Eerden, L. (1998). Impact of gaseous nitrogen deposition on plant functioning. *New Phytologist*, 139. doi: 10.1046/j.1469-8137.1998.00179.x
56. Tripathi, A. K., and Gautam, M. (2007). Biochemical parameters of plants as indicators of air pollution. *Journal of environmental biology*, 28.
57. Unsworth M.H. and Black V.J. (1981) Stomatal responses to pollutants. In: Jarvis P.G. and Mansfield T.A. (Eds.) *Stomatal physiology* pp. 187-203 Cambridge University Press, London
58. Van Buren, J. P., and Robinson, W. B. (1969). Formation of complexes between protein and tannic acid. *Journal of Agricultural and Food Chemistry*, 17. doi: 10.1021/jf60164a003
59. Van der Kooij, T. A. W., De Kok, L. J., Haneklaus, S., and Schnug, E. (1997). Uptake and metabolism of sulphur dioxide by *Arabidopsis thaliana*. *The New Phytologist*, 135.
60. Vázquez-León, L. A., Páramo-Calderón, D. E., Robles-Olvera, V. J., Valdés-Rodríguez, O. A., Pérez-Vázquez, A., García-Alvarado, M. A., and Rodríguez-Jimenes, G. C. (2017). Variation in bioactive compounds and antiradical activity of *Moringa oleifera* leaves: influence of climatic factors, tree age, and soil parameters. *European Food Research and Technology*, 243. doi: 10.1007/s00217-017-2868-4
61. Wang, M., and Liao, W. (2016). Carbon monoxide as a signaling molecule in plants. *Frontiers in Plant Science*, 7. doi: 10.3389/fpls.2016.00572.
62. World Health Organization. (2018). 9 out of 10 people worldwide breathe polluted air, but more countries are taking action. <https://www.who.int/news/item/02-05-2018-9-out-of-10-people-worldwide-breathe-polluted-air-but-more-countries-are-taking-action>
63. Yeshi, K., Crayn, D., Ritmeijert, E., and Wangchuk, P. (2022). Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. *Molecules*, 27. doi: 10.3390/molecules27010313

