Bio -monitoring of Air pollution using Galled and Non galled leaves of *Pongamia pinnata L.* Pierre in three different pollution zones of Chennai

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Abstract

Air pollution is a much bigger issue than any other type of pollution as it has a higher impact of trans national dispersion of pollutants in the entire world. It is been well established that air pollution has a larger impact on the plant species globally. This serious problem should be deeply studied in order to control. There are many systems and models present to identify and address this issues but they are not effective.

Plants are one such immense source to address this problem as they are widely present and also sensitive to identify. APTI studies hence helps to find out the ability of the plant species, whether it is sensitive, or intermediate or tolerant towards air pollution.

Galls are structures formed by plants as a result of interaction with other organisms such as aphids, nematodes, mites, bacteria fungi and viruses. Some studies show that these gall formations gets altered even due to environmental factors such as Air pollution and seasonal changes. There are numerous studies done in medicinal aspects of galls, but consequently, galls cannot be only limited to the medicinal uses they perhaps also are extensively used to observe environmental issues such as air pollution. This study aims in bringing the bio monitoring aspects of galls of *Pongamia pinnata* L.Pierre. Results show that galled leaves decrease in Chlorophyll in high pollution zone and with increase in pH, Relative Water Content and Ascorbic acid.

Introduction

Air pollution is a much bigger issue than any other type of pollution as it has a higher impact of trans national dispersion of pollutants in the entire world. It is been well established that air pollution has a larger impact on the plant species globally. In relation to plant-environment interactions, as plants are more sensitive and resistant species compared to other organisms towards pollutants, they are often used for studying air pollution. Changes in morphology, anatomy, physiology, biochemical levels and phytochemical levels occur as a result of plant-environment interactions. There are also plants that get injured due to high accumulation and absorption of pollutants.

The conception of monitoring of air quality by plants is globally used. Some plants show their high sensitivity when they are exposed to pollutants (Wang *et al* 2019) and therefore they are used for monitoring the air pollution. Lichens and moss species are finest examples in air pollution analysis. But due to its high sensitivity towards air pollutants and contaminants, they cannot be grown and monitored in cities. (Suchita Sinha *et al.,* 2019) Higher plants in pollution studies therefore can serve at its best due to withstanding capacities towards varied climatic conditions and temperature and high range adaptability in cities and its environmental make up.

Plants tend to change their biochemical constituents, especially chlorophyll, ascorbic acid, pH of leaves and finally the relative water content. (Kuddus *et al,* 2011) APTI - Air Pollution Tolerance Index of a plant species can be determined by using these constituents. Air Pollution Tolerance Index (APTI) is the most important indicator which determines the ability of trees or plants for their efficient ability in controlling air pollution. (Zahid, A., *et al.,* 2023) APTI can be used to identify the sensitive, intermediate, and tolerance nature of plants species particularly towards air pollutants and contaminants.

This APTI studies hence helps to find out the ability of the plant species, whether it is sensitive, or intermediate or tolerant towards air pollution. Plants with score of ≤ 11 is considered as sensitive and it can act as an indicator, plants with the score of 12–16, is considered as an intermediate and it can act as a sink for air pollutants and contaminants and finally plant having score of ≥ 17 is considered as sink and recommended for the development of green belt. Perhaps, it is considered that overall plant tolerance increases as response to air pollution at traffic and industrial zones. (S.K. Bharti *et al.,* 2017)

Galls are structures formed by plants as a result of interaction with other organisms such as aphids, nematodes, mites, bacteria fungi and viruses Shrestha S *et al.,* (2013). Some studies show that these gall formations gets altered even due to environmental factors such as Air pollution and seasonal changes. There are numerous studies done in medicinal aspects of galls, but consequently, galls cannot be only limited to the medicinal uses they perhaps also are extensively used to observe environmental issues such as air pollution.

Bio monitoring of air pollution has been done by observing galling mechanism and APTI of *Alstonia scholaris* in Kolkata which reveals that the gall count increases with increase in air pollution. Likewise there are several APTI studies done in avenue trees which serve as a primary test for air pollution. (Suchita Sinha *et al.,* 2019)

This study aims in bringing out bio monitoring ability of non-galled (normal leaves) and galled leaves of *Pongamia pinnata* (L) Pierre growing in three zones namely Residential zone (Anna Nagar), commercial zone (T. Nagar) and industrial zone (Manali) in Chennai, city in Tamil Nadu, India towards air pollution. These zones were selected based on the data provided by Tamil Nadu Pollution Control Board (TNPCB). Here Residential zone is considered as low pollution zone, commercial zone as moderate pollution zone and industrial zone as high pollution zone.

