A STUDY ON EFFECTS OF ENVIRONMENTAL FACTORS IN PLANTS TOLERANCE TO AIR POLLUTANTS

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By

NAVNEET KOUR

Registration Number: 11919210

Supervised By

Prasenjit Adak (23966)

Department of Chemistry (Assistant Professor)

Lovely Professional University, Punjab, India

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

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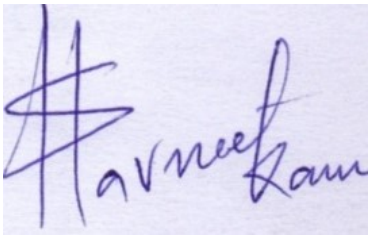
DECLARATION

I, hereby declared that the presented work in the thesis entitled "A STUDY ON EFFECTS OF ENVIRONMENTAL FACTORS IN PLANTS TOLERANCE TO AIR POLLUTANTS" in fulfillment of degree of **Doctor of Philosophy (Ph.D)** is outcome of research work carried out by me under the supervision Dr Prasenjit Adak, working as Assistant Professor in the School of Chemical Engineering and Physical Sciences, of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



(Signature of Scholar)

Name of the scholar: Navneet Kour

Registration No.: 11919210

School of Chemical Engineering and Physical Sciences

Lovely Professional University, Punjab, India

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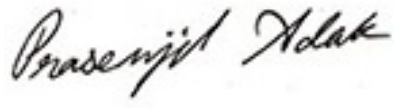

(Signature of Supervisor)

Name of supervisor: Dr Prasenjit Adak

Designation: Assistant Professor

School of Chemical Engineering and Physical Sciences

Lovely Professional University, Punjab India

ABSTRACT

The rapid urbanisation, industrialisation, and various commercial activities are increasing to meet the demands of the rapidly growing population which contribute to air pollution. Urban vegetation has become increasingly important because it improves the local and regional air quality, in addition to social reasons. Plants have long been used as markers of air pollution stress as they respond to air pollution in a dynamic manner. Additionally, they play a crucial role in maintaining and monitoring ecological balance by actively participating in the cycling of gases such as oxygen, carbon dioxide and nutrients. Air pollutants, including both gaseous and particulate are known for their detrimental effects on plants growing in polluted areas. Their atmospheric concentrations vary according to their sources, distribution patterns, weather patterns, and topographical characteristics of the environment. Plants provide a substantial leaf surfaces for absorption and assimilation of air pollutants, thereby mitigating their atmospheric concentration.

The most common effects studied were leaf injury, reduced photosynthesis, mitochondrial respiration, stomatal clogging and early senescence. Plants exposed to polluted environments often respond by changing their morphology, physiology, and biochemistry. The morphological effects are visually observable. Physiological and biochemical processes can be studied by using a common method known as Air pollution tolerance index (APTI). The Air pollution tolerance (APTI) has been considered as a method to assess the tolerance of plant species to air pollutants. The APTI depends on the four biochemical parameters of plants namely relative water content (RWC), pH, and total chlorophyll (TC) and ascorbic acid (AA) for the determination of the APTI value. Higher value of APTI suggests higher tolerance of plants. In the present study, APTI of plants species was estimated from three different locations such as Phagwara industrial area, Phagwara bus stand and Lovely Professional University.

The results showed significant variation in the biochemical parameters and APTI values of the same plants species at different sampling sites. As reported in the previous literature; many factors influence plant tolerance. These factors include morphological parameters, pollutants source, pollutants concentration seasonal change, soil type, surrounding conditions

which are among the most influential factors on plant tolerance and have been reported in previous study. In the current study, an attempt was made to explore the effect of these parameters on biochemical parameters thereby affects the tolerance in plants. Further, three parameters were considered in the current study. Three parameters include Morphological parameters, Environmental factors and pollutant concentrations. For Morphological parameters experiment, Leaf surface texture (LST) and Leaf surface area (LSA) were selected to explore their effect on biochemical parameters. However, ascorbic acid from industrial plants exhibited correlation with LSA but on other sides none of the biochemical parameters from roadside plants exhibited significant results with LSA and LST.

Additionally, another experiment was conducted to study the effects of environmental factors on biochemical parameters. Two plants species (*Ocimum sanctum* and *Mentha piperita*) were selected to grow under controlled condition to study the effects of environmental factors on their biochemical parameters. The environmental factors chosen for the current study were Light intensity, Temperature and Humidity. *Ocimum sanctum* and *Mentha piperita* plant species grown under these environmental factors. The environmental factors were monitored on a daily basis throughout the year. *Ocimum sanctum* and *Mentha piperita* was sampled twice in a month for biochemical analysis. The experimental data was collected throughout the year along with the environmental parameters data. The collected data were statistically analyzed by using different software such as SPSS, MS EXCEL (special package). It was observed that light intensity, temperature and humidity together affect the biochemical parameters. The biochemical of both the plants species exhibited significant relationship with environmental factors. On other side, the effect of air pollutants on biochemical parameters has also been studied. Two air pollutants (Sulphur dioxide (SO_2) and Nitrogen dioxide (NO_2)) have been selected to study their effect on biochemical parameters. The levels of air pollutants of distinct regions of Punjab have been assessed through the online portal of Central pollution control board (CPCB). Also, from CPCB monitoring stations for (SO_2) and (NO_2), leaf samples have been collected to study the effect of these pollutants on plant biochemical parameters.

The experimental data along with the secondary data was meticulously analyzed by using different software such as SPSS, MS EXCEL (special package) to explore the relationship between pollutant concentrations ($SO_2$and NO_2) concentrations with biochemical parameters. It was observed (SO_2) has significant relationship with the TC, AA and RWC excluding pH

and (NO_2) exhibited significant relationship with AA only.

The current study emphasize the significance of considering multiple factors collectively rather than a focusing solely on individual parameters when assessing their impact on biochemical parameters in plants. Moreover, these findings provide a deeper understanding of the complex relationship between biochemical parameters environmental factors and air pollutants. Thus, this led to the conclusion that there is a need for modification in the existing APTI model. So, existing APTI model has been modified as proposed APTI model which includes a correction term (CT) and written as

$$APTI = \frac{AA_m + CT_{AA,EF} + (TC_m + CT_{TC,EF} + pH_m + CT_{pH,EF}) + RWC_m + CT_{RWC,EF}}{10}$$

Here, CT is site specific and can be include environmental factors data from a particular area.

$$APTI = \frac{AA_m + CT_{AA,EF} + (TC_m + CT_{TC,EF} + pH_m + CT_{pH,EF}) + RWC_m + CT_{RWC,\mathrm{AP}}}{10}$$

Here, CT is pollutant concentration specific and can be include any air pollutants concentrations data from a particular area. Using modified model, tolerant and sensitive plant species can be identified more precisely. Tolerant plants can be used for plantations to develop green belts and green microclimates in urban landscaping. Government has also started number initiatives for developing green belts in urban areas. By using secondary environmental and air pollutants data of a particular area, biochemical parameters of a plant can be calculated with the help of modified model minimizing the need of laboratory experiment and resources. Thus, the selection of appropriate plant species for green belts and phyto remediation to improve air quality in urban areas helps to achieve environmental sustainability.

Key words: Air quality, air pollution, air pollutants, plants, tolerance, environmental parameters, biochemical parameters, morphological parameters.

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LIST OF ABBREVIATION

ABBREVIATION	FULLFORM
APTI	Air Pollution Tolerance Index
RWC	Relative water content
TC	Total chlorophyll content
AA	Ascorbic acid
CPCB	Central Pollution Control Board
SO_X	Sulphur dioxide
NO_X	Nitrogen dioxide
O_3	Ozone
PM	Particulate matter
NO_X	Nitrogen oxides
CO	Carbon monoxide
SPM	Suspended Particulate Matter
NO	Nitric oxide
CT	Correction Term
V_a	Volume of air sampled
V_t	Volume of aliquot taken
V_s	Volume of sample
WHO	World Health Organization
NAAQM	National Ambient Air Quality Monitoring
NAMP	National Air Quality Monitoring Programme
USEPA	United States Environmental Protection Agency
ppb	Parts per million
Eq	Equation
LST	Leaf surface texture
LSA	Leaf surface area
LPU	Lovely Professional University
R_P	Pearson correlation coefficient
R_L	Coefficient of Determination for linear regression
R_{NL}	Coefficient of determination for non linear regression
EPA	Environment Protection Agency

ROS	Reactive oxygen species
T	Temperature
LI	Light intensity
H	Humidity
S.E	Standard error
OLS	Ordinary least squares

CHAPTER 1 INTRODUCTION

1.1 Air Pollution

Air pollution has emerged as one of the most important aspects of environmental research, as the progress of human civilization has resulted in significantly degradation of air quality (Patel et al., 2022; Kour and Adak, 2023). The air quality in developing countries has deteriorated alarmingly in the last three to four decades due to excessive development of industrial and motorized vehicles. Over the past three decades, the rapid expansion of industrial and urban areas along with the gradual development of the Indian economy has contributed to the progressive deterioration of air quality in developing countries such as India (Safeena et al., 2021). The rapid urbanisation, industrialisation and various commercial activities are increasing to meet the demands of the rapidly growing population which contribute to air pollution (Safeena et al., 2021).

Urban areas can be viewed as a huge source of anthropogenic pollutant emissions, which can change the chemistry and lifecycle of the atmosphere in its downwind regime for several hundred kilometers (Brauer et al., 2019). It induces a broad range of acute and persistent health effects depending upon the specific characteristics of the pollutant constituents (Cohen et al., 2005). It results in a significant risk to the health of living organisms such as plants, animals and humans. It is estimated that 40,000 Indians die every year due to air pollution, and these deaths can be avoided by reducing the concentration of particulate matter (Ram et al., 2015). In 2012, air pollution caused an estimated 7 million deaths worldwide and more than 3 million premature deaths annually. Globally, 92% of the population inhabits, where quality of air do not meet World Health Guidelines, and another 91% population live in areas where pollutant level surpass the WHO guidelines permitted levels (WHO, 2016).

Despite the decade's progress, Air quality in the US has started declining in recent years, based upon data released by the Environmental Protection Agency (EPA) in the summer of 2019.In some aspects, urbanization has increased the number of city dwellers, while on other side their life span has been significantly reduced due to development-related deterioration in air quality. It can be caused by natural and manmade sources such as smoke emitted from tobacco, solid fuel combustion emitted during cooking, cleaning agents used for homes, insecticides manufacturing industries, vehicular exhaust, ignored environmental regulation,

and fuel burning in vehicles, incinerators, waste disposals, forest fires and fire emanating from stubble burning in farms, is regarded as the principal cause for ill health and death in human and animal population (Popescu and Ionel, 2010).

In developing countries like India, the rise in the concentration of ambient air pollutants is more common due to the lack of technology and resources to fight pollution and maybe a rapid expansion of population as compared to the developed countries. In an urban area, more than 60- 70% of air pollution is caused by automobile exhaust emission and industrialization (Kaur et al., 2017). It has been reported that automobiles account for introducing 10% of sulphur dioxide (SO_2), 30% of Suspended particulate matter (SPM), 30-40% of oxides of nitrogen (NO_x), 50% of hydrocarbons (HC), and 70% Carbon monoxide (CO) in ambient air of urban areas in India(Kaur et al., 2017). 65% of carbon dioxide (CO_2) is emitted from industries and fossil fuels (IPCC, 2014).

Industries releases 9- 26% of (CO_2), 3-7% of nitrous oxide (N_2O), and 4-9% methane (CH_4) and methane are 20 times more overpowering than CO_2 (Munsif et al., 2021). Most cities in India have violated the annual average limit of SPM for industries i.e., 360 $\mu g/m^3$ (Das et al., 2018). Based on the nature of their formation two groups of air pollutants have been identified. Primary pollutants directly emitted from the source (e.g., SO_2, Nitric oxide, CO, etc.) and secondary pollutants that are formed in the atmosphere as a result of chemical reactions between air constituents and primary pollutants e.g., Sulphur trioxide (SO_3), Ozone (O_3), Peroxyacetyl nitrate (PAN) etc.

Anthropogenic activities are the primary sources of air pollution including stationary sources (factories, refineries, and power plants), mobile sources (cars, trucks, buses, etc.) as well as indoor sources (building materials and activities such as cleaning). Fossil fuel combustion, serving as the cornerstone of global energy production, stands as the primary catalyst for anthropogenic greenhouse gas emissions (GHG).