Figures

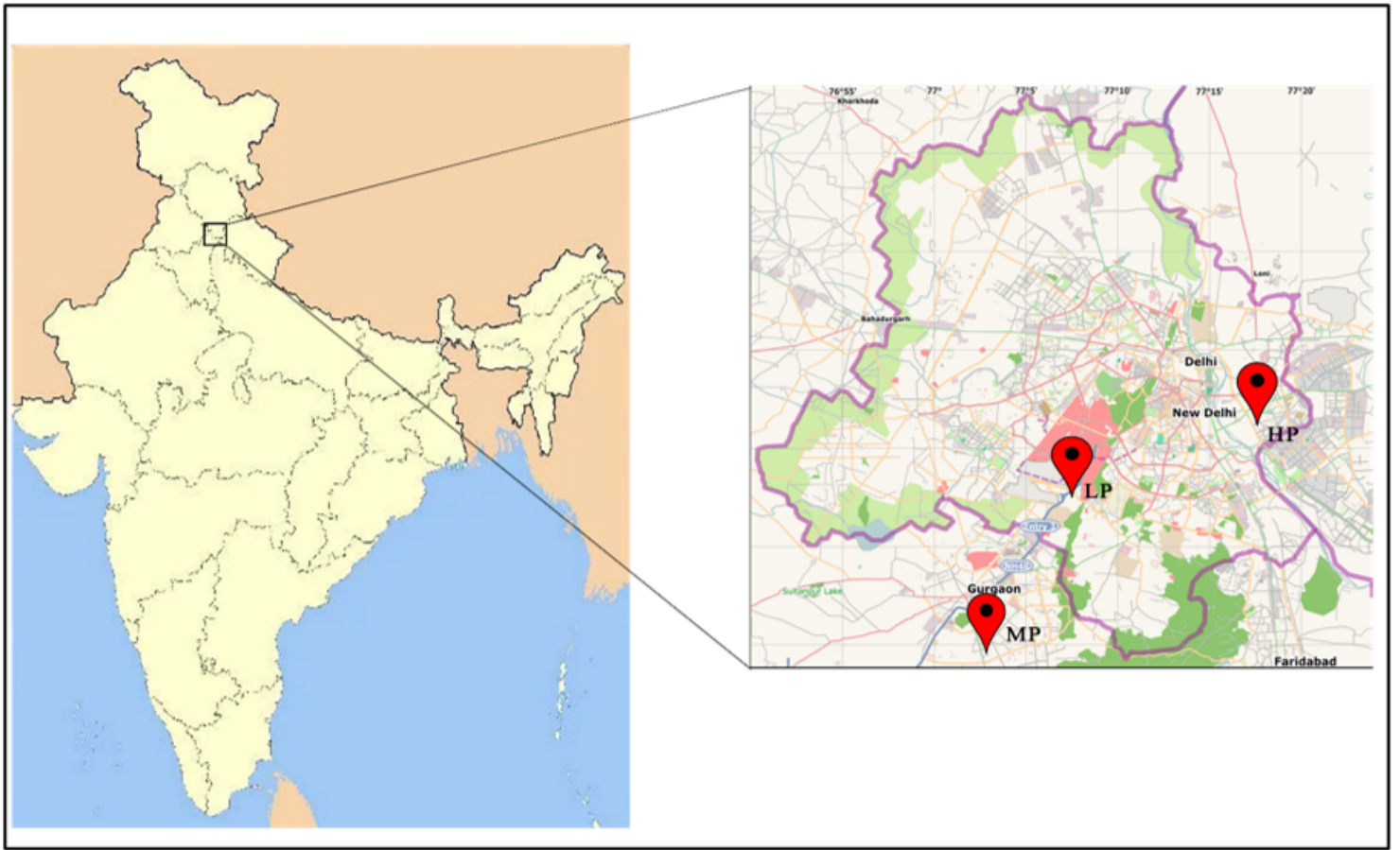


Figure 1

(left) Map of India and (right) Map of Delhi showing sites of investigation

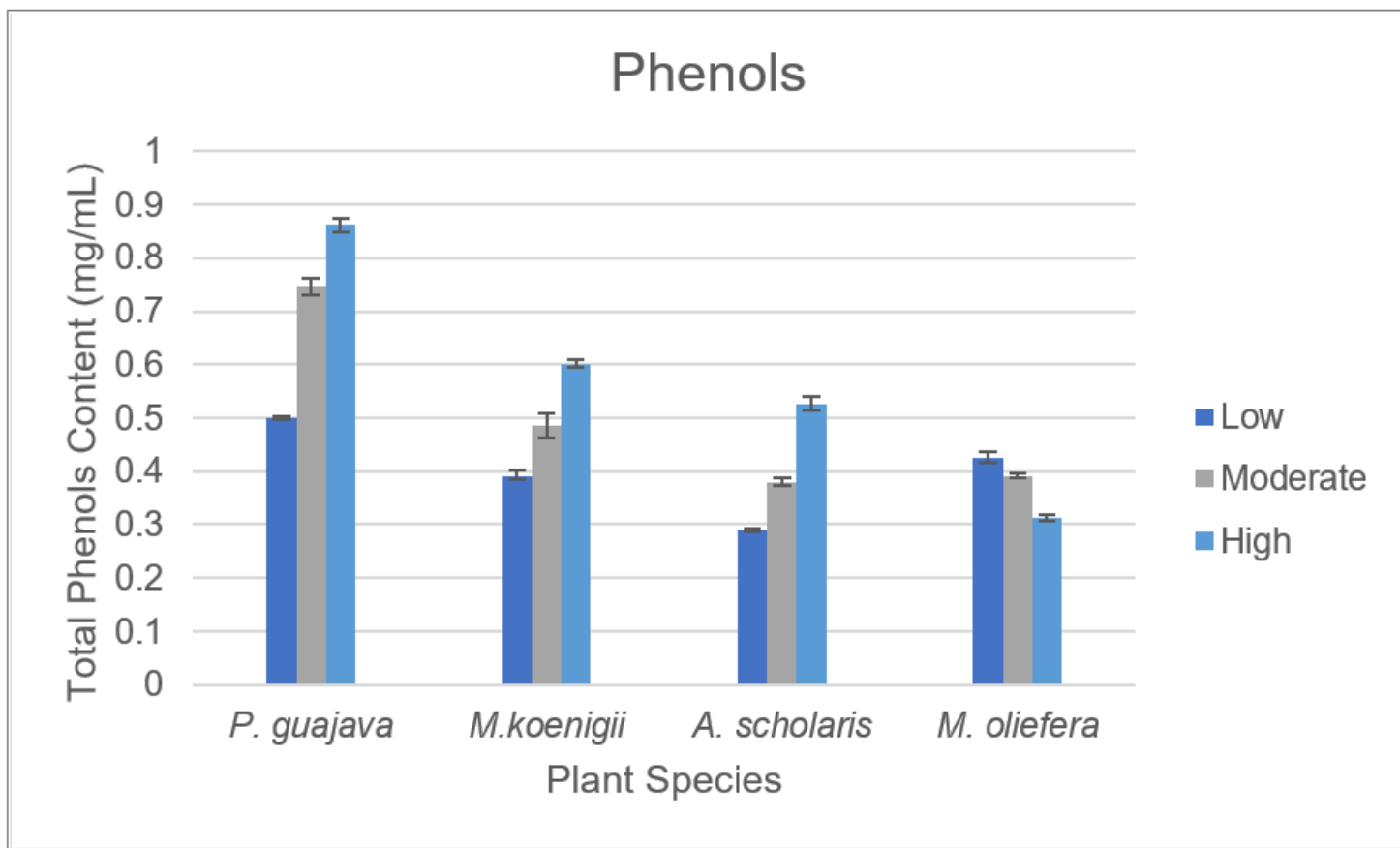


Figure 2

Mean Concentrations of Total Phenols at three different study locations for four different plants (Values are expressed as mean $\pm$ SEM. Means within columns are significantly different ($p < 0.05$) at different sites)

