

A Morpho-Biochemical Approach to Evaluate the Tolerance Index of *Psidium Guajava* L. in The Coal Fly Ash Affected Area, Yamuna Nagar, Haryana India

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ABSTRACT

The plant species which are grown in close surroundings of Thermal power plants are worst affected by the higher emission rate of air borne fly ash & gradually becomes static Eco-sinks for harmful thermal emissions. So, the present study used Air pollution tolerance Index (APTI) to assess the response of commonly grown fruit tree *Psidium guajava* L. towards fly ash emissions in Rattanpur village situated in close vicinity of thermal power plant in Yamuna Nagar city. Leaf samples of selected plant individuals of fly ash affected area and control area were analyzed for the assessment of foliage dust load, macro-morphological leaf traits and biochemical attributes, including Leaf extract pH, Relative water content (RWC), chlorophyll content and Ascorbic acid content. The APTI results of the selected plant

species were found to be in sensitive category due to the declined level of ascorbic acid and chlorophyll content with respect to fly ash emission. However, results indicates that *P. guajava* L. can be considered as bioindicator plant in affected area but should not be solely recommended for the development of “Green belt” in fly ash affected village.

Keywords Coal fly ash, *Psidium guajava* L., Bioindicator, APTI, Green belt.

INTRODUCTION

In the recent decade, India has witness tremendous increase in energy demands to fulfill the requirements in the industrial, domestic, and agricultural sector. According to the major statistics of Ministry of coal, among all the power generation sectors, coal based Thermal power plant contribute around 75% of total installed capacity of electricity generation in India (Coal.nic.in).

However, the unregulated dispersal of air borne fly ash due to coal combustion has become the biggest challenge in the area adjacent to these thermal power plants. This is because higher emission rate of air borne particulate matter in the local environment contributes high level of fly ash dust load on the available surfaces (Hariram *et al.* 2022). Foliage surface of naturally grown plants due to their specific macro-morphological traits (Santamaria *et al.* 2022) offers large surface area for particulates to settle

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down. Consequently, variable physiological impact on different plants species were reported due to the deposition of these air borne fly ash dust particles on leaves surface (Qadir *et al.* 2019).

Although, fly ash has been utilized efficiently in different sectors such as an ecofriendly soil amendments (Ansari *et al.* 2023) cement industries, bricks production, buildings & roads construction (CEA. 2021). But main cause of concern is the unregulated emission of air borne fly ash in the surroundings which is reported to poses harmful impact on the people's health & nearby grown plants (Mahlangeni *et al.* 2025, Gupta *et al.* 2024).

Majority of the plant species with highly dust laden foliage is very common sight in vicinity of TPP. And it is a known fact that plants act as natural dust scavengers in any natural environment but the dust capturing capabilities of these plants depends on some specific plant traits such as phyllotaxy, leaf texture, surface features, size and shape of the leaves (Santamaria *et al.* 2022, Steinparzer *et al.* 2023). Although these macro-morphological features help in greater retention of dust particles (Erkebaev *et al.* 2021) but chemical nature of deposited dust reported to cause many lethal effect on plants survival in stressed environment. Specifically, the perennial habits of vegetation make them more prone to physiological stress due to the long-term exposure to air borne particulate matter in polluted environment (Ghosh *et al.* 2023). Various physiological and biochemical parameters such as pigments concentration, relative water content, antioxidant level, leaf extract pH were reported to affected adversely by the stress caused by the prolonged retention of dust on leaf surface (Sahu *et al.* 2023).

Although several field studies observed the physiological changes to be highly specific to plant species and pollution sites (Hariram *et al.* 2022) but significant alteration were reported to occur in (Upadhyay *et al.* 2025) plants specifically in highly fly ash contaminated site. Furthermore, these affected plant species can be classified as sensitive or tolerant based on their response towards a specific stress causing pollutant (Bharti *et al.* 2018). Thus, it becomes quite imperative to monitor the response

of stress tolerant & bio-indicator plant species in polluted sites which could be further projected as a sustainable alternative for the development of "Green Infrastructure barrier" (Grover *et al.* 2025) along with some socio-economic benefits (Singh *et al.* 2023). Indigenous (Enitan *et al.* 2022) and fast-growing trees with some socio-economic importance due to their wide adaptability & long growth period should be the main prioritization for their potential role in the development of green belt around thermal power plants (Qadir *et al.* 2020) as well as open fly ash dumping areas to mitigate the impact of air borne fly ash pollutants in the surrounding residential areas.