Study Site

Chennai is the state's largest city in Tamil Nadu, India. Study is done in three different zones such as Residential zone (Anna Nagar), commercial zone (T. Nagar) and industrial zone (Manali) in Chennai, city in Tamil Nadu, India. These zones were selected based on the data provided by Tamil Nadu Pollution Control Board (TNPCB). Here Residential zone is considered as low pollution zone, commercial zone as moderate pollution zone and industrial zone as high pollution zone during the rainy (June-November) and summer (March-May) seasons.

Collection of plant sample:

Fresh leaves of *Pongamia pinnata L.* Pierre were collected in paper covers before sunrise along the road side from the three above mentioned zones. Collected leaf samples were washed with tap water, rinsed with distilled water and then stored for further studies.

Estimation of Total Chlorophyll (Arnon's Method -1949):

- 1gm of fresh leaves of *Pongamia pinnata L.* Pierre collected from three different sites during Rainy and Summer seasons were washed separately with water and air dried at room temperature, then macerated with 80% acetone in a mortar and pestle.
- A pinch of calcium carbonate was added to the material to prevent deterioration of chlorophyll. The leaf extract was centrifuged at 2000rpm for 15 min, and filtered.
- The Optical density (OD) of the filtrate was measured using spectrophotometer at 645nm and 663nm.
- The total chlorophyll content was calculated by Arnon formula and tabulated.
    - Total chlorophyll=20.2(OD645) $\pm$8.02(OD663)

Ascorbic acid analysis (Bharti,KS *et al* ., 2017):

- 1gm fresh leaves of *Pongamia pinnata L.* Pierre collected from three different sites during Rainy and Summer seasons were homogenized separately in 4ml oxalic acid -EDTA extracting solution, 1 ml of orthophosphoric acid, 2ml of 5% sulphuric acid followed by 4ml of ammonium molybdate and 3ml distilled water.
- The solution was kept undisturbed for 15min, the absorbance was measured by digital spectrophotometer at 760 nm and tabulated.

Measuring pH of leaf extract (A.Singh – 1977):

- 5g fresh leaves of *Pongamia pinnata L.* Pierre collected from three different sites during Rainy and Summer seasons were homogenized separately in 10ml of distilled water.
- Leaf extract was filtered by Whattman filter paper .

- Then the pH was determined after calibrating pH meter with the buffer solution 4,7 and 9.

Relative Water Content (Barr and Weatherly-1962):

- Fresh weight was measured by weighing the fresh leaves of *Pongamia pinnata L.* Pierre collected from three different sites were immersed overnight in the water bottled ,dried and then weighed to get turgid (being in a state of distension)weight.
- The leaves were kept to dry overnight in an oven and reweighed to obtain the dry weight.
- RWC were calculated by following formula of Barr and Weatherly.
    - RWC(%)=[(FW-DW)]/[(TW-DW)]x100

Where,

DW=Dry weight

TW=Turgid weight.

Air Pollution Tolerance Index determination (Singh and Rao - 1983):

The air pollution index was determined by the following method by Singh and Rao

APTI=[Ax(T±P)±R/[10]

Where,

A=Ascorbic acid

T=Total chlorophyll

P=pH

R=Relative Water Content

Air Pollution Tolerance Index (APTI)

Estimation of chlorophyll

Estimation of chlorophyll was done by collecting the leaves from all three different pollution zones such as namely Low polluted - Residential zone (Anna Nagar), moderately polluted commercial zone (T. Nagar) and highly polluted industrial zone (Manali) in Chennai, city in Tamil Nadu, India for two different seasons such as Rainy and Summer

Galled leaves in both rainy and summer season contained lower amount of chlorophyll compared to that of Non Galled leaves. Minimum amount of chlorophyll was recorded in the Galled leaves of **high pollution zone** in summer season as **(2.83mg/ml).**

Total Chlorophyll content during Rainy Season and Summer Season

Total Chlorophyll content during Rainy Season and Summer Season (mg/ml)				
S.NO	**Leaves**	**Study Site**	**Rainy season**	**Summer Season**
1.	Galled Leaves	Low Polluted Zone	8.522±0.002	5.428±0.004
		Moderate Pollution Zone	5.214±0.003	3.727±0.003
		High Pollution Zone	4.034±0.002	2.831±0.001
2.	Non Galled Leaves	Low Polluted Zone	13.274±0.003	14.357±0.003
		Moderate Pollution Zone	9.794±0.002	10.441±0.001
		High Pollution Zone	7.203±0.002	9.732±0.003