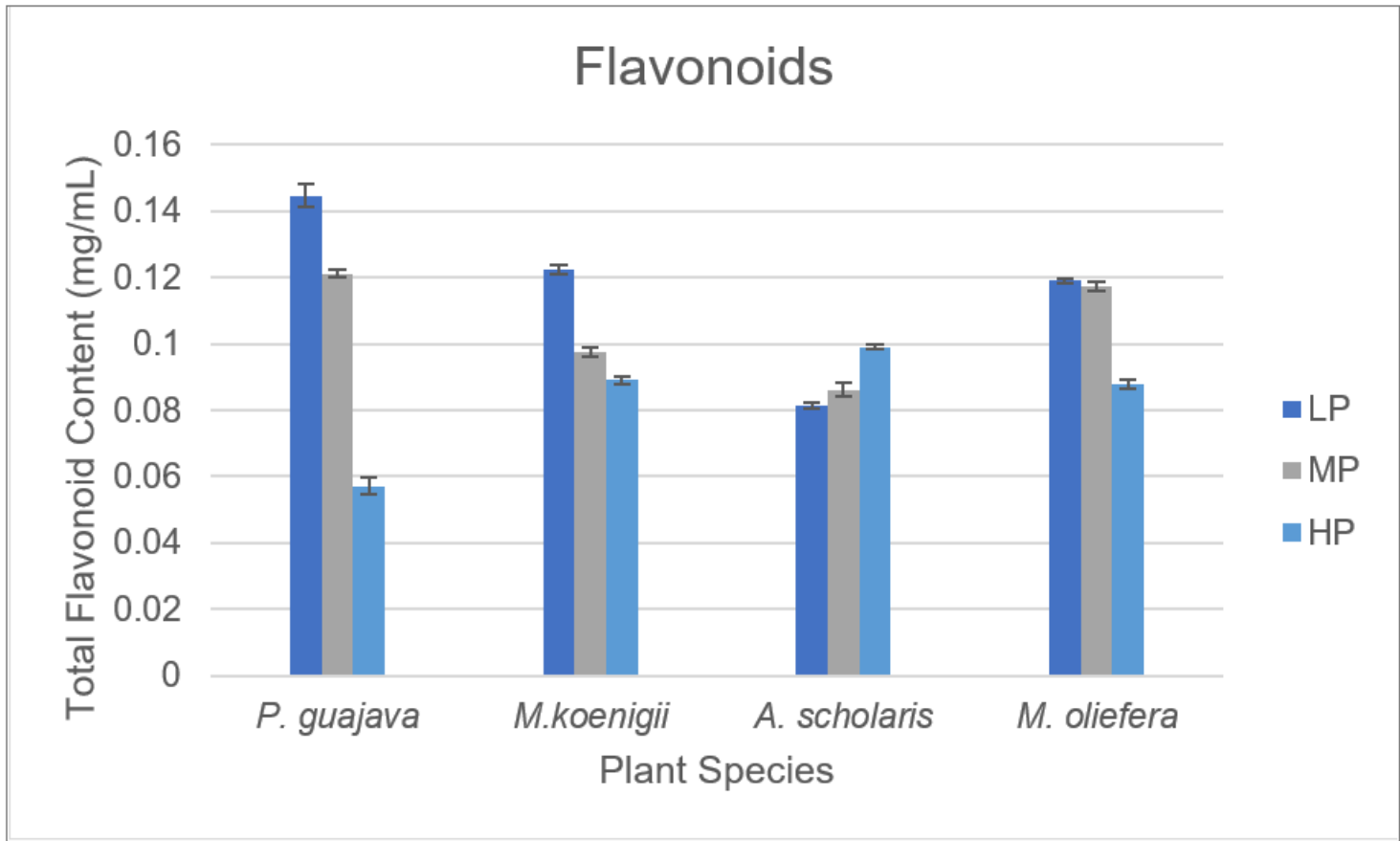


Figure 3

Mean Concentrations of Total Flavonoids at three different study locations for four different plants (Values are expressed as mean $\pm$ SEM. Means within columns are significantly different ($p < 0.05$) at different sites)