Thus, Air pollution tolerance Index (APTI) developed by (Singh *et al.* 1983) was the most potential and widely used expeditious tool (Molnar *et al.* 2020) used for the recommendation of best tolerant and bio-indicators plants for developing green belts in affected areas. APTI has been extensively used in phyto-monitoring field studies to determines the tolerance level of plants species from very basic biochemical parameters of leaves (Goswami *et al.* 2022 ; Molnar *et al.* 2020) such as pH, Total chlorophyll content (TCC), Ascorbic Acid (AA) & Relative moisture content (RWC). However, some field studies suggested that physiological attributes in plant were never be found consistent ; as mostly observed to be specific to plant species and variable study sites (Hariram *et al.* 2018). Therefore, evaluation of physio-biochemical parameters along with some vital macro-morphological leaf attributes of the cultivated and naturally grown plants were considered obligatory in the stressed environment before the development of sustainable green belt around the affected area in close proximity of thermal power plants.

Although previous research works were extensively reported the impact of road side and industrial dust load on vegetation efficiency (Rahul *et al.* 2023) but very limited studies were available that describe specifically the effect of air borne coal fly ash on the morpho-biochemical attributes of plant species of some socio-economic importance. Furthermore, there was no study conducted regarding the fly ash tolerant plant species of socio-economic importance in the Yamuna Nagar district of Haryana ;

Table 1. Description of Plant Species selected for the present study.

Species Name	Common Name	Family	Habit	Leaf Shape	Leaf Texture	Phyllotaxy
<i>Psidium guajava</i> L.	Guava	Myrtaceae	Tree	Oblong	Rough	Opposite

therefore, the present study was designed to evaluate the impact of Coal based fly ash dust load on the survival of most commonly & widely distributed plant species of *Psidium guajava* L.

This plant species was selected as test crop due to its wide adaptive mechanism and physiological response (Aguh *et al.* 2025). *Psidium guajava* L. (Guava) is a most common plant of Myrtaceae family (Table 1) grown widely in tropical & subtropical region with abundant phytochemicals in leaves (Tomar *et al.* 2021). Moreover, during the preliminary assessment of vegetation in the vicinity of the thermal power plant this fruit trees were observed to be flourished well in most of the households of affected village.

Thus, to evaluate the tolerance potential of *P. guajava* L. abundantly grown in the fly ash affected village, following morphological & biochemical analysis were undertaken in the present study.

1. Comparative assessment of the Dust Load and Macro-Morphological leaf traits of *Psidium guajava* L.
2. Analysis of major Physio-biochemical parameters such as Leaf extract pH, Relative water content (RWC), essential pigments content & Ascorbic acid concentration (AsA).
3. Estimation of Air pollution tolerance index (APTI) to assess the suitability of *Psidium guajava* L. plantation in fly ash affected village.

MATERIALS AND METHODS

Description of the Study Site

The present study was conducted in twin city Jagadhri–Yamunanagar, famous Brass City of Haryana situated in the North-Eastern part of Haryana. Total geographical area of the district is 1756 sq.

km situated between 29°55':30°31'N Latitudes and 77°00':77°35'E Longitudes. The climate of Yamuna Nagar district observed as sub-topical monsoon, mild and dry winter and hot summer. The hot weather season starts from mid- April to last week of the June followed by the Southwest monsoon which lasts up to September. The transition period from September to mid-November forms the post monsoon season. The winter season starts late in November and remains up to March. The two sampling sites were selected within the city were designated as fly ash affected site and control Site for the present study (Fig. 1).

Sampling sites and pollution source

The preliminary Phyto-diversity survey was conducted to assess specifically the woody perennial trees in the close vicinity of Thermal power plant during the month of October 2024. But due to the fall season at that time, sampling was conducted in the month of March & April 2025. Fly ash affected site was located in close vicinity of Thermal power plant is known by the name of Rattanpur Village. Railway Colony area of Yamuna Nagar city with maximum green cover and without any anthropogenic pollution was chosen as a control site which is situated about 15 km from the Deen Bandhu Chotu Ram Thermal power plant (DBCR, TPP) Yamunanagar city.

During preliminary survey of polluted site, cultivated and naturally grown vegetation was observed to be fully laden with coal fly ash (Fig. 2). Dry loading of fly ash dumper trucks that carried coal fly ash to the dumping site as well as direct emission of air borne particulate matter from thermal stacks across the boundary wall of Thermal power plant was observed to be the major pollution source in nearby surrounding areas of the affected village.