According to previous literature, one fifth of global fossil fuel expenditure by transportation segment contributing 26% of global green house gas emissions (Louisa et al., 2021). Motor vehicles contribute significantly to air pollution in major cities of industrialized countries. More than half of the world's emissions of particulate matter (PM), CO_2, HC, SO_2, NO_x, and CO released from fossil fuel combustion by vehicles and industries (Bolaji et al., 2010).

These six critical air pollutants including particulate matter with a diameter of 2.5μm ($PM_{2.5}$) and an aerodynamic diameter smaller than 10 μm (PM_{10}), SO_2, NO_2, CO_2, and O_3

cause a detrimental effect on the environment and humans as well (Ghaffarpasand et al., 2021).

Forest fires generally generate an abundant amount of carbon black and release it into the atmosphere. Besides, lighting in the sky produces NO_x in enormous quantities; hydrogen sulfide is largely produced from oceans algae, and marshy methane Volcanic eruption disperses a huge amount of SO_2, hydrogen fluoride (HF), and greenhouse gases in the ambient air together with particulates, smoke which generally increases the ambient temperature (Munsif et al., 2019). Mobile sources are most leading sources of CO and NO_x. The petrol and diesel engines emit similar type of pollutants but their proportions are varied due to difference in the mode of operation of the two types of engine. In petrol engines, the fuel and air is mixed homogeneously and combusted in high temperature, the exhaust gas is almost colorless. The improper mixing and combustion in lower temperature produces more smoke with white, blue or black (Colvile et al., 2001).

Diesel engines can also contribute significant particulates in the ambient air as the diesel combustion process results in soot. Diesel and petrol-powered vehicles generate an extensive variety of contaminants with concentrations and relative proportions of contaminants based on their technology and their operational circumstances (Ghaffarpasand et al., 2021) .

The complete combustion of carbon constituted fuel (coal, fuel oil, wood, natural gas) produces CO and HC. The internal combustion engines do not allow the fuel to burn completely to produce CO_2 and water; some unburned amount of fuel gets exhausted with CO as an integral component.

When the amount of excess fuel or unburned fuel is high in fuel mixtures, the CO concentration in the exhaust remains high. On the other hand, CO emissions are very low for weak fuel mixtures and hence they are not generally considered as important. It is important to monitor vehicular emissions of CO_2 from a climate perspective. Another leading pollutant NO_x is emitted mostly due to road transportation activities which constituted about 41% of harmful emissions of NO_x, NO, and NO_2 are usually grouped as NO_x emissions (CPCB, 2020). Highway transportations have long been identified as major sources of nitrogen oxides and particulate matter. NO is the main oxide of nitrogen produced inside the engine cylinder. NO_x is produced by the reactions of free nitrogen and oxygen of air during the combustion of fossil fuels contained in motor fuel at high temperatures. Under typical photochemical conditions in the urban atmosphere, NO_2 quickly converts to NO, making it critical to measure NO emissions in addition to NO_2 (Ghaffarpasand et al., 2021). It has been

reported that 92% of CO and 65% of hydrocarbons is due to transport activities which are also responsible for 4% of Sulphur oxide, 14% of particulates, and 42% of NO_x present in the atmosphere resulting in an overall proportion of about 43.4% of pollutants from only transport activities (CPCB, 2020).

The factories, refineries, and the power plant are directly or indirectly dependent on fossil fuel and produce CO and CO_2, So_X and SPM. Industrial stacks emit SO_2 as fuels used in the industries contain a higher concentration of Sulphur which gets oxidized during the combustion and produces SO_2 that resides 10 days in the air. Besides, some of the industrial processes release a huge amount of CO and hydrocarbons into the air. Six primary air pollutants commonly found in industrialized nations has been identified by World Health Organization such as NO_x, SO_2, CO, and SPM (Munsif et al., 2021).

The emission of SPM from power plant stacks is regulated based on concentration levels, which vary according to boiler size. For instance, plants with a generation capacity exceeding 210 MW must adhere to a PM concentration limit of 150 mg/Nm^3, whereas those with a capacity below 210 MW have a limit of 350 mg/Nm^3 (Guttikunda et al., 2014). Mainly SPM is released from the cement industries and the VOCs from petroleum and chemical industries in the environment. The largest share of SO_2 in the atmosphere comes from power generation from coal and oil followed by industrial combustion generation. Power plants constitute 21% of the global SO_2 emission, followed by industries and non–road vehicles which contribute about 16% and 13% emissions of air pollutants respectively. It is also observed that Sulphur content in Indian coals is much less than those found in the United States (1.0 - 1.8%) and China (0.5 - 1.0%). Agriculture activities include the use of nitrogen fertilizer whichgenerates NO, ammonia (NH_3), and greenhouse gases such as CH_4 and CO_2. During flooding, organic matter gradually reduces the water and oxygen in the soil, and CH_4 is produced by anaerobic decomposition and aerobic produce CO_2 (Sivaramanan, 2015). Methane is 20 times more potent than CO_2 as a greenhouse gas (CPCB, 2020).

Plume plays an essential role in the dispersion of air pollutants. A plume can be described as an air space that is inhabited by a stack emitted gaseous stream.

When the plume travels, it expands and disseminates, hereby diminishing the concentrations of surroundings pollution even so the cross- sectional mass of the plume remains the same (CPCB, 2020). At what speed and height, a plume will achieve are affected by the various parameters such as mixture of emission velocity and temperature, vertical and horizontal air flow.

So, as the plume expands, its concentration goes down. As soon as the air toxics have come into symmetry with the surrounding conditions, atmospheric and meteorological factors predominantly impact the dispersion and transport of air pollutants. Nitrogen can be dissolved into NO and maybe in NO_2 with the help of an enormous amount of air. In the regions of intense solar transmission or radiation, these emissions are extremely important, which can stimulate reactions leading to the evolution of photochemical smog (CPCB, 2020).

Various climatic and meteorological factors such as wind speed and direction influence air toxics scattering and transport. A large number of pollutants are often released at a relatively high velocity from stacks or vents, which depending on meteorological conditions, can also help to move pollutants higher in the atmosphere. Depending on weather conditions, this can also assist in moving pollutants higher in the atmosphere (CPCB, 2020). The dispersion of the ambient air pollutants can be affected by many factors which have been discussed below.

Ambient temperature - A plume parcel that is much warmer than the surrounding air will generally increase the distance over which pollutants will be transported. The physical form of pollutants is also gets affected by the temperature and pollutants (CPCB, 2020).

Release height - At various heights, pollutants are emitted into the atmosphere. The higher the release height, the greater the dilution of pollutants in the air. Release height is one of the important essences in evaluating the local effects on air transport, such as building downwash (CPCB, 2020).

Greater release heights commonly result in increased pollutant dilution in the atmosphere, lower ground-level concentrations, and a longer distance to peak ground-level concentrations.

Time of release - The dispersion and transport of pollutants are determined by the timing of their release in specific meteorological conditions.

When a vehicle is moving at high and low speeds, the exhaust flow is also fluctuating high and low respectively, while emission of partially oxidized compounds is higher. Thus, on a volumetric basis, the highest emission takes place in deceleration; this is eventually due to low exhaust flow and low air-fuel ratio. There are particularly three parameters upon which damage is dependent:(a) to what extent does a pollutant subsequently disperse or scatter from the source (b) for how much time it will remain in the environment and (c) the process of the chemical reaction which it withstands (CPCB, 2020).

When the pollutants are released from sources in the form of particles and the rate at which the pollutant is removed from the atmosphere to surfaces (e.g. plants, soils, surface water) is to be controlled or determined upon the size of the particle (CPCB, 2020) .

Dry Deposition- The settling or sorting out of particles (aerosols, sea salts particulate matter, and adsorbed reacted gases) on vegetation due to gravity is known as Dry Deposition. Therefore, because of the lack of other removal mechanisms (e.g., condensation and/or aggregation to form larger particles), particles of smaller size gravitate to remain in the ambient air for longer hours.

According to different meteorological conditions, fine particles may remain in the surrounding atmosphere for days or weeks and travel hundreds or thousands of miles from their origin. There are tremendous health issues produced by these particles as many of them fall in the Respirable range i.e., they can reach very deep in our respiratory system and cause damage to our internal organs (Bolaji et al., 2006; Kour and Adak, 2021).

Wet Deposition – The removal pollutants from the air such as rain, snow, or hail is known as Wet deposition. It affects both particulate and vapor-phase pollutants. Precipitation events are very much helpful in removing pollutants from the air and settling them on the earth's surface for larger particles and vapor phase pollutants which possess the property of being soluble in water(It depends on the occurrence of precipitation events; it is better described over long periods(e.g., seasons or years). The importance of precipitation in removing pollutants from the air relies on the climatic conditions in the areas affected by pollution (CPCB, 2020). In addition to deposition, chemical reactions may take place that reduces air pollutant concentrations in the atmosphere. Air pollutants can be destroyed with the help of sunlight, through reactions with chemical pollutants.

To estimate the ambient air concentration associated with pollutant releases, it is mandatory to consider chemical reactions as well as the physical removal processes. However, more harmful pollutants may get formed due to these chemical reactions (e.g., formation of secondary air pollutants like PAN) (CPCB, 2020).

Goyal and Sidhartha (2002) noted that the monthly average concentrations of SO_2 exhibited consistent seasonal fluctuations in Delhi, India. These variations showed reduced in concentration in monsoon season and maximum concentration in winters (Goyal, 2002). Additionally, other factors such as in sufficient or non-implementation of environmental regulations, use of inefficient production technologies, worsening traffic congestion and lack of policy to keep older and air polluting vehicles off the road, are adding to our woes. It is

therefore, important to identify and monitor major polluting industries and develop and utilize technologies that allow site specific mitigation of the air pollutants.

1.2 Historical Background

A strong atmospheric inversion was found in the Meuse Valley in 1930, resulting in the death of at least 63 people and the development of respiratory complications in many others due to the trapping of effluents in the stagnant atmosphere. One of the largest disasters caused by air pollution was observed in Donora, Pennsylvania (USA) in October 1948, inwhich17 people died and 43% of the city's residents became ill. Photochemical smog occurred in the early 1950s due to the interaction of oxidants and hydrocarbons in the presence of sunlight to form toxic pollutants such as ozone and PAN, causing eye irritation, reduced visibility, damage to crops and breakdown of rubber.

In London alone, over a span of about five days, more than 4,000 deaths were attributed to the addition of air pollutants in the city. Consequently, the Beaver Committee on degrading the air quality was established in 1953, leading to the passing of the Clean Air Act inBritainin1956.Today, London is recognized as one of the cleanest cities globally.

Conversely, one of the most significant industrial disasters resulting in severe air pollution occurred in Bhopal. On the night of December 3, 1984, highly toxic methyl isocyanide gas accidentally released from Union Carbide's pesticide manufacturing plant. At least 5000 people were killed and 50,000 were seriously affected in this accident. The impacts of the disaster on humans and environment are still felt today (Chameides et al., 1994).

The last few decades have seen rapid emissions of gaseous pollutants due to rise in population, vehicles and thermal power plants. In fact burning coal/petro fuels to generate electricity in thermal power plants or to run vehicles have high environmental costs due to the release of harmful gaseous air pollutants like So_X and NO_X, which are considered harmful for environment and humans as well (Chameides et al., 1994). However, the influenced of air pollution on plants and humans can depend on the emitted concentrations of harmful gases, prevailing weather conditions, and sensitivity differences between biological components.

1.3 Effect of air pollutants on plant species

Depending upon the expanse of damage, Plant symptoms caused by air pollutants are indicated as chronic or acute. A whole tissue is mainly killed by a chronic injury or maybe all the portion of leaf or needle is damaged by a chronic injury. An acute injury is defined as an injury restricted to certain areas only which may result in dark, pigmented spots are seen on a leaf. Sometimes affected plants are dwarfed and usually found in disinfected areas. Low levels of pollution are the main cause of acute injury that can cause little injury over a brief period, or if a plant has some resistance to the pollutant. Some symptoms of acute injury are yellowing, bleaching, dwarfing, or growth loss without visible symptoms (Nouchi et al., 2002). Inside the leaf SO_2 is oxidized into Sulphur trioxide (SO_3), which further combines with water to form sulfuric acid (H_2SO_4). Thus, the acid formation in plants affects physiological processes and leads to a reduction in the productivity of the plant (Das et al., 2018). Moreover, it displaces the magnesium ion from the chlorophyll molecule and degrades it into a pheophytin molecule (non photosynthetic brown pigment).