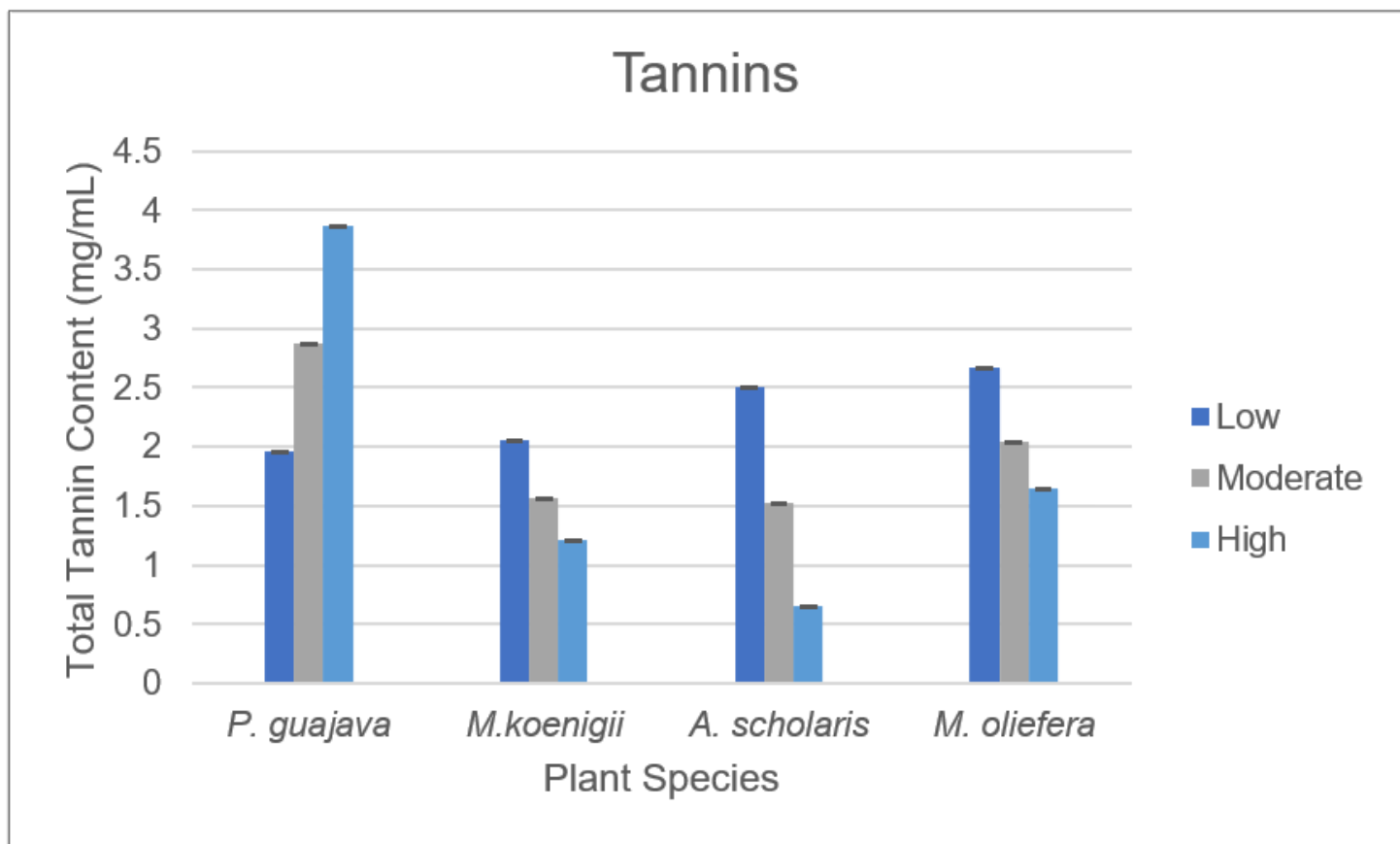


Figure 4

Mean Concentrations of Total Tannins at three different study locations for four different plants (Values are expressed as mean $\pm$ SEM. Means within columns are significantly different ($p < 0.05$) at different sites)