Leaf sample collection

Fly ash dust loaded leaves of five individual Guava

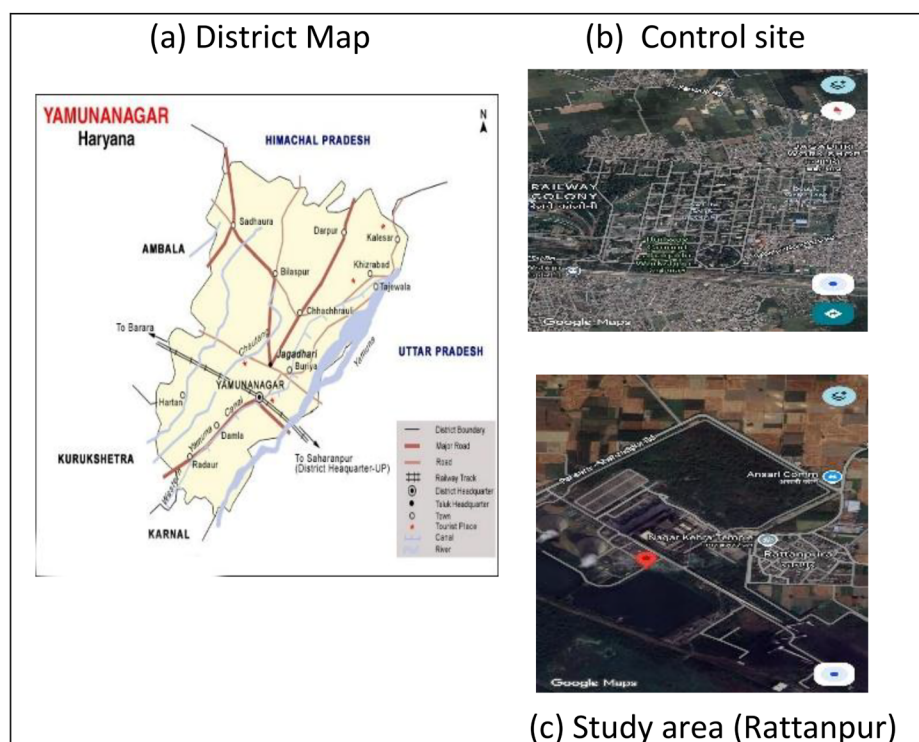


Fig. 1. Location of sampling sites in Yamuna Nagar District.

plants from each site were collected in the month of March. Socio-economic importance, abundance, wide distribution & commonness in the both the selected sites were the major selection criteria for the plant species. Five leaf samples from three different branch-

es of each individual plant species were collected in early morning hours. 15 leaf samples were collected from each individual tree of both the site thus making total 150 leaf sample for the analysis. Fully expanded and healthy foliage samples from each branch were



Fig. 2. Selected Plant species of *Psidium guajava* L. from fly ash affected & control area.

kept in carefully in polythene bags & marked separately and then transferred to the laboratory for further analysis. The fresh weight of each individual leaf sample from all the branches was taken within one hour of sampling. The composite mixture of leaf samples in triplicate was taken for biochemical analysis.

Foliage dust load capacity

Five matured leaves of three different branches of each tree were randomly selected from the exposure side and used to estimate the dust load on the leaf samples from both sampling sites (Hariram *et al.* 2018). The leaves with the dusts were weighted with the help of a digital balance. The dust adhering on the leaf surface was then cleaned carefully with the help of a brush and distilled water and the leaves were thoroughly cleaned with cotton cloth and reweighed thereafter, in order to determine the amount of dust present on the surface of leaf. The leaf area of all samples was calculated with the help of a graph paper conventional method. Finally, the amount of dust (in mg) deposited per cm² of leaf area was calculated by using the following equation 1.

$$W = \frac{w_1 - w_2}{A}$$

Where, W = Dust Load (mg/cm²) Eq. (1)

W₁ = Initial weight of the leaf with dust

W₂ = Final weight of the leaf without dust

A = Total Leaf Area (cm²)

Morphological leaf traits

Five leaves from three different branches of each tree in fly ash affected & control site were collected & brought to laboratory for the study of following vital macro-morphological leaf traits.