The morphological symptoms caused by the introduction of SO_2 in plants are marginal, bronzed, or necrotic areas, interveinal chlorosis, and dull coloration. Similarly, NO_x gets absorbed by the leaves and reacts on cell walls to form nitrous acid (HNO_2) and nitric acid (HNO_3), which lowers the cellular pH, inhibits the metabolism and free radical formation leads to toxicity, growth suppression (Sharma et al., 2017).

The morphological symptoms caused by NO_x are discolored spots or light brown color and bleached or necrotic spots in interveinal areas of leaves. Although fluorine is found in different environmental components such as soil, water, air and its availability in the air is more than that in water and soil. It is released into the atmosphere due to various anthropogenic activities such as combustion of fossil fuels, smelting of ores like bauxite, and reduction of phosphate rocks in fertilizers manufacturing. Then it enters the leaf via stomata and intercellular spaces of mesophyll cells and diffuses further into vascular tissue. Fluoride damage mostly occurs on the leaf tips and margins (Nouchi et al., 2018).

Injury symptoms from Ammonia are blackening and bleaching of leaves, lesions between veins, spotting, and color change of the fruits. When plants exposed to ozone shows a variety of symptoms such as tissue collapse, interveinal necrosis, stipple, flecking, mottling, chlorosis, bleaching. Stunted growth and flowering, bud formation suppressed. Chlorine effects are similar to those caused by SO_2 and fluorides. Two different types of injuries can

occur; plants with broad leave, necrotic, bleached, or brown areas that tend to be near the leaf margins, tips, and between the veins (Das et al., 2018).

The most toxic Phyto air pollutant next to O_3causes bronzing, or silvering which develops in bands, the thickness of the leaf blade which results in collapse, bleaching and transverse bands, senescence, chlorosis, growth is stunting and premature leaf fall. It is considered as most toxic to small and young plants (Kour and Adak, 2021). Ethylene also influenced the plant hormones and growth regulators' activities that affecting mature tissues and normal organ development without generating leaf-tissue collapse and necrosis. Bud abscission, epinasty which is downward curling of the leaves and shoots are the injuries to broad leaved plants thereby affecting the overall growth of plants (Nouchi et al., 2002).

Among various air pollutants, SO_2 and NO_2 are most toxic to plants (Hamid and Jawid, 2009). Over the past few decades, emissions $SO_2$and NO_2 into the atmosphere have increased in many nations, particularly in some Asian nations. The NO_x and So_x concentrations will continue to rise, and the standard levels of these pollutants will continue to be exceeded (Sheng and Zhu, 2019). The SO_2 was one of the first air pollutants to be shown to harm plants and the environment. The amount of SO_2 in the air has significantly increased due to the combustion of fossil fuels (Wei at al., 2017).

1.3.1 Sulphur dioxide

Sulphur dioxide, a short-lived, colorless, and foul-smelling poisonous gas, has garnered classification as a "criteria pollutant" by both the European Commission in 2015 and the US Environmental Protection Agency in 2016. This noxious gas primarily originates from the combustion of fossil fuels. It affects human health and global ecosystems (Wei et al., 2017). As the major precursor of sulphate aerosols, it has significant impacts on global and regional climate by altering radiative forcing and reducing visibility. It also contributes to acid deposition which harms aquatic and terrestrial ecosystems.

Anthropogenic SO_2 emissions, particularly those from fossil fuel combustion, significantly exceed natural emissions on a global basis due to the high concentrations of sulfur contained in fossil fuels (Smith et al., 2011). For several decades, SO_2 emissions and acid deposition have been posing a significant problem worldwide (Bytnerowicz et al., 2007). It is considered a major atmospheric gaseous pollutant and occurs in the environment in the

following concentration range i.e. ambient level 2 µg m -23 µg m; High level > 50 and critical level > 75 SO2µg m (Yadav et al., 2019). The concentration of SO_2 above the ambient threshold limits are likely to affect plants and animals due to (i) changes in enzyme systems (ii) cellular changes in chemical components and (iii) Physical structure (iv) metabolic changes that slow growth and reduce productivity and (v) Immediate tissue degeneration depending on the sensitivity of the crop (Mazid et al., 2011).

Plants that close their stomata at night can better resist SO_2 during that period. It has been studied that conifers are considered more susceptible in spring and early summer as the new needles are growing longer. After absorption through stomata, it combines with water to form a poisonous sulfite, but it is gradually oxidized to relatively harmless sulphate. Thus, the toxicity of SO_2 depends on the rate at which it is absorbed by the plant and faster absorption of SO_2 through stomata will cause greater damage (Sha et al., 2010).

The effects of SO_2 on a plant occur through stomatal absorption and trapping in mesophyll chloroplasts and vacuoles at a level proportional to the SO_2 concentration in the air (Mazid et al., 2011; Baciak et al., 2015).The effect of SO_2 is the best-known example of a direct phytotoxic effect on plants (Bytnerowciz et al., 2006, Yadav et al., 2019).

The rate of penetration through stomata is influenced by environmental factors like solar radiation, humidity, and temperature. After entry into leaf cells, sulfur dioxide undergoes oxidation to sulfites, causing a reduction in leaf pH and upsetting the oxidation-reduction equilibrium in plant tissues. Consequently, chlorophyll loss occurs, disrupting the photosynthesis process at the enzymatic level and impeding electron transport, ultimately leading to decreased CO_2 absorption (Baciak et al., 2015). The breakdown of SO_2 produces bisulphite and sulphite ions although sulphite is toxic, but in low concentration it is converted into sulphate by chloroplast which is not toxic (Rahul and Jain, 2014).

In light of the fact, that Sulphur (S) is an essential nutrient and also limited in soil. The ability of plants to use SO_2 to meet S requirement may prove to be an important strategy for adaptation and mitigation of atmospheric SO_2 (Lee et al., 2017). The most common anatomical effects of sulphur deficiency in plants include growth retardation, alternation of photosynthesis, stomatal movements, enzymatic activities, protein synthesis, interference in various stress conditions, membrane functioning etc (Mazid et al., 2011). However, ambient SO_2 also helps to assimilate and produce amino acids containing sulphur for instance cysteine and methionine. The potential impact of SO_2 on growth and development of plant is

determined via level of exposure. High levels of SO_2 are perceived by the plant as abiotic stress and activate a succession of biochemical and metabolic responses for the adaptation and survival of plantspecies under SO_2 stress. Biochemical changes under SO_2 stress produces wide range of reactive oxygen species which result in oxidative stress in plants (Muneer et al., 2014).

Differences in sensitivity of plants to SO_2 stress can be controlled by optimized production of antioxidants such as ascorbic acid etc. and activity of anti-oxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), etc., which remove harmful reactive oxygen species and protect the plant from oxidative damage (Chauhan and Joshi, 2010).The most common morphological injuries in leaf are interveinal necrosis followed by chlorosis (Baciak et al., 2015). Carmichael et al. (2003) conducted a study that involved measurements of gaseous pollutants at 50 sites across Asia, Africa, South America, and Europe. In India, a research was conducted in Agra, that recorded the highest concentration of sulfur dioxide (SO_2), indicating significant contributions from anthropogenic sources like power plants, industrial boilers, and heating and cooking activities (Gupta et al., 2008).

1.3.2 Nitrogen oxides

The atmosphere consist several number of nitrogen oxides (NO_x), including nitric oxide, nitrous oxide (N_2O), nitrogen trioxide (N_2O_3) and NO_2. Once released, it rapidly dispersed into the atmosphere and also contributes in the formation of ozone (O_3) and nitric oxide (Okasnen and Kontunen- Soppela 2021). It has severe impact on plants either directly after being deposited on plants, soil or water, or indirectly through chemical reactions in the atmosphere. When dissolves in cells it produces nitrite ions (NO_2, which can be toxic at high concentrations leading to cell acidification (which results in generation of reactive oxygen species) and nitrate ions (NO^{3-}).

In spite of this, it reduces the plant growth in high concentration and also inhibits photosynthesis (Hamid and Jawaid, 2009; Kour and Adak, 2024). NO_x are one of the most widely emitted pollutants in the world, and yet little is known about their effects on agriculture. The direct damage to crop cells and growth inhibition can occur due to the promotion of ozone (O_3) and aerosol formation by nitrogen oxides (NO_x). Among the oxides of nitrogen, NO and NO_2 act as phytotoxins directly impacting plant growth and reducing yields. NO_x can affect plant health indirectly through two main pathways. Firstly, NO_x serves

as a significant precursor to troposphere ozone (O_3) formation, which is another phytotoxins known to decrease crop yields. Particularly in regions with elevated levels of volatile organic compounds (VOCs), variations in NO_2 closely correlate with changes in O_3 levels. Secondly, NO_x acts as a precursor to particulate matter aerosols. In the presence of ammonia, NO_x can lead to increased concentrations of ammonium nitrate aerosol (NH_4NO_3) and may also oxidize sulfur dioxide (SO_2), generating ammonium sulfate aerosols [$(NH_4)_2SO_4$] (Lobell et al., 2022). Nitrogen oxides are also among the essential macronutrients of plants that improve plant growth (Kour et al., 2024). It enters as nitrate (usually through the roots) and is reduced to NH_3before being used by plants to form of amino acids (Mansfield et al., 1982, Wei et al., 2017).

Additionally, when ambient NO_x concentrations are high they form HNO_3, HNO_2 and NO in plants. The overall efficiency of uptake of various chemical species into cells through stomata, cell wall and plasma membrane is an important factor that determines the ability of plants to absorb NO_2, and thus explains the variation between plant taxa (Morikawa et al., 2003). Plant absorbs gaseous NO_2 more rapidly and NO_2 is considered more toxic than other oxides of nitrogen. Large, irregular brown or black spots are the most obvious symptoms of NO_2.When present at equal concentrations, the absorption of nitrogen dioxide per unit leaf area has been studied to be approximately 3 times higher than that of nitric oxide (NO). Consequently, NO_2 is considered more toxic than NO.

However, the phyto toxicity of NO_2 is rare and significantly lower compared to sulfur dioxide (SO_2) and ozone (O_3). When NO and NO_2 dissolves in the extracellular solution of leaves, and combine to form equal amounts of nitrite and nitrate, along with protons (H^+). Nitrate (NO_3) serves as a valuable nitrogen source for plants, similar to when it is absorbed through the roots (Hamid and Jawaid, 2009; Kour and Adak, 2024). Despite general understanding of the potentially harmful effects of NO_x; few studies have attempted to measure its effects on crops on a large scale. Several studies have examined pollution levels near an urban area and plant health as well (Agrawal et al., 2003). For example, the WHO's g/m^3guidelines suggest "no effect" levels of 15 to 20 for vegetation for annual average NO_2 [about 8 to 11 parts per billion (ppb)] whereas the level of NO_2 in most areas generally exceed these values (Cersosimo et al., 2020).

1.4 Effective Air quality management

For all origins of pollutants, various regulatory agencies develop strategies and enact appropriate action plans. Source complication and their effect on receptors are interlinked with source, strength, meteorology, release height, atmospheric transformations, and other factors (CPCB, 2020; Kour and Adak, 2021).

The initiation of the National Clean Air Program (NCAP) is anticipated to bring about substantial reductions through effective management of ambient air pollution, following recent successes observed in China and the remarkable low emissions of air pollutants witnessed North America and Western Europe (Brauer et al., 2019).

To improve air quality, a wide range of interventions have been implemented. Efficient management of air quality comprises three key elements:

1) Quantification and detection of key pollution sources.

2) Implementation of non regulatory and regulatory measures to mitigate cause emission.

3) Measurement and mitigation of ambient air quality to aid in cause detection assess growth towards quality of ambient air and evaluate the efficacy of emission reduction efforts.

They also facilitate comprehensive comparisons beside international or national thresholds and guidelines. Also, it aids in assessing the efficiency of emission reduction measures, thereby enabling the monitoring of overall progress. Traditionally, ambient air quality measurements involve the deployment of multiple fixed- location monitors at strategic sites to evaluate regional or urban background concentrations, as well as to discern the influence of particular sources like industrial, vehicle exhaust, thermal plants etc. They are equipped with vigorous, high-quality instruments capable of providing real- time or estimations of common air of such as sulfur dioxide (SO_2), carbon monoxide (CO), nitrogen oxides (NO_x), and PM. In many regions, data from these monitoring stations are made publicly available in real-time through websites and are included into air quality index to serve as vital open information accessing tool.