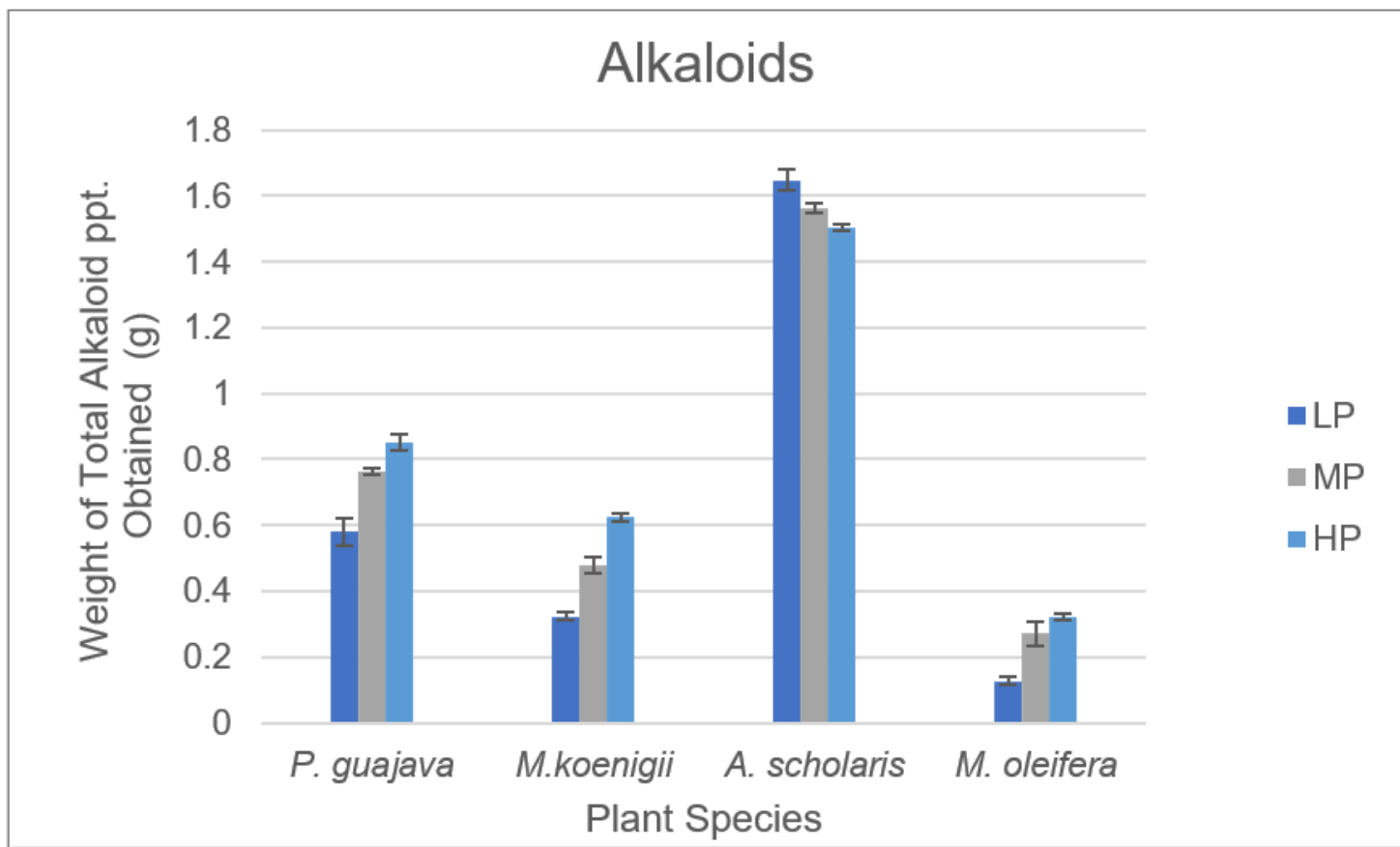


Figure 5

Mean Concentrations of Alkaloids at three different study locations for four different plants (Values are expressed as mean $\pm$ SEM. Means within columns are significantly different ($p < 0.05$) at different sites)