Leaf area

Leaf area measurements were done by using conventional method of graph paper (Singh *et al.* 2019). A leaf outline was drawn carefully on the graph paper. Then the area of the leaf was calculated by counting the number of bigger and smaller squares. Average

leaf area of all samples (expressed in cm²) from each individual tree of both the sites was taken for further analysis.

Petiole length

Shakeel *et al.* 2021 method of growth marker analysis was used for the measurement of Petiole length using a measuring scale and the average mean value was expressed in cm.

Length of internodes

Length of internodes was calculated in cm by using scale at different regions from the 3rd node in each branch from the selected individual tree of both sites and the average value of three branches of each tree was taken for comparative analysis (Shakeel *et al.* 2021).

Biochemical parameters

pH of leaf extract

Fresh leaf sample (1 gm) was used to estimate the leaf extract pH of all samples from both the sites. Sample was crushed carefully in thin paste followed by dissolving in 10 ml distilled water. After centrifugation for 5 minutes at 2500 rpm, supernatant was taken to measure the pH by using pre-calibrated digital pH meter (Singh *et al.* 1983).

Relative water content (RWC)

RWC was estimated after taking the fresh weight (FW) of five leaf sample of each branch. Overnight water immersed leaves were blotted dry and weighed foliage turgid weight (TW). The leaves were later dried in the oven at 70 for 24 hours and reweighed up to the constant weight for foliage dry weight (DW). The average relative water content (RWC) of leaves was then calculated by the given using equation 2 (Singh *et al.* 1983).

$$RWC = \frac{FW - DW}{TW - DW} \times 100 \quad \text{Eq. (2)}$$

Where, FW = Fresh weight, DW = Dry weight, TW = Turgid weight

Total chlorophyll content

The chlorophyll content of leaf samples from both the selected sites was measured by taking 100 mg of fresh leaf material from the plants of both sites (Arnon 1945). Leaf Samples were homogenized gently with mortar and pestle and extracted with 10 ml of 80% ice-chilled acetone till the whole leaf content becomes colorless. The leaf extract centrifuged at 5000 rpm for 5 minutes, filtered and supernatant was taken for further dilution. Absorbance was taken at 645 nm and 663 nm wavelength by using Systronic -166, Vis- Spectrophotometer.

Following equations (4 to 6) were used to calculate the chlorophyll concentration in mg /gram of FW.

$$\text{Chlorophyll a} = [(12.7 \times \text{O.D } 663 \text{ nm}) - (2.69 \times \text{O.D } 645 \text{ nm})] \times V / 1000 \text{ W mg/g} \quad \text{Eq. (4)}$$

$$\text{Chlorophyll b} = [(22.9 \times \text{O.D } 645 \text{ nm}) - (4.68 \times \text{O.D } 663 \text{ nm})] \times V / 1000 \text{ W mg/g} \quad \text{Eq. (5)}$$

$$\text{Total Chlorophyll (mg/g)} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad \text{Eq. (6)}$$

Where, V= Total Volume of chlorophyll solution (ml) and W=Weight of leaf tissue (g).

Ascorbic acid content

The modified method of (Bajaj and Kaur 1981) was used to determine the content of Ascorbic acid in the foliage of both sites. The calibration graph of standard L-Ascorbic acid was prepared by taking 0.1, 0.2, 0.3, 0.4, 0.5 ml aliquots of it into separate calibrated brown flasks. Total volume of 25 ml was prepared by adding sufficient amount of oxalic acid –EDTA solution accordingly followed by the further addition of 1ml of Ortho –phosphoric acid, 1 ml of 5% concentrated H_2SO_4 , 2 ml of Ammonium Molybdate reagent and 16 ml of distilled water. After an hour-long incubation period, the absorbance at 760 nm was taken against a reagent blank prepared in the same way but without L-ascorbic acid.

Similar protocol of calibration graph was used for the estimation of Ascorbic acid by taking 1gm of leaf

Table 2. Standard Category levels on the basis of APTI Values.

Sl. No.	Category	APTI values
1	Sensitive	≤ 11
2	Intermediate Tolerant	12-16
3	Tolerant	≥ 17

sample of selected plant species, followed by estimation of exact concentration from the calibration graph.

Air pollution tolerance index (APTI)

Air pollution tolerance index (APTI) of *P. guajava* L. in both the selected sites was calculated by integrating the above biochemical parameters into the widely used empirical formula in stress physiology studies (Singh *et al.* 1983) according to the following equation.

$$\text{APTI} = \frac{[A \times (T + P)] + R}{10}$$

Where, A- Ascorbic acid (mg/g); T- Total Chlorophyll content(mg/g) ; P- leaf extract pH ; R- Relative Water Content (%).