Additionally, ambient air evaluations provide crucial assistance in air quality forecasting and models describing variation within regions. However, it's important to note that setting up and maintaining such monitoring stations incurs significant costs. This financial aspect may be essentially significant in swiftly rising economies like India, where a various range of small sources of pollution exists (Brauer et al., 2019). Many scientific abatement measures

have been taken at the source but the most effective and sustainable way is plantation and development of green belts (Sharma et al., 2017; Panda et al., 2018).

Plants act as natural filters on earth to ameliorate pollution as they captured a large amount of carbon dioxide and store it as food material. Any alteration in atmospheric conditions has a direct impact on the physiology and biochemistry of plants and causes various physiological changes before showing noticeable injury to the foliage (Kour et al., 2021).

A leaf is most sensitive and continuously exposed to air pollutant which absorbs, accumulate and integrate pollutants to mitigate their level in ambient air (Kour and Adak, 2021; khanoranga and khalid, 2019; Chandawat et al., 2011; Agbaire and Esiefarienrhe 2009; Joshi and swami 2007; Tanee et al., 2014; Bora and Joshi 2014; Vyankatesh and Arjun, 2013; Pradhan et al., 2016; Sharma et al., 2019; Kumar et al., 2017; Pradhan et al., 2016).

In stress conditions plants are considered as tolerant, if they experience low damage and as sensitive if they exhibited higher injuries. The tolerance and sensitive behavior of plants towards pollutants may provide a simple tool of monitoring air pollutants and air quality of an area (Subramani and Devananda, 2015; Bakiyaraj and Ayyappan, 2014).The method used to assess the response of plant against pollutants is known as air pollution tolerance index (APTI) which can be employed for abatement of air pollution in an urban environment. Since, APTI depends on the biochemical parameters of leaf such as chlorophyll, ascorbic acid, leaf extract pH, and relative water content.

These parameters are analyzed and computed together in an APTI formula and obtained APTI value. Plant with higher APTI value can be used as sink in polluted sites. The climate condition and the physicochemical characteristics of air pollutants, their residence time in the surrounding have effect on plants and animals (Pradhan et al., 2015). To assess the susceptibility inside the plants, biochemical parameters act as a key indicator and more than one parameter needs to be assessed for better reliable results. The concentration of air pollutants in plants is directly related to the changes in the biochemical parameters (Das et al., 2010).

Chlorophyll is one of the important biochemical parameters which represent the main core of energy production in green plants. It performs a major function in growth and development of plant. Determination of chlorophyll content is considered to be one of the significant methods to assess the effect of air pollution on plant. The photosynthetic activity of plants is regulated by their chlorophyll levels, which in turn promote the growth and development of plant. The variation in chlorophyll content within plants is determined by factors such as

species type, air pollution levels, leaf age etc (Ghafari et al., 2020; Anake et al., 2022). Also, chlorophyll content in plants is influenced by various abiotic factors such as temperature, drought, light intensity, and salt stress.

It is the primary site of attack of air pollutants such as SPM, SO_2, and NO_x (Wei et al., 2017; Kour and Adak, 2024). High levels of SO_2 gas can lead to the destruction of chlorophyll structure, transforming it into pheophytin through the displacement of Mg^{++} ions by two hydrogen atoms. The decrease in chlorophyll concentration may also be due to the disruption of thylakoid membranes within the chloroplasts. The enzymatic activity of enzyme chlorophyllase may also increase due to increased concentration of air pollutants that might be responsible for the destruction of chlorophyll (Geeta and Namrata, 2014; Rai and Puneet, 2021).

Ascorbic acid is another important biochemical parameter for the estimation of APTI. It is an antioxidant and present in greater amount throughout the plant. Also, it provides tolerance to unfavorable environmental conditions, air pollution and any other stress (Keller and Schwager, 1977; Pathak et al., 2011; Rai and Panda, 2014). It is a strong reductant which maintains the cell membrane stability and cell division under pollution stress conditions and also protects the chloroplast against SO_2 induced pollution by free radicals Hydrogen peroxide (H_2O_2), Oxygen (O_2), Hydroxide (OH) accumulation (Sharma et al., 2017 ; Zhang, et al., 2016 and Rai 2019).

High ascorbic acid content results from high production of reactive oxygen species during stress conditions in plants. Thus, higher ascorbic present in the plants considered higher tolerant plant towards air pollutants (Karmakar et al., 2021). For instance, when plant absorbed high amount of SO_2, it increases the amount of ascorbic acid (Tripathi and Gautam, 2007; Rai and Panda, 2014). Joshi et al., 2009 studied when plants exposed to pollution or various other stresses, ascorbic acid reacts with hydrogen peroxide and protects carotenes and tocopherols.

Besides, in response to water stress ascorbic acid in plants protects thylakoid membrane from oxidative damages. Variation in ascorbic acid content stands out as a significant contributing factor to the diverse tolerance capacities observed among trees when confronted with various air pollutants (Bandara et al., 2021).

The pH exhibited a significant role in deciding, plant's tolerance level against pollution and particularly regulates the SO_2 sensitivity in plants (Gharge and Menon, 2012). Low pH in leaves influences the stomatal activities, including respiration, transpiration and

photosynthesis (Liu and Ding, 2008; Khanoranga and Khalid, 2019). As Ahmad et al., 2019, Karmakar et al., 2021 found that low pH is responsible for reduced photosynthesis in plants. Previous literature explained the strong relationship between pH and air pollutants (Kaur and Nagpal, 2017; Banerjee et al., 2018; Singh et al., 2020). Alkaline pH during stress condition considered that the plant is tolerant and can be used as sink to air pollution (Khanoranga and Khalid, 2019; Sen et al., 2017; Rai and Panda, 2014). The variation in leaf pH to acidic is generally considered due to acidic pollutants such as NO_2 and SO_2 in the ambient air.

When specifically SO_2 absorbs by plants with the help of stomata, it absorbs in water and produce bisulphate, sulphites and their ionic species thereby releasing protons that affect the pH. Consequently, it shifts pH towards acidic across a majority of plant species (Ahmad et al., 2019; Achakzai, et al., 2017).

Higher pH in leaves provides better tolerance in plants against pollutants.The leaf relative water content is the amount of water present in it, to sustained physiological balance under stress conditions and high transpiration rate (Pandey et al., 2015). It is mainly attributed with protoplasmic permeability. The higher amount of relative water content maintains the physiological balance under stress condition. Due to air pollution, RWC in leaves gets reduced as a result of which the overall physiological condition of the plant becomes unstable. High amount of air pollutants increased the permeability of cell and decreased the plant nutrients (Karmakar et al., 2022; Kour and Adak, 2021). Ghafari et al., 2020 reported that reduced water content due to air pollution results in closure of stomata, and decrease in leaf transpiration. A higher Relative Water Content (RWC) in plants not only serves to low pH within the cell but also confers resistance against drought conditions (Sen et al., 2017; Karmakar and Padhy, 2019; Rai, 2016).

Shrestha et al., 2021 reported in their study that transpiration competence of the plant can be determined by leaf water content. Depending upon the environmental factors and plants species, relative water content varies. Higher RWC increase tolerance to air pollutants where as decrease in RWC influenced stomatal conductance and carbon dioxide absorption in plants (Roy et al., 2020, Jain et al., 2019 and Pathak et al., 2019, Punit et al., 2021). The high relative water content in leaf show tolerant behavior of plant against pollutant (Sahu and Sahu, 2015). Similar findings were reported by Manjunath and Reddy in their study that higher RWC had better air pollution tolerance.

1.5 Environmental Factors

Environmental factors have been considered to be one of the most influential factors affecting biochemical parameters. Among various environmental factors, the following environmental factors have the strong influencing effects on biochemical parameters.

1.5.1 Effect of Temperature

It is one of the major ecological variables determining the natural distribution of plants. Like almost all other growth processes, photosynthesis also predominates affected by temperature (Berry and Bjorkman, 1980). It also plays a fundamental role in biological systems, as chemical reaction rates are intricately linked to tissue temperature. This relationship is crucial because the energy required to initiate reactions, known as activation energy, is directly influenced by temperature (Moore et al., 2021). However, antioxidant ascorbic acid is also strongly influenced by temperature.

Schonhof et al., (2007), found relationship between temperature and light intensity with ascorbic acid. Since, as an antioxidant ascorbic acid implicated in photo protection and provides tolerance to environmental stresses. Also, Evers (1994) found that low temperatures metabolized low carbohydrates which results in higher ascorbic acid amount in plants. Interestingly, pH is directly related to enzyme function. On other side, temperature is one of the factors which affect the enzyme function. Thus, temperature indirectly affects the pH also including ascorbic acid. Conversely, higher temperature disrupts metabolic processes, photosynthesis, vapor pressure deficit and leaf water status (Urban et al., 2017; Merilo et al., 2017). High VPD increases the water loss which affects evapotranspiration, nutrient uptake and plant water status (Mott and Peak, 2013). Besides, higher temperature affects the photochemical reaction occurred in thylakoid membranes and carbon metabolism in the chloroplast (Yamori et al., 2008). Other side, low temperature also disturbs the photosynthesis process including electron transport, carbon reduction cycle and stomatal operation (Alen and Ort, 2001).

Hou et al., (2016) found in their study that chlorophyll content decreases when plants are subject to temperature stress. Schonhof et al., (2006) also studied that change in plant metabolism caused by different temperature and light intensity levels lead to changes in phyto chemical and ascorbic acid contents.

Sanghi et al., (2015) studied in their experiment when plants exposed to different temperatures for several days. They are more sensitive at low temperatures as compared to high temperature. With increase in temperature, transpiration tends to increase because the vapor pressure difference between leaf surface and air increases with increases temperature. High transpiration rate influences reduction in relative water content which causes loss of water and dissolved minerals, which eventually alter the tolerance index of plants (Tibbitts, 2014). Every biochemical parameter considered for tolerance studies is affected by temperature (high or low) according to previous literature. This cannot be overlooked when calculating the tolerance index of plants.

1.5.2 Effect of light intensity

Light intensity, or the amount of light reaching a given surface area, stands as the primary determinant of the rate of photosynthesis in plants, as studied by Chapman and Carter (1976),Taiz and Zeiger (2002), and Blankenship (2014). Radiation, a critical environmental factor influencing plant survival, growth, reproduction, and distribution, affects numerous physiological and morphological processes in plants (Keller et al., 2005; Kumar et al., 2011).

Despite its significance, controlling light intensity remains one of the most challenging environmental factors. Dole et al. (2004) discovered in their study that plants exposed to high light intensity absorb excessive light energy, leading to the deactivation of the photosynthetic apparatus or the loss of chlorophyll-containing reaction centers within chloroplasts. Conversely, Dai et al. (2009) observed in their research that under low light intensities, insufficient Adenosine triphosphate (ATP) production results in reduced carbon absorption and plant growth.

Furthermore, Correet al. (1983) and Kumar et al. (2013) studied hat low light intensities cause a reduction in stomatal conductance and photosynthesis rate, thereby decreasing the overall plant growth rate. Chloroplasts within plant cells contain light-absorbing pigments known as chlorophyll, essential for capturing light energy during photosynthesis (Mirkovic et al., 2017). Plants grown at high temperature conditions with moderate light and adequate water supply were highly sensitive (Juhren et al., 1957). Earlier, Duggar et al., (1962) observed in their experiment that in light preconditioning ascorbic acid levels increase due to which plants experienced less damage, and injury due to ozone decreases. Moreover, it was estimated ascorbic acid concentration in leaf was 2.5 to 3.3 times higher while ozone damage decline to approximately 50%. Later, Menser et al., (1963) also observed light influences

resistance in plants from ozone by changes the level of ascorbic acid concentration and it is more effectively seen in mature leaves than young leaves.

Similarly, Eskling and Akerlund, 1998 observed higher amount of ascorbic acid content in leaf has been accredited to light intensity stress and later Smrinoff and Wheeler, (2000) reported as an antioxidant ascorbic acid is implicated in photo protection and provides tolerance to environmental stresses. Heck et al., (2012) also reported that, in light exposures plant were 5 times more susceptible to SO_2 than dark exposures. When plants are exposed in light are more susceptible to phytotoxic air pollutants than in dark. Darkness period prior to exposure and the extent of exposure is the two factors that may also affect the sensitivity of plants. Light is also one of the important environmental parameters and it is necessary to explore their relationship with biochemical parameters.