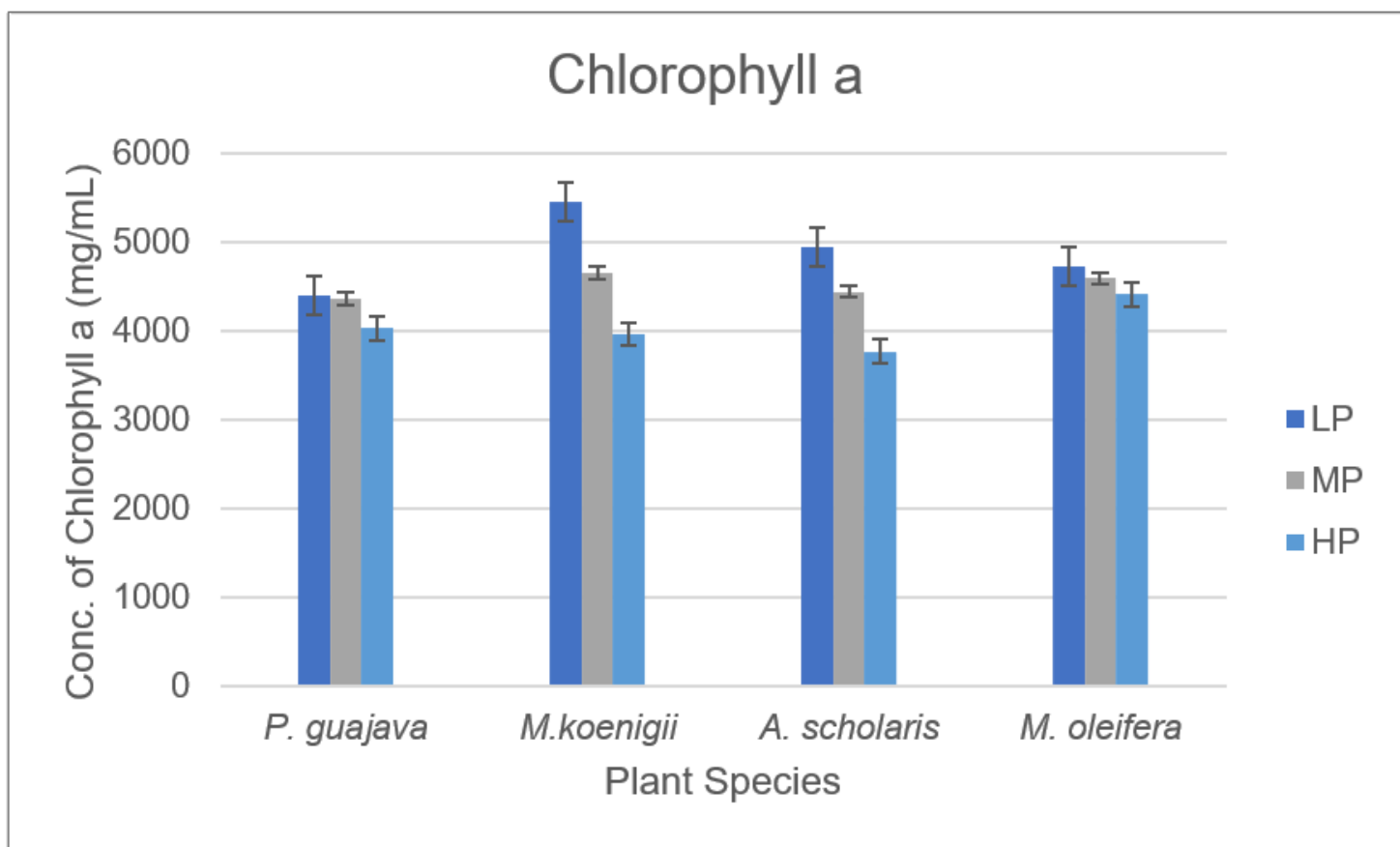


Figure 6

Mean Concentrations of Chlorophyll-a at three different study locations for four different plants (Values are expressed as mean $\pm$ SEM. Means within columns are significantly different ($p < 0.05$) at different sites)