Further different categories of plant tolerance response of both sites were classified (Bharti *et al.* 2018) on the basis of estimated APTI values (Table 2).

Statistical analysis

Data analysis was performed by using two sample t-tests (assuming unequal variance) in Microsoft Excel (2024) to identify the significant difference between the mean values of morpho-biochemical parameters of *Psidium guajava* L. in fly ash affected village as compared to control site. Percent change (%) in the foliage of fly ash affected plants species against control site plants was calculated by using equation 5.

$$\text{Percent Change (\%)} = \frac{X_p - Y_c}{Y_c} \times 100 \quad \text{Eq. (5)}$$

Where, X_p =Observed Polluted site value and Y_c =observed value of control site.

RESULTS AND DISCUSSION

The present study evaluated the effect of air borne



Fig. 3. Dust loaded and Cleaned Foliage of *Psidium guajava* L.

coal fly ash on the morpho-biochemical parameters and air pollution tolerance index (APTI) of commonly found fruit tree of *Psidium guajava* L. (Guava) in the close vicinity of Thermal Power Plant, Yamuna Nagar, aiming to establish its fly ash tolerance potential. The results of the present study summarized in Fig. 3 to Fig. 6, revealed that due to the observed value of APTI (typical value ≤ 11), the selected plant species of *P. guajava* L. can be classified in sensitive category and thus cannot be recommended suitable for the development of sustainable green belt around thermal power plant.

Visible impact of coal fly ash on *Psidium guajava* L.

All the plants adjacent to thermal power plants were noticed to cover with grey colored air borne coal fly ash particulate matter due to which all leaves were

observed dull greenish in color. The leaves margins specifically in polluted site were noted to be slightly curved. The selected plant species in polluted site was recorded to retain significant amount of dust load on its upper surface due to its rough surface with a negligible amount of dust on its lower surface. This might be due to the close location of affected plant with coal ash loading site or due to the development of some specific morphological leaf traits modification observed in fly ash stress condition. Noticeable color difference was observed after the removal of dust from the foliage of selected plant species (Fig. 3).

Variation in Macro-morphological parameters

Prolonged exposure of air borne coal fly ash was reported to induce significant morpho-biochemical alterations in naturally grown and cultivated plant species in fly ash affected sites in comparison to control site. Table 3 reflects the values of vital leaf traits such as leaf area, petiole length and internodal length of *P. guajava* growing in both fly ash affected village and control sites. All the observed leaf traits except internodal length indicated the declining trend in fly ash affected area. Significant variation in leaf area, petiole length and internodal length reflecting a variation of 7.69 %, 25 %, and 7.14% between control and polluted site respectively (Fig. 4). Similar to our work, specific leaf traits such as specific leaf area (SLA), leaf length (LA) and leaf width (LW) from fly ash affected site were also reported to show reduction in *Pithocellobium dulce* (Roxb) Benth, and *Withania somnifera* (Qadir *et al.* 2019, Qadir *et al.* 2020).

Table 3. Average values of foliage Morpho-biochemical attributes of *P. guajava* L. grown in two different study sites. ($\pm$ SD, n=5).

Parameters	Sites	
	Control	Fly ash affected
Dust Load (mg/cm ²)	0.822 $\pm$ 0.09	2.410 $\pm$ 0.25
Leaf Area (cm ²)	50.82 $\pm$ 2.13	47.8 $\pm$ 2.58
Petiole Length (cm)	0.774 $\pm$ 0.05	0.614 $\pm$ 0.09
Internodal length (cm)	4.22 $\pm$ 0.24	4.46 $\pm$ 0.20
Chlorophyll-a (mg/g)	1.978 $\pm$ 0.299	1.651 $\pm$ 0.296
Chlorophyll-b (mg/g)	0.616 $\pm$ 0.125	0.460 $\pm$ 0.06
Total Chlorophyll content (mg/g)	2.594 $\pm$ 0.374	2.112 $\pm$ 0.296
Chl-a/Chl-b Ratio	3.270 $\pm$ 0.53	3.589 $\pm$ 0.932
pH	6.66 $\pm$ 0.18	6.93 $\pm$ 0.02
RWC (%)	86.30 $\pm$ 5.50	96.1 $\pm$ 2.24
Ascorbic Acid (mg/g)	1.980 $\pm$ 0.784	1.467 $\pm$ 0.594
APTI	10.462 $\pm$ 0.53	10.936 $\pm$ 0.87