1.5.3 Effect of Humidity

Humidity (atmospheric moisture) is another important environment factor for plant growth and development. The most common effect of humidity is to control the rate of transpiration in leaves. A decrease in ambient humidity increases the VPD between the air and moist leaf surfaces and increases transpiration in leaves (Tibbitis, 1979). Humidity directly affects the opening of stomata (Bunce, 1982). Amin et al., 2023 found that exposure to low temperature and high humidity stress can hinder plant growth and development by reducing enzymatic antioxidant activities, triggering the production of reactive oxygen species (ROS), inducing lipid per oxidation, suppressing chlorophyll biosynthesis, and impairing the photosynthetic system. They also found enzyme activity was increased in cucumber at different levels of low temperature and high humidity. On other side, Lysenko et al., (2023) reported that the role of atmospheric humidity is ambiguous; its effect on the photosynthesis is unexplored.

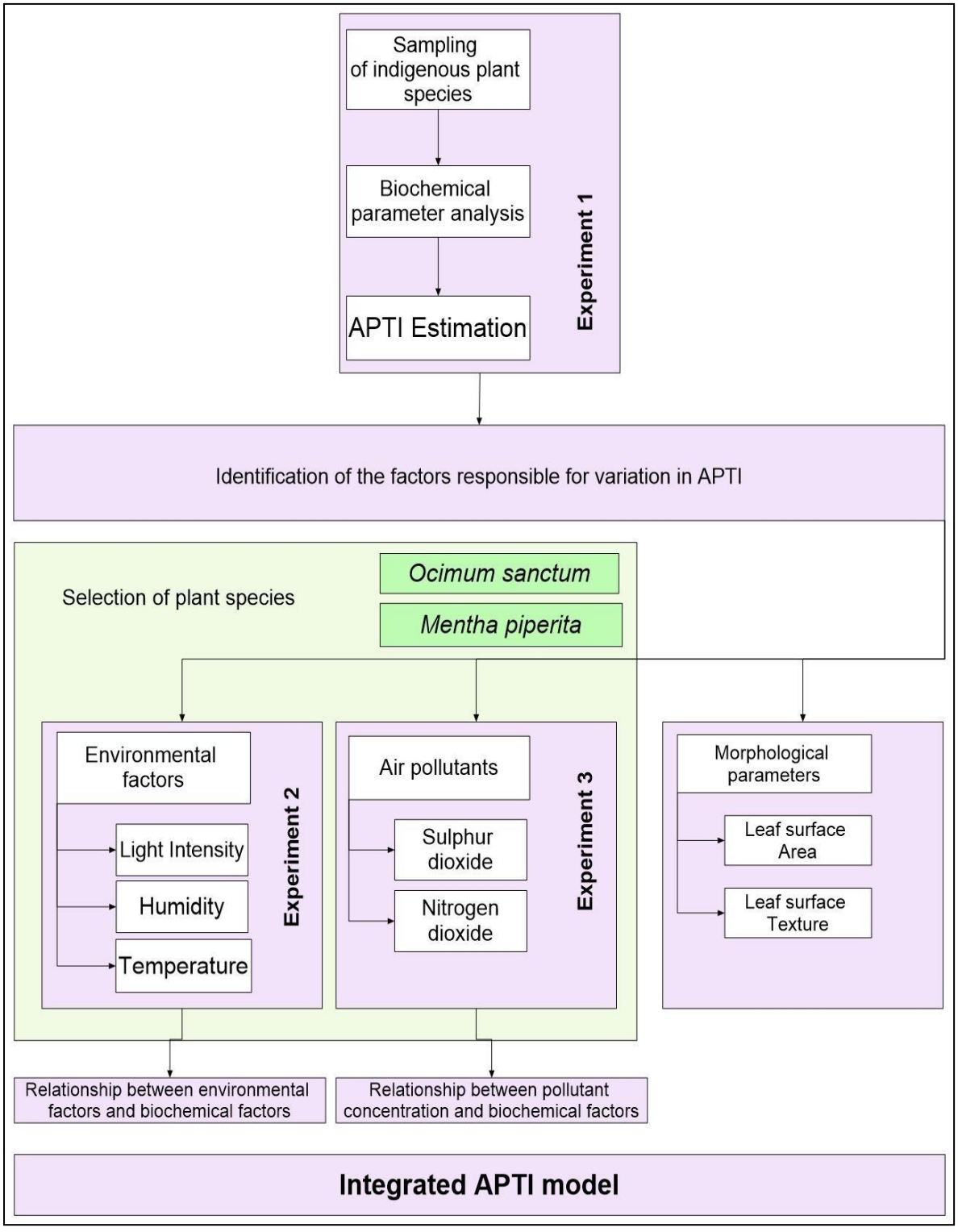
In the present study, it is assumed that the three environmental factors selected (Humidity, Light intensity and Temperature) exhibit interdependence and explicitly influences plant metabolism, physiology, morphology and biochemistry. This assumption was indispensable to consider insensitive plants' resilience against stress. Another assumption is considered to be the relationship between biochemical parameters with environmental parameters and atmospheric pollutants (SO_2 and NO_2).

1.6 Objectives of proposed work

a. To study the effect of environmental factors such as temperature, humidity and light intensity on plants tolerance against air pollutants.

b. To develop an integrated approach for inclusion of SO_2 and NO_x concentration for plants tolerance calculation.

1.7 Organization of the proposed work



1.7.1 Sampling of plant species

The current investigation examined 15 plants sampled across three distinct regions (Industrial, Roadside and Control) of Phagwara to assess their APTI (as explained in the chapter 3). The study encompassed three seasons: winter summer and Monsoon. The selected plants species included *Ficus benghalensis; Ficus religiosa, Murraya koenigii, Cascabela thevetia, Melia azedarach, Psidium guajava, Ziziphus mauritiana, Ocimum sanctum, Mentha piperita, Syzygium cumini, Mangifera indica, Polyalthia longifolia, Morus alba, Alstonia scholaris and Moringa oleifera* were sampled from each sampling site. The inclusion of multiple species enhances the likelihood of identifying plants resilient to pollution compared to relying on a single or few plant species. Fresh mature leaves from these sampled plants were collected for APTI estimation.

1.7.2 Biochemical parameter analysis

Prior studies have elucidated the influence of atmospheric pollutant on various physiological and biochemical attributes of plant leaves including ascorbic acid content, relative water content, total chlorophyll content, and pH, stomatal conductance. In the current study, we focused on evaluating the impact of air pollution on these biochemical parameters (Detailed explained in chapter 3). These parameters were selected to comprehensively assess plant's response to the prevailing air pollution conditions. By considering multiple physiological parameters, our current study aimed to gain a holistic understanding of plants adaptability and resilience to environmental stress.

1.7.3 Air pollution tolerance index estimation

The Air Pollution Tolerance Index (APTI) was calculated by integrating four key biochemical parameters of the leaf, namely, ascorbic acid, leaf extract pH, total chlorophyll content, and relative water content. According to Singh and Rao (1991), APTI serves as a quantitative measure of a plant species resilience or susceptibility to atmospheric pollutants. In the present investigation, APTI was assessed across three distinct seasons (summer, monsoon, winter), revealing significant variations in the APTI values of each selected plants

species (Detailed explanation has been discussed in chapter 4). For instance, During Monsoon season, the measurement for APTI in *Melia azedarach* at industrial site has been observed to be 10 and at roadside was 11.1 while at LPU, it has been observed 17.5. For the summer season, the measurement for APTI in Melia azedarach at industrial site and roadside has been observed to be11.3and 11. 2 respectively while at LPU, its APTI value has been observed to be 16.4. For the winter season, the measurements for APTI in *Melia azedarach* at LPU has been observed to be11.2 while at industrial and roadside; its values have been observed to be7.3 and 8.2 respectively. Similar variations were observed for each plant species, concerning their biochemical parameters and APTI values.

1.7.4 Factors responsible for variation in APTI

The significant differences in APTI values of different plant species may arise due to myriad of factors encompassing environmental factors, pollutants, soil composition and morphological parameters governing tolerance mechanisms (Explained in chapter 4). In the current study, the following parameters were examined to explore their effects on plant tolerance.

1.7.4.1 Morphological parameters

Two morphological parameters, Leaf surface texture (LST) and Leaf surface area (LSA) were selected to explore their effect on biochemical parameters. However, relative water content and ascorbic acid exhibited correlation with LSA and LST, definitive conclusions regarding their significance could not be ascertained (Detailed explained in chapter 4).

1.7.4.2 Air pollutants (SO_2 and NO_2)

Previous scientific studies have underscored that (NO_2) and Sulphur dioxide (SO_2) is primarily responsible for causing damage to plants in polluted area. In the current study, NO_2 and SO_2 were specifically considered to investigate the influence on biochemical parameters. Recognizing that the previously selected areas were insufficient for providing decisive insights, six other areas in Punjab was selected to further explore the effects of air pollutants on the biochemical parameters of plant species (Detailed relationship discussed in chapter 6).

1.7.4.3 Environmental Factors

Environmental factors are known to profoundly affect plant tolerance responses. Hence, in the current study, the influenced of temperature, light intensity and humidity on plants biochemical parameters were investigated. These factors were selected due to their indispensable roles in modulating key physiological and biochemical processes essential for plant growth and development, as explained in the previous literature. The biochemical parameters of the two plant species (*Mentha piperita* and *Ocimum sanctum)* were examined under controlled environmental conditions (Detailed explained in chapter 5).

1.7.5 Relationship of biochemical parameters with environmental factors and air pollutants

The experimental data were acquired and meticulously analyzed to explore the relationship between environmental factors (Temperature, Light intensity and Humidity) and pollutant concentrations ($SO_2$and NO_2) concentrations with biochemical parameters. In the current study, an attempt was made to modify APTI model to deepen our comprehension of plant resilience mechanisms under stress.

CHAPTER 2 REVIEW OF LITERATURE

2.1 Background

This chapter aims to provide a comprehensive review of research conducted on air pollution and its effects on plants. Additionally, it explores the methodology of identifying tolerant plants using the Air Pollution Tolerance Index (APTI) method, and other parameters role in variation of biochemical parameters including environmental factors and air pollutants. The general objective of the present study is to understand the response of plant's biochemical parameter to environmental factor and air pollutants. However, the detailed reason for the variation of biochemical parameters has not been explained in detail in the previous studies done herewith. The assessment of impact of air pollution on morphological, anatomical and biochemical parameters of plants has been done by various workers. Several workers around the world have also monitored the ambient air quality of various places using APTI method.
In this chapter, review of the work of previous studies conducted by various researchers for the estimation of ambient air quality and effect of ambient air pollution on the plant species has been compiled and discussed.

2.2 Air quality

In developing countries, the air quality has witnessed a concerning decline over the past 3 to 4 decades, largely due to rapid industrial expansion and increase in transport emissions. The rapid pace of urbanization, industrialization, and various commercial activities has significantly contributed to the escalation of air pollution (Kaur et al., 2017; Kour and Adak, 2021).

In the last three decades, the gradual rise of the Indian economy, as well as rapid expansion in the industrial and urban sectors has resulted in a progressive degradation in air quality in developing countries like India (Safeena et al., 2021). It results in a significant risk to living organisms including plants, animals, and humans (Agbaire and Esiefarienrhe, 2010; Karmakar et al., 2021) It has become major environmental threat human health, environment and economy as well. Air pollution is a relative term, encompassing situations where the air contains substances at concentrations that pose harm to human health and detrimentally impact ecosystems, as well as socially valued materials and structures.

Paul et al., (2009) defined air pollution as when solid and gaseous pollutants discharged anthropogenically in the environment beyond the threshold limits.

Frank et al., (2015) and Ritchi et al., (2017) studied the history of London air pollution which gives us an idea of the future of today's rising megacities cloud. Azam et al. (2016) defines air pollution as encompassing all the detrimental effects stemming from any sources contributing to the pollution of the atmosphere and/or the deterioration of ecosystems. The data obtained have been compiled, statistically analyzed and released in two 10 years volumes, the first for 1967-1977and the second for 1978-1987. Air pollution and its study by one way or other run parallel with the developmental activities at global level. The WHO expert committee report on Air Quality Criteria and Guidelines for Urban Air Pollution is one of the authentic documents in this direction (WHO, 2000). In recent decades, many developing countries, including India, have witnessed a concerning decline in air quality. In response to this pressing issue, the Government of India took proactive measures by enacting the Air (Prevention and Control of Pollution) Act in 1981. This legislation aimed to regulate and mitigate air pollution, laying the groundwork for more comprehensive action in subsequent years.