Discussion

A study done by M.M Hosssain *et al.,* (2014) shows that galled leaves were recorded with lower amount of chlorophyll compared to that of Non galled leaves. Samson *et al.* (2012) states the level of chlorophyll dropped down in galled leaves of some species in hilly regions of Japan. Lower level of chlorophyll content in galled leaves is mainly due to the loss of chlorophyll pigments that occurs during galling process, which also lowers the photosynthetic performances of leaves. Factors like anatomical alterations, such as disruption of palisade and spongy parenchyma, nutrient redistribution and redirection away from normal leaf growth may also affect chlorophyll levels. Decreased amount of chlorophyll occur mainly because of air pollutants, fly ash and dust emissions. Dust deposition on leaves hinders in stomatal functioning and damages palisade cell which are said to be the primary reason. Disruption in magnesium ions which are the primary factors of chlorophyll production may also be an important factor for reduced chlorophyll content.

Estimation of Relative Water Content

Relative Water Content estimation was done by collecting the leaves from all three different pollution zones such as namely Low polluted - Residential zone (Anna Nagar), moderately polluted commercial zone (T. Nagar) and highly polluted industrial zone (Manali) in Chennai, city in Tamil Nadu, India for two different seasons such as Rainy and Summer

Relative Water Content (RWC) of **Galled leaves** in both rainy and summer season was higher compared to that of Non Galled leaves. Maximum RWC was recorded in the Galled leaves of **high pollution zone** in summer season as (97.47 %). Percentage of RWC decreased with increase in pollution.

Relative Water Content during Rainy Season and Summer Season

Relative Water Content during Rainy Season and Summer Season (%)				
S.NO	**Leaves**	**Study Site**	**Rainy Season**	**Summer Season**
1.	Galled Leaves	Low Polluted Zone	64.01±0.023	80.22±0.002
		Moderate Pollution Zone	77.32±0.004	89.52±0.024
		High Pollution Zone	83.89±0.003	97.47±0.832
2.	Non Galled Leaves	Low Polluted Zone	72.51±0.166	60.47±0.003
		Moderate Pollution Zone	64.83±0.002	54.01±0.310
		High Pollution Zone	58.22±0.017	47.71±0.002

Discussion

This study corresponds to this investigation done by Denis C. Oliveira *et al.*, (2017) which states that galled leaves of *Matayba guianensis* induced by *Bystracoccus mataybae* (Eriococcidae) has higher RWC compared to that of normal non galled leaves. It was recorded that RWC of galled leaves were 96.88% and non galled leaves were 67.78%. Study done by Ogburn and Edwards, 2012 also states that RWC %increases in galled leaves than that of non galled leaves in many plants species.

RWC (Relative Water Content) and leaf succulence are totally dependent upon thickness of the leaves and increase in the RWC may also be an indication that more utilization of water is done by gall mites/ aphids that are present in galls. As number of gall increases, more water is taken in and thus leading to high RWC. Alterations in the RWC occur mainly due to the stomatal closure. When the air pollutants like SO2, NO2 and Particulate matter deposits on the leaf surface, stomatal conductance and stomatal density is heavily affected. Elevated CO2 may also have a negative impact on plant water relation. Oxidative stress created by the environment can also damage cell membrane and disrupts water balance.

pH of leaves during Rainy and Summer Season

pH study was done by collecting the leaves from all three different pollution zones such as namely Low polluted - Residential zone (Anna Nagar), moderately polluted commercial zone (T. Nagar) and highly polluted industrial zone (Manali) in Chennai, city in Tamil Nadu, India for two different seasons such as Rainy and Summer

pH of **Galled leaves** in both rainy and summer season was acidic (low pH) compared to that of Non Galled leaves. Lowest pH level was observed in Galled leaves of **high pollution zone** during summer season as 4.23±0.002.

pH of leaves during Rainy Season and Summer Season				
S.NO	**Leaves**	**Study Site**	**Rainy Season**	**Summer Season**
1.	Galled Leaves	Low Polluted Zone	6.35±0.002	5.79±0.001
		Moderate Pollution Zone	5.88±0.002	5.69±0.003
		High Pollution Zone	4.45±0.001	4.23±0.002
2.	Non Galled Leaves	Low Polluted Zone	7.11±0.002	6.98±0.002
		Moderate Pollution Zone	6.78±0.004	6.27±0.005
		High Pollution Zone	6.00±0.001	5.52±0.003

Our study converges with the study done by Antoine Guiguet *et al.,* (2023) states that the *A. nubilipennis* galls had pH level below 3 which can be seen only in citrus fruits. Our study also aligns with findings done by Etienne A *et al.,* (2013) which states that galled tissues had low pH compared to that of non galled normal leaf tissues. Findings of Freund M, *et al.,* (2022) and Gilbert KJ *et al.,* (2021) states that the pH of galled tissues was lower compared to that of non galled leaves. Considering the pH of non galled leaves, lowest pH in the Non Galled leaves was observed in of high pollution zone during summer season. Galled leaves contain lower pH than that of normal leaves present in the same plant. This major difference in pH may be due to the increased amount of acids such as malic and gallic acid. Also more fluid secretion, secreted by the mites/ aphids inside these galls is acidic and this may lower the pH.