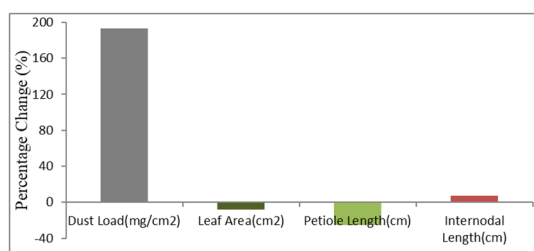


Fig. 4. Percentage change in Morphological Attributes of *P. guajava* L.

Significant alterations in morphological leaf traits generally reflects a disruption of normal growth patterns of plants due to physiological stress. Higher pH and lack of N₂ were reported (Yadav *et al.* 2021) to be significant in causing alteration in the morphological attributes of naturally grown plants. Other than this, higher rate of senescence, decreased leaf production and interference with the cell elongation mechanism were reported to be some plausible explanations linked to the reduction in leaf attributes (Qadir *et al.* 2019). Shorter petiole length in polluted *P. guajava* leaves was observed parallel to the fly ash affected stolon length of *Eichhornia crassipes* (Upadhayay *et al.* 2025) to facilitate the greater retention of particulate matter on leaf surface by restricting the movements of leaves as compared to the plants with longer petiole length (Hariram *et al.* 2022).

Variation in foliage dust load

Average values of dust load on the foliage of selected plant species in both the sites are presented in Table 3 and Fig. 4. Lower net dust retention on the foliage of *Psidium guajava* L. due to the dry weather and moderate dusty wind velocity was observed in both the study sites.

Statistical analysis showed that there is significant variation of dust load between study sites ($P < 0.01$). The enhanced foliar dust load was observed specifically more on the plant foliage of immediate polluted surroundings due to the heavy emission of fly ash from the stacks of thermal power plant of Yamuna Nagar. Rough lamina, slightly curved leaf margins and short petiole length of the leaves of *Psidium*

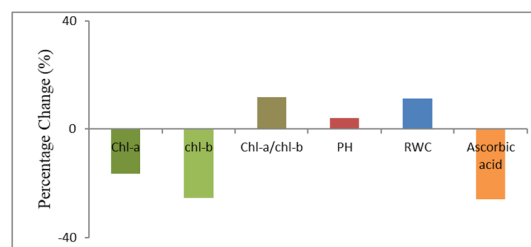


Fig. 5. Percentage Change in Biochemical Attributes of *P. guajava* L.

guajava L. in the fly ash affected area were observed to be significant reasons for the more retention of dust load in fly ash affected area compared to control site. Similar results of the noticeable difference between the dust loads due to specific foliage macro-morphological features were also reported by (Rahul *et al.* 2023, Erkebaev *et al.* 2021) for dust capturing capabilities of the vegetation. Similarly (Vashist *et al.* 2023) suggested the potential of individual leaf traits as significant factors in efficient removal of particulate matter.

Variation in foliage pigment content

Average mean values and percent change in the chlorophyll content of *Psidium guajava* in fly ash affected area and control site are presented in Table 3 and Fig. 5 respectively. The significant percentage decrease of 16.5% and 25.3% was observed in the chlorophyll –a and chlorophyll-b content of foliage respectively. The chlorophyll –b content was found to be proportionally less compared to the chlorophyll –a content at both the study sites similar to the results reported in *Lolium multiflorum* L. (Borowiak *et al.* 2018). This might be possible due to the reason that some specific stress tolerant or sensitive plant species at polluted site maintain or up regulate the chlorophyll content as part of their tolerance and survival in affected area.

The present investigation indicated significant decrease of 18.6% in the total chlorophyll content (TCC) in fly ash affected site supported by the similar observations in Wheat and Pumpkin plants (Wang *et al.* 2024, Ahmad *et al.* 2021). However, low to moderate level of particulate matter (<2.4% of leaf

surface) was observed to stimulate the chlorophyll biosynthesis in *P. guajava* L. (Aguh *et al.* 2025) revealing threshold type of response while examining the impact of particulate matter on the physiology of *Psidium guajava* L. plant.