The responsibilities outlined in the Air Act were further reinforced under the Environment (Protection) Act of 1986, underscoring the government's commitment to addressing environmental concerns comprehensively. Subsequently, in line with these legislative mandates, the Central Pollution Control Board initiated the establishment of the National Ambient Air Quality Monitoring (NAAQM) network during 1984-1985. This network was designed to systematically monitor air quality across various regions of the country, providing crucial data for policymakers and environmental agencies to evaluate the extent of air pollution and formulate useful strategies for its control and mitigation. The programme was later named as National Air Quality Monitoring Programme (NAMP) and under this, 290 stations spread over 90 cities / towns have become operational (CPCB / NAAQMS/14/1999 - 2000) (Nandini and Dayal, 2000).

When the pollutants released in to the urban atmosphere, the rate of transport, transformation, dispersion and deposition of pollutants depends upon the prevailing meteorological conditions of an urban atmosphere. However, the concentration of pollutants will comparatively be higher at commercial and industrial sites than the residential areas (Glen et al., 2018).

2.3 Air pollutants and its effect on plants morphology and physiology

Ninova et al. (1983) studied anatomical and morphological characters of *Platanus acerifolia* at various levels of air pollution. They reported the reduction in the leaf lamina and petiole size due to air pollutants.

Jahan and Iqbal (1992) evaluated the effect of air pollution on morphological and anatomical characters on leaves of various roadside plants affected by automobile exhaust. They stated that there was no visible change seen on morphological and anatomical characters. Some reductions in the leaf's characters were observed in plants which collected from the city polluted site. There was a significant reduction in leaf length, leaf area, length of the petiole and anatomical characters of *Guaiacum officinale* from the polluted site.

Tiwari et al. (2008) conducted a study on the impact of air pollution on the foliar morphology of two species of Cassia in Indore city, India. Their findings revealed a decrease in various parameters examined, including leaf size, fresh and dry weight of leaves, as well as the number of stomata, stomatal index, and size, dry weight, length, and breadth ratio in plants growing in polluted habitats.

Prajapati and Tripathi (2008) assessed the efficiency of dust absorption ability of selected plants species. They evaluated the effect of dust deposition on biochemical parameters of plants. They had found that in winters, plants exhibited maximum dust deposition followed by summer and rainy season. The chlorophyll content is inversely proportion to dust deposition whereas, chlorophyll exhibited direct relation with dust deposition. Higher dust deposition was recorded in *Dalbergia sisso* and *Dendrocalamus strictus*

Gostin (2009) studied the leaf morphological characters of the plants around the industrial areas and roadside. He reported that plants absorb the pollutants on their leaf surface. Leaf thickness, height and length of epidermal cells, stomatal cells, stomata length and stomatal index showed differences in a polluted and unpolluted environment.

Seyyednejad et al. (2009) investigated the impact of air pollution on certain morphological and biochemical factors of *Callistemon citrinus* in the petrochemical zone located in the southern region of Iran. They reported a reduction in the morphological parameters affected by the air pollution such as length of vein and leaf, breadth of leaf and leaf area.

Pourkhabbaz et al. (2010) investigated the effect of environmental pollution on leaf properties of urban plan tree, *Platanus orientalis*.

They concluded that urban conditions affect the leaf properties and led to reduction in photosynthesis due to reduction in leaf area, lower stomatal densities and pore widths. The internal anatomy was not affected.

Chauhan and Joshi (2010) studied the effect of ambient air pollutants on wheat and mustard crops growing near urban sites and industrial areas of the Haridwar district in Uttar Pradesh. They reported that the plant height of wheat and mustard plant at four 31 sites showed significant variations at the industrial sites in comparison with their control sites respectively due to elevated air pollutants.

Loganathan et al., (2012) studied the effect of dust pollution on morphology and histology of some medicinally important plants. They concluded that cement dust pollution has harmful effects on fresh and dry weight of leaves, leaf area, leaf length, stomatal index, petiole length, saturated weight, RWC, pH.

Leghari and Zaidi (2013) conducted a study to assess the influence of air pollutants on leaf morphology across various plant species. Results revealed a significant reduction in leaf length, width, area, and petiole length across all plant species in polluted areas compared to control sites. Additionally, notable variations in morphological parameters were observed among different seasons, with the highest reduction percentage recorded during summer, followed by autumn, while the lowest reduction percentage was observed during the spring season.

Seyyednejad and Koochak (2013) studied morphological and biochemical responses in *Prosopis juliflora* growing in the area surrounding one of the oil fields in the southwest of Iran. They reported that the leaf area, length of the petiole, chlorophyll and carotenoid content and soluble carbohydrate contents reduced in the polluted area as compared with the plants growing at the control site.

Nandy et al., (2014) assessed the morphological damages to leaf length, breadth, Leaf length/breadth ratio and visible injuries in plants due to vehicle exhaust growing at roadside in Kolkata, India.

They reported that *Ficus bengalensis*, *Alstonia scholaris* and *Neolamarckia cadamba* were exhibited higher tolerance. *Ficus religiosa* was observed as a less tolerant as it is may be due

to resistance capability of the leaf surface. They concluded that there was an adverse effect of air pollution, mainly morphological damage. However, the present study focused on two air pollutants, SO_2 and NO_x among all the common air pollutants.

2.3.1 Oxides of Nitrogen (NO_x)

The nitrogen oxides are usually used to represent the composite atmospheric concentrations of the gases such as a NO and NO_2. These two, under urban atmospheric conditions, are primarily involved in air pollution (Dohmen et al.,1984). The United States Environmental Protection Agency (USEPA) only regulates NO_2, because it the most common type of NO_x produces anthropogenically (USEPA, 1999). It has severe impact on plants either directly after being deposited on plants, soil or water, or indirectly through chemical reactions in the atmosphere (Oksanen& Kontunen-Soppela, 2021). Further, it absorbs in cells and produce nitrite ions cell acidification and nitrate ions In spite of this it reduces the plant growth in high concentration and also inhibits photosynthesis (Hamid and Jawaid, 2009; Sheng and Zhu, 2019). The NO_2 uptake was reported to be about three times higher than NO per unit leaf area. When concentrations of both these two gases are equal, NO_2 is considered more toxic than NO (Wei et al., 2017; Sheng and Zhu, 2019). Since the late 70's plants are being used as accumulators as well as biomarkers of air pollutants, especially for detection, recognition, and monitoring of the occurrence of various air pollutants. Plants are non-motile and continuously exposed to air pollutants that may serve to raise the alarm for the possible effects on human beings due to air pollution. Hence the use of plants is often advisable for the determination of the air pollution load and its effects in different areas.

2.3.2 Oxides of Sulphur (SO_x)

Sulfur oxides include major oxides as sulfur dioxide (SO_2) and sulfur trioxide. It is a colourless gas having characteristic pungent, irritating taste and odour (Pan, 2011). Sulfur dioxide can gradually react with the oxygen of the atmosphere to form Sulphur trioxide (SO_3) and as it is unstable compound immediately tends to react with water to form sulphuric acid (H_2SO_4). The combustion of sulfur-containing fossil fuel prompts the development of oxides of sulfur. Among all oxides of sulfur, SO_2 is the most common gas and exceptionally noxious to living life forms including plants and animals as stated by Schlesinger et al. (2000).

The SO_2 was first identified among other pollutants to cause harm to environment (Hamid and Jawaid et al., 2009; Kour and Adak, 2023). The amount of SO_2 in the air has significantly increased due to the combustion of fossil fuels (Wei et al., 2017). Due to its global ubiquity, ozone has been documented to induce visible injuries such as interveinal chlorosis and necrosis on leaves (Hamid and Jawaid, 2009). In the era of 1970s, still SO_2 was thought to be a major contributor to acid rain which damages the forests. However, in 1980s when the Clean Air Act came into effect the decrease of SO_2 in the ambient air led to sulfur deficit in crops plants. This may be because plants are injured at high SO_2 concentrations which incorporate SO_2 and H_2S into low sulfur pools such as cysteine and sulphate (SO^{2-}) (Wei et al., 2017; Kour and Adak, 2023).

In plants, after entering through the leaf stomata, SO_2dissolves in the cells and oxidized to bisulphite (HSO^{3-}) and sulphite ions (SO_3^{2-}). SO^{2-} is highly toxic; chloroplasts convert small amounts of it into SO^{2-} (Hamid and Jawaid, 2009). If they ($SO^{2-}$and SO^{2-}) accumulate in high concentration it causes SO_2 toxicity by inhibiting photosynthesis and energy metabolism (Wei et al., 2017; Baciak et al., 2015).Generally, SO^{2-}being 30 times more toxic than SO^{2-} (Thomas et al., 1943). As a result, plants experience, chlorotic spots, intervenial chlorosis and necrosis in leaves and brown tips in pine conifers (Sharma et al., 2017; Kour and Adak, 2023).

2.4 Air Pollution Tolerance Index (APTI)

After prolonged exposure to various air pollutants, plants experience morphological and physiological damage or injuries that can be studied with the help of the APTI. It is a method that evaluates the plant's response to air pollutants. It was reported that plants with a higher amount of APTI can be used as a sink in polluted areas. Therefore, APTI of the plants is an important parameter to be monitored for evaluating the tolerance and sensitivity of the plant species so that the development of green belts in polluted areas can be planned accordingly. APTI is estimated with the help of four biochemical parameters such as chlorophyll, relative water content, ascorbic acid, and leaf pH equally contribute to plants' tolerance against air pollution. These parameters were computed together in a single formula (Eq. 1.1) to obtain an empirical value that signifies the air pollution tolerance index of plant species.

$$APTI = \frac{A(T+P)+R}{10} \quad \text{..... (Eq.1.1)}$$

Where,

A is considered as ascorbic acid,
T is considered as total chlorophyll content
P is pH and
R is relative water content.

Singh (1993) analyzed the effect of pollution on plants. They reported a marked gradation as the pollutant load decreased from polluted to the control areas. According to him APTI varies from plant to plant depending on the ability to withstand the effect of pollutants without showing any external damage.

Shannigrahi et al., (2004) studied the tolerance of plant species in and surrounding an industrial site. They reported that *Mangifera indica*, *Moringa pterygosperma*, *Cassia renigera* and *Ailanthus excela* had the highest APTI values. Control areas had more APTI values than the polluted areas.

Karthiyayini et al., (2005) studied twenty-seven plant species and determined their APTI values. They reported that *Azadirachta indica* was the most tolerant as compared to other tree species. *Ricinus communis* in shrubs, *Amaranthus viridis* and *Cucurbita pepo* in herbs were most tolerant species.

Tiwari and Tiwari (2006) reported the air pollution tolerance index of some plants growing nearby Raigarh, India. They found that APTI was minimum in *Acacia nilotica* (5.21) and maximum in *Ficus glomerate* (15.02).

According to them plants having low APTI values were sensitive while the plants with high APTI values were tolerant to air pollution. Therefore, *Acacia nilotica* can serve as sink and *Ficus glomerate* can be considered as bio indicator for industrial area.

Joshi and Swami (2007) evaluated some economically important trees for their physiological responses to air pollution. They investigated the effect of automobile exhaust on plants such as *Eucalyptus citriodora*, *Mangifera indica*, *Tectona grandis* and *Shorea robusta* from the roadside. They recorded the highest APTI in *Shorea robusta* (9.02) and lowest in M. indica (6.76).

The high concentration of SO_2 and particulate matter present in and around the coal-fired industries influenced the distribution pattern of plants. Only tolerant plant species survived under stress conditions. Sensitive species were not able to survive in the polluted conditions.

Dwivedi and Tripathi (2007) investigated the plants in the surrounding area of brick industries at Varanasi. They studied ninety-nine plant species and reported that *Ricinus communis* with APTI 81.10 is the most resistant wild species uniformly distributed in the polluted sites. *Lepidium sativaum* with APTI 5.27 was found to be the most sensitive plant present only at control or less polluted sites.

Lakshmi et al., (2008) studied the industrial area of Vishakhapattanam. They evaluated air pollution tolerance index of twenty-four tree species. Twenty tree species showed APTI values less than 16. Tree species that showed APTI values above 18 like *Ficus religiosa, Zizyphus jujuba, Phyllanthus emblica* and *Cassia fistula* showed moderate responses by changing their biochemical contents.

Liu and Ding (2008) informed that a variety of Physiological parameters gave more reliable results. Some species exhibit air pollution tolerance index variation due to change in ambient temperature and water status of plants. The outcome showed that during the growing season's APTI was important.