Ascorbic content during Rainy Season and Summer Season

Ascorbic acid estimation was done by collecting the leaves from all three different pollution zones such as namely Low polluted - Residential zone (Anna Nagar), moderately polluted commercial zone (T. Nagar) and highly polluted industrial zone (Manali) in Chennai, city in Tamil Nadu, India for two different seasons such as Rainy and Summer

Ascorbic acid content of **Galled leaves** in both rainy and summer season was higher compared to that of Non Galled leaves. Higher amount of ascorbic acid was observed in Galled leaves of **high pollution zone** during summer season as 0.471±0.033mg/ml

Ascorbic content during Rainy Season and Summer Season (mg/ml)				
S.NO	**Leaves**	**Study Site**	**Rainy Season**	**Summer Season**
1.	Galled Leaves	Low Polluted Zone	0.321±0.024	0.357±0.021
		Moderate Pollution Zone	0.379±0.031	0.393±0.048
		High Pollution Zone	0.421±0.005	0.471±0.033
2.	Non Galled Leaves	Low Polluted Zone	0.381±0.042	0.322±0.001
		Moderate Pollution Zone	0.331±0.020	0.310±0.003
		High Pollution Zone	0.284±0.003	0.196±0.001

Discussion

This study corroborates with the findings of Anand, P.P and Ramani, N. (2021) which states that antioxidant capacity of matured gall of *Pongamia pinnata* was higher than that of non galled leaves which were taken as control. Also it states that antioxidant capacity increases as the size of the gall increases. Also our findings goes well with the study done by Anitha Rajasekaran and Mercy Mahimaidoss (2016) which states that the total antioxidant capacity of leaf galls of *Mangifera indica* was 15% higher than non galled leaves.

Changes in the Ascorbic acid contents may occur due higher exposure to acidic pollutants which can bring down the pH levels. This, in turn reduces the efficiency of converting hexose sugar to ascorbic acid. Also, lower levels of ascorbic acid were directly proportional to the pH levels. Therefore decrease in pH decreases the levels of ascorbic acids. Ascorbic acid levels are higher in galls due to stress created by the galling insects. Ascorbic acid is known as the well know stress oxidant enzyme and it is proved true. Also as the ascorbic acid is involved in cell division and differentiation, it can be present in higher amounts in galled levels.

APTI value during Rainy and Summer Season

APTI was calculated for the galled and non galled leaves from all three different pollution zones such as namely Low polluted - Residential zone (Anna Nagar), moderately polluted commercial zone (T. Nagar) and highly polluted industrial zone (Manali) in Chennai, city in Tamil Nadu, India for two different seasons such as Rainy and Summer.

APTI of **Galled leaves** in both rainy and summer season was higher compared to that of Non Galled leaves. APTI of **Non Galled leaves** in both rainy and summer season were lower compared to that of Galled leaves. Both galled and non galled leaves fall in the 'intermediate" level.

APTI value during Rainy Season and Summer Season

S.NO	Leaves	Study Site	Rainy Season	Summer Season
1.	Galled Leaves	Low Polluted Zone	11.17	12
		Moderate Pollution Zone	11.90	12.59
		High Pollution Zone	11.87	13.02
2.	Non Galled Leaves	Low Polluted Zone	14.96	12.86
		Moderate Pollution Zone	11.88	10.56
		High Pollution Zone	9.54	7.68

Discussion

Galled leaves exhibited more adaptability and physio chemical changes in air polluted zones Levels of Chlorophyll, pH, Relative Water Content and Ascorbic acid changed in highly polluted zones compared to that of low polluted zones.

Conclusion

From the present study it can be stated that the galled leaves of *Pongamia pinnata* collected from high pollution zone during summer exhibited high APTI with decreased chlorophyll content, higher RWC, pH and ascorbic water content. Here, the pollution along with seasonal changes gives out so many alterations in physiological aspects. APTI of **Galled leaves** in both rainy and summer season was higher compared to that of Non Galled leaves. Hence this gall formation can be considered as an indicator of air pollution. Furthermore, APTI determination provides a reliable method for monitoring the plants with respect to air pollution.

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