Lower total chlorophyll content observed in current study in fly ash affected site primarily supposed to be an indicator of an adaptive response of *P. guajava* L. for reimbursing the less light due to retained dust and adaptation to the stress environment. Moreover, by increasing the proportion of Chlorophyll – a relative to Chlorophyll–b, plant may be prioritizing the efficient utilization of available light energy for the process of photosynthesis (Qadir *et al.* 2019) thus might be mitigating the adverse impact of air borne fly ash stress on pigment content.

Chl-a/Chl-b Ratio reduction in fly ash affected area could be was investigated as significant marker of pollution resistance of *Psidium guajava* plant. The 11.77% percent increase was observed in chl-a/chl-b ratio of the selected plant species in the fly ash affected individual plant species. Higher chl-a/chl-b ratio in polluted site may be associated with plants adaptive response to stress (Hariram *et al.* 2022), may be to optimize its photosynthetic efficiency under fly ash stress. Thus, Chlorophyll a/b ratio serves as a sensitive bio-indicator of abiotic stress in the selected species also supported by the work of (Shrestha *et al.* 2025).

Variation in pH, relative water content and ascorbic acid concentration

Fig. 5 demonstrated the relative percent changes in the biochemical attributes of selected plant species in polluted site with respect to plants in control area. The significant difference ($P < 0.05$) was observed between the average values of pH and alkaline nature of fly ash was observed to shifts the pH of foliage extract of plant grown in affected area towards somewhat near neutral category as presented in Table 3. The plausible interpretation of near neutral PH observed in fly ash affected foliage was might be due to the solubilization of carbonates and hydroxides salts of Mg and Ca. The percent increase of (4.05%) was observed in the leaf extract pH in fly ash affected site

contrary to the leaves extract of control area plants which maintains slightly acidic pH to facilitate normal metabolic functions. However, in spite of the evident difference between the pollution levels the outcomes for the pH were not drastically different in both the study sites.

Earlier studies have suggested that high leaf extract pH, specifically in polluted sites such as fly ash stress conditions increased their tolerance level for acidic pollutant as well as particulate matter (Kostić *et al.* 2024, Qadir *et al.* 2019). Slightly alkaline PH as observed to be linked with greater tolerance (Setu *et al.* 2024) contrary to the acidic pH associated with increased susceptibility of the plants to external stress by inhibiting the synthesis of ascorbic acid (Sapkota *et al.* 2024).

The RWC of selected plant species also showed a statistically significant difference between the two sites ($P < 0.05$) indicating that fly ash has significantly enhanced the RWC of foliage of fly ash affected foliage as compared to foliage of control site. The assessment of RWC showed the percent increase of (11.77%) in the foliage of plant species in polluted site. Similar, to our results RWC was consistently reported to be higher (81–95%) in fly ash contaminated site as compared to low contaminated site in aquatic hydrophytes (Upadhayay *et al.* 2025). This study reported the variation in RWC to be varied with plant species and pollution site. Similarly, earlier worker also determined the higher values of RWC of specific plant species such as *Pithecellobium Dulce*, *Carica papaya* and *Cassia fistula* in polluted sites (Qadir *et al.* 2019 ; Setu *et al.* 2024) which reflect the plant's ability to maintain water status in stress environment.

Generally, both the prevailing climatic factors of early summers in the present study were linked to the higher transpiration rate but possibly due to the stomatal plugging by fly ash deposition, transpiration rate get restricted resulting in more water retention by foliage. Secondly, fly ash also reported to enhance water holding capacity and micro-porosity of surrounding soil (Panda *et al.* 2018) which might results in higher moisture availability at the roots and thus help the plant to maintain physiological

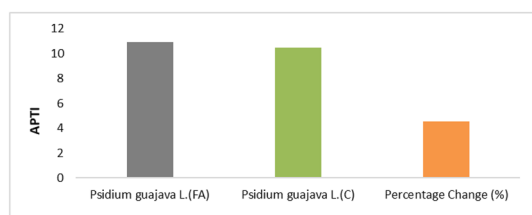


Fig. 6. Variation in APTI of selected plant species.

balance under fly ash stress (Sahu *et al.* 2023). The higher relative water content can be interpreted as an adaptive mechanism as also reported by earlier work (Sapkota *et al.* 2024) which suggested higher values of RWC in *Jacaranda mimosifolia*, *Buddleja asiatica*, and *Cinnamomum camphora* as probable resilience markers to contribute as potential vegetative green barriers.

A highly significant difference in ascorbic acid content was observed between the plant species of both sites revealing a strong influence of fly ash ($P < 0.001$) on the selected plant species. A percent decrease of 25.9% of ascorbic acid content was observed in the foliage of fly ash affected site which indicated high stress due to the fly ash load on the available foliage surface.