Sulistijorini et al., (2008) estimated the tolerance index of plants selected from polluted areas of Jagorawi highway and control site of Sindang barang field in Indonesia. They reported that out of the eight plants studied, *Cinnamomum burmanii* was sensitive species and *Delonix regia, Pterocarpus indicus, Swietenia macrophylla* was found to be less tolerant species. *Lagerstroemia speciosa* was found to be more resistant to air pollutants.

Agbaire and Esiefarienrhe (2009) studied the APTI of six plant species in the surrounding area of Otorogun gas plant in Ughelli, South local government area of Delta State. The susceptibility of plants to air pollutants had found to be related to their index values. It could also be linked to the responses of plants to air pollutants.

Tripathi et al., (2009) studied the APTI of ten plant species from three locations i.e. residential, industrial and commercial areas of Moradabad city. They reported that brass and allied industries are the main cause of the rise in the level of air pollutants in the industrial site.

Jyothi and Jaya (2010) analyzed the APTI of plant species growing nearby National Highway passing through Thiruvananthapuram district and developed the use of plants as bio indicators. They reported that Polyalthia longifolia showed highest APTI value of 13.61 and was considered to be tolerant to air pollution. In the case of shrubs, they found *Clerodendron fortunatum* with APTI value of 7.34 to be more tolerant to air pollution.

Begum and Harikrishna (2010) examined the APTI of seventeen plant species growing in the area surrounding the three industrial sites of Bengaluru 16 city. Among the plant species studied *Syzygium cumini* considered as most tolerant.

Das and Prasad (2010) evaluated the seasonal variation in APTI for twenty common plant species according to the variation in seasons. The highest value of APTI was found in *Azadirachta indica* (16. 57) in the rainy season. This was followed by winter season (16.54) and summer season (11.93). The highest APTI value was observed in *Mangifera indica* during the rainy season (15.42) followed by winter season (14.41) and summer season (13.64).

Seyyednejad et al., (2011) evaluated the APTI of four plant species from the industrial zone of South Iran. They found that the APTI values of the unpolluted area were slightly higher than that of the polluted areas. They recorded the APTI values of 8, 8, 7 and 5 in *E. camalduensis*, *A.lebbeck*, *C. salignus* and *P. juliflora* respectively.

Thambavani and Sabitha (2011) studied five plant species growing near a sugar mill. They evaluated the API of the plants species and they reported that *Ficus benghalensis* and *Ficus religiosa* were considered the most tolerant plant species that can be suitable for industrial areas and could be proved best for green belts development.

Kuddus et al., (2011) studied the APTI of seven plants growing in the urban industrial region in Allahabad. These plants were also economically important. Among the plants studied and evaluated Mangifera indica (18.51) was considered as comparative tolerant species and *Artocarpus sp* (8.75) was found to be the most sensitive species to air pollution.

Thambavani and Maheshwari (2012) studied the APTI of fifteen tree species from semi-urban area of Virudhu nagar, Tamil Nadu. Based on API score they identified *Ficus religiosa* and *Mangifera indica* with the highest scoring (69%) to be most tolerant and suitable for heavy traffic areas to be planted along the roadsides. Species like *Azadirachta indica, Eugenia jambolana* and *Ficus religiosa* scoring > 60% can be planted in and around the industrial areas.

Bhattacharya et al., (2012) assessed the air pollution status of Anand city in Gujarat. They also considered the effect of the pollution on the dominant vegetation in that area. They identified three common species *Azadirachta indica*, *Peltophorum pterocarpum* and *Polyalthia longifolia* from eleven sampling sites. The order of tolerance was found to be *Polyalthia longifolia* (6.57– 10.22) > *Peltophorum pterocapum* (6.81-8.43) > *Azadirachta indica* (6.01-7.59).

Randhi and Reddy (2012) assessed the APTI of sixteen plant species across various zones including residential, traffic, industrial, and peri-urban areas in Hyderabad, Andhra Pradesh. They categorized the selected plants on their tolerance index values into sensitive, intermediately tolerant, moderately tolerant, and tolerant groups. Species such as *Delonix regia*, *Peltophorum pterocarpum*, *Alstonia scholaris*, *Ficus religiosa*, *Samania saman*, and *Azadirachta indica* exhibited high APTI values, indicating their tolerance to air pollution. They are suitable sinks to mitigate air pollution. Species like *Syzygium cumini*, *Terminalia catappa*, *Swietenia mahagoni* and *Saraca indica* can serve as bio-indicators of air pollution. They reported that in monsoon season APTI was high. It helps to unclog the stomata which increase the photosynthetic activity.

Mahecha G.S et al. (2013) examined the responses of the three plants and has determined the APTI of plants that include the *Annona squamosa*, *Ficus racemosa* and *Santalum album* by examining the biochemical and physiochemical parameters which are grown around in the Madri industrial area in Udaipur. The results clearly show that plant species *Santalum album* L having higher value of APTI.

Deepalakshmi A. P et al. (2013) studied the nature of plants exposed to some of the pollutants discharged from vehicles and carried out the assessment of air pollution tolerance index of ten wild plant species along the Bangalore roads that were busy. The results indicated that maximum reduction was seen in *Bougainvillea spectabilis*. *Ageratum conyzoides* was considered very much sensitive. *Peltophorum pterocarpum* and *portulaca oleraceae* are considered as tolerant to air pollution.

Bhattacharya et al. (2013) carried out an evaluation of air pollution tolerance index of six different plant species from nine stations of Baroda city. *Polyalthia longifolia*, *Mangifera indica* and *Azadirachta indica* showed highest tolerance value irrespective of three seasons namely winter, summer and monsoon in the study.

Bora and Joshi (2014) studied the APTI and API of *Azadirachta indica* (12.98), *Eucalytus sp* (12.61), *Ficus religiosa* (12.61), *Saraca indica* (13.71), *Shorea robusta* (12.64) and *Tectona grandis* (13.33). According to the API score, all the plant species were found to be tolerant to air pollution.

Rai et al., (2014) assessed five different plant species commonly growing along the roads of Aizwal, Mizoram. They computed the APTI values and reported that *Artocarpus heterophyllus* had high APTI value (9.3) and was tolerant. *Lagerstroemia speciosa* was found to have the lowest APTI value (6.6) which was considered as a sensitive plant. It can serve as a bioindicator for air pollution.

Vyankatesh and Arjun (2014) studied the APTI values of different roadside plants in the Nanded city, Maharashtra, India. They determined the physiological response of plant species for tolerance. They stated that plants with APTI score < 8 can be considered as sensitive species. APTI value between 8 to 10 can be considered as intermediate species and value > 10 to be considered as tolerant species. They reported that *Azadirachta indica, Eugenia jambolana, Moringa oleifera* and *Tamarindus indica* were tolerant species. *Acacia nilotica, Dalbergia sissoo, Delonix regia, Ficus bengalensis, Leucaena leucocephala, Mangifera indica* and *Polyalthia longifolia* were intermediate species. Species like *Eucalyptus, Ficus religiosa, Ficus glomerata,* and *Phyllanthus emblica* were identified as sensitive to air pollution.

Madan and Chauhan (2015) determined APTI and API of *Azadirachta indica, Ficus religiosa, Mangifera indica, Polyalthia longifolia* and *Syzygium cumini* growing in Haridwar city. They reported that Mangifera indica had the highest APTI value and Polyalthia longifolia had the lowest APTI value. The API score showed that *Ficus religiosa* was found to be highly effective for growing in the polluted areas.

Dhankar etal., (2015) assessed the APTI values of *A. lebbeck, A. indica, A. scholaris B. variegate, E.oblique, F. benjamina, F. religiosa, F. virens, F. benghalensis, M. indica, P. guajava, P.glabra, Saraca* asoca, *Syzygium cumini* and *Z. mauritiana* around Rohtak city. From the fifteen plants studied they suggested *F. virens* and *E. oblique* for green belt development.

Nayak et al., (2015) evaluated APTI of five plant species growing around the industrial area and Navsari agriculture university campus. They reported that in the industrial site *Casssia fistula* showed highest APTI value. This was followed by *Saraca asoca* and *Syzygium cumini*

which can be considered as tolerant to air pollution.

Tectona grandis and *Terminalia catappa* were found to be sensitive to the pollution. Hence, they recommended that *Casssia fistula, Saraca asoca* and *Syzygium cumini* should be planted in the industrial site for abatement of the air pollution. They also suggested that a green belt should be developed using these plant species.

Akilan and Nandhakumar (2016) computed the APTI values for *Azadirachta indica*, *Nerium oleander*, *Pongamia pinnata* and *Tamarindus indicus*. They selected Arcot, Ranipet and College farm located in Vellore district, Tamil nadu.

Gholami et al., (2016) investigated APTI values for six plant species growing in Ahvan, Iran. They reported that *Myrtus* (7.21) showed the highest APTI value and *Prosopis* (4.57) the lowest. The results also showed that to reduce air pollution plants with higher APTI can be planted. The plants with the lower APTI can be used to use as indicator species to identify air pollution.

Joshi et al., (2016) studied 30 plants species to study the significant tolerance and susceptible plant species. Among 30 plant species, *Putrannjiva roxburgii* (14.85) is highly tolerant to air pollution followed by *Mangifera indica* (10.03) and *Nyctanthes arbor-tristis* (6.87) is most sensitive to air pollution followed by *Bauhinia purpurea* (6.92).

Aasawari and Kakde (2017) analyzed APTI values of plants from polluted and control sites in Thane city. They reported higher APTI values at the control site as compared to the polluted site. The minimum APTI value was observed in *Tectona grandis* (5.2 ± 0.32) and the maximum was in *Azadirachta indica* (13.5±0.44).

Yousafzai et al., (2017) evaluated APTI for seven tree species commonly growing in Chiang Mai city, Thailand. They studied comparatively in the dry and polluted season. They reported that Mangifera indica, *Ficus religiosa* and *Butea monosperma* were tolerant, whereas *Lagerstromia speciosa*, *Polyalthia longifolia* and *Plumeria rubra* were intermediately tolerant species. *Alstonia scholaris* was found to be sensitive species.

Kour and Raina (2017) the study included seven plant species selected from roadside and reference location. The biochemical parameters and APTI values were examined across two seasons (monsoon and winter) to conclude the impact of air pollution and the plant's tolerance levels. Results showed a decline in biochemical parameters and APTI values for plants in polluted areas as compared to the reference location.

Skrynetska et al., (2018) the objective of the study was to assess the environmental condition in Sosnowiec, southern Poland, using APTI. The APTI values ranges from 4.4 to 9.42, suggesting that the studied species are sensitive to air pollution. Consequently, all selected species are deemed suitable bio indicators for environmental pollution in the area.

Zouari et al., (2018) the study aimed to evaluate the APTI of 4 plant species in Sfax Tunisia, around both polluted and non polluted industrial sites. Consequently, *Olea europaea* (APTI = 20.09) and *Phoenix dactylifera* (APTI = 17.1) exhibit the highest tolerance and *Ficus carica* (APTI = 8.8) and *Morus alba* (APTI = 7.4) considered as most susceptible.

Alhneswai et al., (2018) reported in their study that tolerant plant species serve as effective sinks for mitigating air pollution. They investigated seven plant species in industrial, urban and rural sites within Kerbala city, Iraq for APTI. As a result they observed *Olea europaea* and *Eucalyptus camaldulensis* demonstrated high tolerance to air pollution.

Banerjee et al. (2019) highlighted the vital role of plants as natural air purifiers. The study evaluated the APTI of 36 plant species and providing a comprehensive evaluation of plant suitability for green belt development in industrial areas like Durgapur,West Bengal, India. *Largestromeia speciosa* (Jarul), *Schleicheraoleosa* (Kusum) and *Thespesia populnea* (Pipal) emerges as notable performers demonstrating high APTI values.

Manjunath et al. (2019) studied the comparative study of APTI in plants from the polluted and non-polluted area from Bengaluru city. Based on their study, *Ricinus communis* from the polluted and non-polluted area showed a difference in water holding capacity.

Plant from the polluted area showing a reduction in relative water content while that of a non-polluted area. In addition to that other biochemical parameter like pH and stomata index showing the slight difference between plants of polluted and non-polluted species but there was the potential influence was seen in chlorophyll content of *R. communis* from the polluted site as compare to non-polluted site.

Sharma et al., (2019) conducted a study in Himachal Pradesh for APTI of 6 commonly found temperate and sub temperate plant species along National highway 5. *Grevillea robusta* exhibited the highest APTI value (12.8).