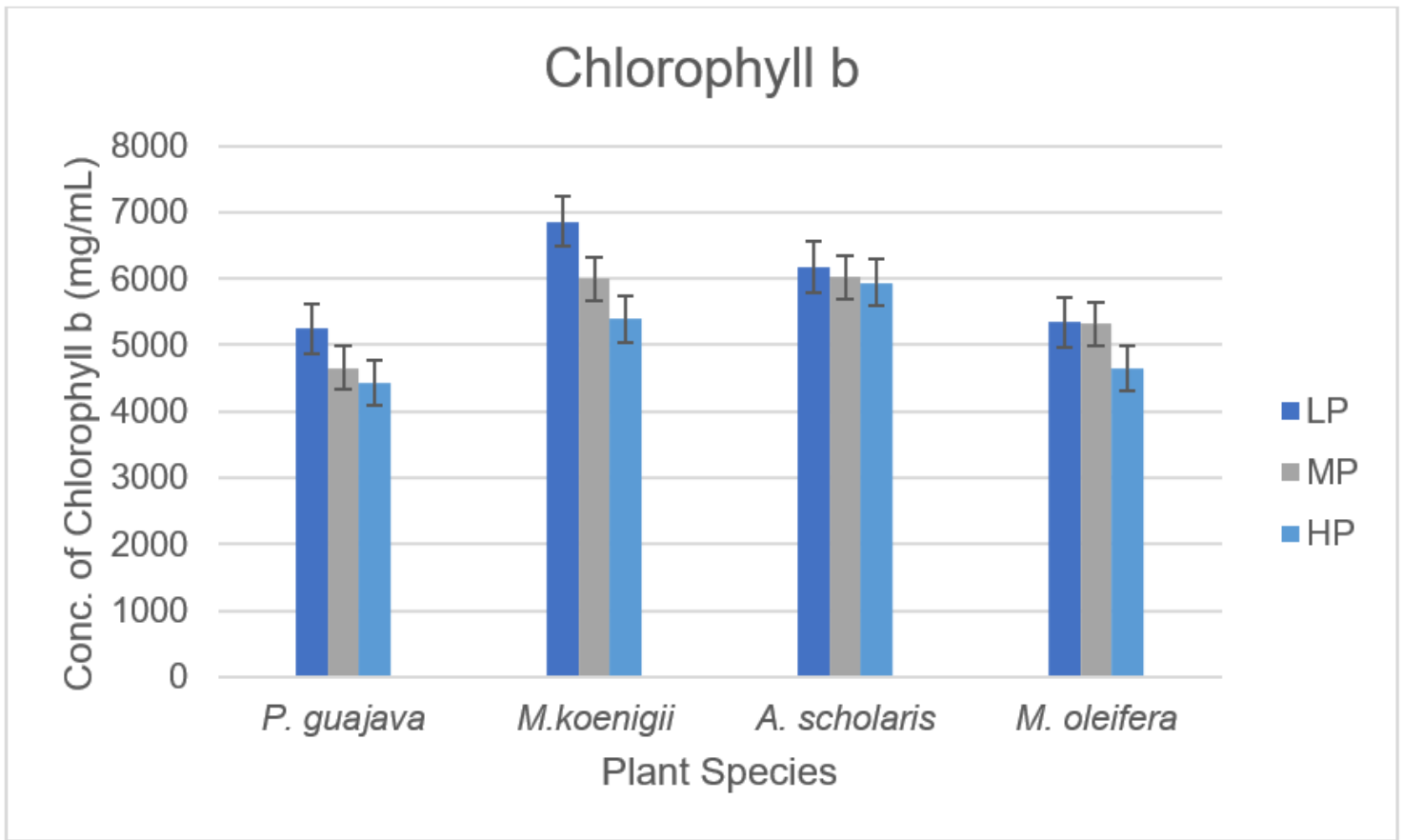


Figure 7

Mean Concentrations of Chlorophyll-b at three different study locations for four different plants (Values are expressed as mean $\pm$ SEM. Means within columns are significantly different ($p < 0.05$) at different sites)