Ascorbic acid is reported to be a universal non-enzymatic biochemical antioxidant in plants which plays vital role of potential free radical scavenger (Akram *et al.* 2017) in abiotic stress tolerance. Similar to our work, reduced ascorbic acid content was reported in aquatic macrophytes of high fly ash polluted site such as *Eichhornia crassipes* and *Pistia stratiotes* (Upadhyaya *et al.* 2025) indicating that plants in higher fly ash contaminated sites do not inevitably depend only on higher ascorbic content in foliage. Sharma *et al.* 2022 also reviewed that sensitive species possess low level of ascorbic acid content as compared to tolerant. Although elevated ascorbic acid concentration in *Withania somnifera* and *Pithecellobium dulce* was reported due to the fly ash stress (Ansari *et al.* 2023 ; Qadir *et al.* 2019) but it was also suggested, that prolonged fly ash exposure can decline the antioxidant content as a sign of physiological exhaustion (Enitan *et al.* 2022). And might be the abundance of phenolics and flavo-

noid content in *P. guajava* L. leaves also contribute to antioxidant power (Tomar *et al.* 2021;) during the depletion of ascorbic acid concentration in fly ash affected foliage.

Air pollution tolerance index (APTI) of *Psidium guajava* L.

The estimated APTI of selected plant species in both fly ash and control site is presented in Fig. 6. Average APTI of *P. guajava* L. was observed to be slightly higher in fly ash affected surroundings (10.936) than in control site (10.46). The study found the marginal increase of 4.5% in the APTI of *P. guajava* L. in fly ash affected site as compared to control site which reflects a successful physiological response to fly ash affected environment.

Although the observed value of APTI classified the plant as Sensitive species (typical value ≤ 11) but slight increase indicated that biochemical parameters of the plant modified with fly ash exposure to withstand the fly ash pollution load in its immediate surroundings (Sapkota *et al.* 2024) Similar results were also reported by (Hariram *et al.* 2022) while investigating the impact of fly ash of Bokaro Thermal Power Plant on *P. guajava* (APTI=9.34) thus, classified it as sensitive plant species. Although, some studies (Lahiri *et al.* 2023 ; Enitan *et al.* 2022) recommended the *P. guajava* L. for the green belt development due to its higher APTI in polluted area and identified it as tolerant species. But due to the declined content of ascorbic acid and chlorophyll specifically in fly ash affected foliage, the present work observed less APTI value reflecting a physiological exhausted defense mechanism. While it might be possible that plant initially increased these parameters as reported in rice crop (Chhuria *et al.* 2025), where being a rich source of essential micronutrients such as Mg and Fe, moderate level of fly ash was reported to trigger their synthesis.

Based on its less APTI score, the present study observed the selected plant species (*P. guajava* L.) as a bioindicator plant species which can be further exploited to provide the significant initial warning symptoms of stress in plants species grown in fly ash dumping sites. The present study demonstrated

the anomalous state of sink despite stress, where prolonged fly ash stress overwhelmed the normal metabolic capacity of naturally grown plants in close proximity of thermal power plants.

CONCLUSION

Significant variations were observed in morpho-biochemical attributes of *P. guajava* growing in fly ash affected village in close vicinity of thermal power plant in Yamuna Nagar, Haryana. While an observed low APTI value in the present study, classified the *P. guajava* as sensitive species but slight increase in APTI reflects that biochemical parameters of the plants of fly ash affected area get modified with prolonged fly ash exposure to combat the thermal emissions stress. However, despite all these morpho-biochemical alterations, *P. guajava* L. observed to be display high morphological resilience in spite of its biochemically sensitive profile.

Despite its sensitivity, the plant observed to be higher dust accumulator due to its rough surface and slightly curved leaf margins. Moreover, spreading dense canopy clearly observed to maintain biomass and its survival in fly ash affected area which might be caused counterbalance of some physiological stresses in affected site under the influence of fly ash nutrient for its growth.

Although the suitability of any plant species for the green belt development is suggested to be highly species and site specific, but the present study has not recommended the *P. guajava*, as a reliable species for the development of sustainable green belt barrier in prolonged fly ash exposure sites. However, in spite of biochemical depletion in its antioxidant level, the selected plant species can act as an effective bioindicator for reporting the initial stress symptoms of fly ash in affected area.

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