Viradiya et al., (2020) stated that plant possess a remarkable ability to mitigate and endure air pollution, which can be quantified using APTI. They conducted their study in two industrial sites in Rajkot city namely Samrat and Metoda industrial area. The study evaluated the APTI of 20 plant species from both locations to assess their tolerance to pollution. *Delnoix regia* and *Azadirachta indica* exhibited the highest APTI values in the Samrat industrial area while, *Casia fistula* and *Ficus rumphii* exhibited highest APTI values in the Metoda industrial area.

The findings of Molnar et al., (2020) underscore APTI efficacy as a method in mitigation air pollutants and for development of green belts. They reported that APTI act a aid in informed urban planning strategies aimed at improving air quality and fostering sustainable urban environments. They conducted their study in Debrecen, Hungary on two plants species.

Fatima et al., (2020) focused on using APTI to select plant species capable of withstanding air pollution in Hyderabad. Plants samples were collected from the campus of research institution for screening their response to air pollution.

The outcome of a study by Bandara et al., (2021) indicated that in both polluted areas, *Madhuca longifolia* exhibited the highest APTI values followed by *Peltophorum pterocarpum, Terminalia catappa,Cassia fistula* and *Pongamia pinnata* and it may be the most suitable species for mitigating air pollution in both environmental settings and also potential for roadside planting in humid tropics like Colombo, Sri lanka.

Shrestha et al., (2021) studied nine plants species and *Cinnamomum camphora* considered as most tolerant to air pollution based on the APTI. Also, it can be used as the most suitable species for roadside plantation in vegetation traffic barriers to air pollutants in Kathmandu valley.

Banerjee et al., (2021) aimed to identify pollution responsive variables in four selected plant species in Durgapur, West Bengal, India. Results showed that *Lagerstroemia speciosa* exhibited the highest APTI (183.5). Besides, a significant positive correlation was observed between APTI and ascorbic acid with the levels of air pollutants, indicating different biochemical responses in the same species in different environmental conditions.

Salisabila et al., (2022) studied species diversity and their APTI in household, industry and transportation areas in East Java. Using the APTI method, *Pseuderanthemum reticulatum* exhibited the highest tolerance to air pollution. Their findings provided insights into the environmental conditions of green open spaces in various sectors and offer recommendations

for sustainable management and mitigate the effect of climate change caused by air pollution.

The study by Mondal and Singh (2022) aimed to assess the APTI and anticipated performance and metal accumulation capacity of 15 common tropical plant species at the Jharia Coalfield (JCF) and Reference sites. APTI values were highest at JCF for *Ficus benghalensis*, *Ficus religiosa*, *Alstonia scholaris*, *Mangifera indica*, *Azadirachta indica* and *Moringa oleifera* and can be used as sink to air pollutants.

In the study conducted by Anake et al. (2022), the objective was to identify plant species suitable for green belt development in polluted areas of Ado-Odo, Ota Ogun State, Nigeria. This was achieved by assessing both the APTI and API of eight plant species sourced from industrial and non-industrial locations. The findings revealed that all screened plants exhibited sensitivity to air pollution, thus serving as valuable bio indicators of environmental degradation. Notably, *Ficus auriculata* emerged as the most sensitive plant species in the non-industrial area. Furthermore, regression analysis and two-way variance analysis unveiled a significant relationship between biochemical parameter and APTI. Remarkably, RWC was identified as exerting the highest influence on APTI, highlighting its pivotal role in assessing plant tolerance to air pollution.

Patel et al., (2023) conducted a research to evaluate the ability of 16 commonly occurring tree and shrub to capture atmospheric dust and resist abiotic stress triggered by dust deposition in urban areas. *Ficus religiosa*, *Ficus benghalensis*, *Alstonia scholaris*, *Dalbergia sisso* and *Terminalia arjuna* exhibited higher APTI values. Plants with broad canopies and rough leaf surfaces with perforated veins were found to be more suitable for greenery development to improve air quality in urban areas like Delhi.

Verma et al., (2023) studied APTI of selected roadside tree species during summer and winter season at Punjab agricultural university and roadside area. The APTI of selected trees ranges from 7.6 to 11.1 with *Cassia fistula* exhibited the highest tolerance. Their findings provide valuable insights into the tolerance levels of roadside tree species to air pollution and their potential performance under different environmental conditions.

Niami et al., (2023) evaluated *Ziziphus spinachristi's* tolerance to air pollution by using APTI in two contrasting environments. Results indicated that *Ziziphus spinachristi's* exhibited tolerance to air pollution in Qatar as evidenced by it calculated APTI value. They reported that plants tolerance to air pollution can effectively contribute to improving air quality mitigating environment contamination

Singh et al., (2024) estimated APTI of seven common trees from polluted and non-polluted locations in Delhi. *Ficus religiosa* exhibited the highest APTI value (23.2) during the winter season at polluted site while *Pongamia pinnata* showed the highest APTI (20.8) at the non-polluted site during the same season. *Polyalthia longifolia* was identified as the most susceptible species to air pollution with lowest APTI values at both the areas and seasons. *Ficus religiosa* was considered the best performer and a suitable option for landscaping in polluted and non- polluted areas. These findings underscore the importance of selecting appropriate tree species for urban landscaping to effectively battle air pollution and promote environmental sustainability in urban areas like Delhi.

Saidah et al., (2024) studied the tolerance of the tree species to air pollution in the petrochemical industrial area by analyzing the APTI. The results revealed that *Filicium decipiens* (Feran leaf tree) exhibited an APTI value of 5, indicating its sensitivity to air pollution in the industrial area.

Zheng et al., (2024) they conducted a study to assess the sensitivity of four evergreen tree species to air pollution in Xi'an city (China) by calculating their APTI. The findings indicated that *Cedrus deodara* and *Sabina chinesis* exhibited the highest APTI values, suggesting their high tolerance to air pollutants.

Bibi et al., (2024) assessed the sensitivity of tree species to air pollution along and urbanization gradient in Vienna, Austria using APTI. Leaf samples were collected from three different locations representing urban, suburban and rural areas. Based on the APTI values, the studied species were identified as sensitive indicators for air pollution monitoring.

Shaukat et al., (2024) studied 10 common tree species in both polluted and unpolluted areas of Karachi city. Overall, the results showed that in the polluted habitat, levels of biochemical parameters were higher as compared to unpolluted habitat. The APTI values indicated that *Azadirachta indica*, *Ficus benghalensis*, *Ficus religiosa*, *Conocarpus erectus* and

Peltophorum pterocarpum were tolerant to air pollution as they exhibited higher APTI values in polluted area. Also, the study suggests that incorporating additional parameters such as free amino acid levels and stomatal characteristics into the computation of APTI could enhance its accuracy.

The effects and risk of air pollution on vegetation are largely determined by the environmental parameters; therefore these parameters should be included in the research works that involve the monitoring of plant tolerance. Three main parameters such as plant adaptability, pollutants exposure, and environmental parameters cannot be ignored as these are the factors that ultimately regulate the air pollution tolerant behavior of the plants (Kour and Adak, 2021; Kour and Adak, 2023).

It was observed that, under the same environmental conditions, different plant species possess different APTI values. However, it was also observed that some plant species possess different APTI values under the same meteorological conditions (Dwivedi and Tripathi, 2006). Singh et al., (1983) took and analyzed plant species from the Varanasi region and from adjoining areas of the same meteorological conditions. However, the APTI values for the same plants or different plants species are not same.

Several other researchers studied plants species such as *Psidium guajava*, *Syzygium cumini*, *Albizzia lebbeck*, *Dalbergia sissoo*, *Artocarpus heterophyllus*, *Polyalthia longifolia*, *Ficus religiosa*, etc. and reported same plant species respond differently under different environmental conditions (Lakshmi et al., 2008; Lalitha et al., 2013; Madan and Verma, 2015; Sharma et al., 2013). It has also been observed that the combined effect of air pollutants and the environmental condition of the plant's habitat may alter the plant-environment relationship on a regional scale (Kuddus et al., 2011). Similarly, several researchers including Paulsamy and Senthilkumar (2009), Govindaraju et al. (2012), Thambavani and Prathipa, 2012; Singare and Talpade (2013) observed a similar trend in their study.

2.5 Environmental factors and its effect on plants

Seasonal variation causes change in environmental conditions which in turn, alters the biochemical parameters of the plants. During the monsoon season, pollutants may get washed away from the leaf surface which may increase the chlorophyll and ascorbic acid content in plants' bodies. Chlorophyll content in plant may considerably be changed by the

environmental effects on plant metabolism. Summer and winter seasons may exhibit a wide range of variations in plant tolerance towards air pollutants. The high content of chlorophyll was noticed in plant species like *Quercus leucotrichophora* (3.01 mg/g), while low total chlorophyll content was observed in the case of *Hypericum oblongifolium* (1.76 mg/g) during the summer season (when the temperature is at peak). In an arid climate, dust deposition interferes with photosynthesis and decreases relative water content in plant leaves, each plays a key role in assessing their sensitivity towards air pollution (Kour and Adak, 2021).

Low chlorophyll content during the winter season may be attributed to various factors including high pollution levels, temperature stress, low sunlight intensity, and shorter photoperiod. Regardless of the study area, *Polyalthia longifolia* and *Clerodendrum* exhibited higher levels of total chlorophyll among the evergreen trees examined. During the summer, monsoon, and winter seasons, an increasing trend was observed in the concentration of ascorbic acid. The highest values of ascorbic acid were recorded during the summer season (2.63 mg/g), followed by the winter season (2.20 mg/g), and then the monsoon season (1.62 mg/g).In contrast, the chlorophyll content showed variability across seasons.

The highest chlorophyll values were observed during the monsoon season (2.48 mg/g), followed by a decrease during the winter season (1.94 mg/g), and the lowest values during the summer season (1.65 mg/g) (Jyothi and Jaya, 2009).Plants react differently to different air pollutants under different environmental conditions, depending on the plant's ability. Somewhere it suggests that biochemical parameters are not sufficient to evaluate the tolerance of plants. Moreover biochemical makeup of plants, as well as their ability to tolerate air pollutants, may be affected by high concentration of gaseous pollutants. Hence, the biochemical parameters, existing meteorological conditions and ambient air quality, cannot be ignored, because these are the factors that eventually control the internal atmosphere of plants and its cells (Kour and Adak, 2023).

Environmental factors such as light, temperature, and humidity significantly influence the sensitivity of plants to pollution. While light serves as the primary energy source for photosynthesis, it can also act as a stress factor. Plant responses to light vary depending on factors such as lighting conditions, season, cultivation practices and genotype. While plants are subjected to high light intensity stress along with various abiotic stresses like, the reducing power (NADPH), energy supply (ATP) and drought generated through photo systems and the electron transport chain can surpass the requirement for physiological processes involved in carbon-fixing reactions.

Koritz and went (1953) studied, when tomato plant were placed in the dark and exposed to low levels of ozonated hexane, they exhibited no depletion in growth whereas when the plants were exposed to the light for 1.5 hours period with the same level of ozonated hexene after the dark period, they exhibited definite growth depletion.

Heck (1964) examined that during a 4- hour dark exposure, there was no significant injury to endive plants, cotton, or tomato from an NO_2 propylene mixture but during 4 hours of light exposure in the presence of the same pollutant, severe damage was noticed. Dugger, et al. reported that plants can be completely protected from PAN pollutants when placed in 30 minute dark exposure but show no effect on O_3, injury. Ascorbic acid concentration increases in tobacco plants due to light preconditioning. The increased amount of ascorbic acid represents the developmental stages of rapid leaf expansion. The increased amount of ascorbic acid concentrations in leaves due to light was 2.5 to 3.3 times higher as compared to the pollutant damage. Light initiates the resistance while pollutant decreases the resistance inside the plant. Both the amount of light inside the plant and the amount of pollutant that enters inside are inversely correlated with each other. Therefore, it was elucidated that, depending upon the plant's adaptability; some plants show tolerance under light conditions and some in shade regions (Heck, 1964).

Plants are most susceptible to Phyto-toxic air pollutants (Smog) during warm moderate conditions (79°F during day and68°F) and they are less sensitive in a moderate cool environment (68°F during the day and 57°F during night time). Juhren et al. (1957) observed that, if plants are relocated from a warm (79°F) to hot (86°F) environment, then they lose their sensitivity within three days. Menser et.al (1963a) examined four Tobacco varieties and recorded their tolerance while the plants were kept at 77°F and 41°F separately. It was observed that the plants kept at 77°F were more susceptible than those kept at 41°F. Light intensity was also observed to modify the response of plants towards pollutants.

Taylor et al. (1961) studied the tolerance of the pinto bean plant to O_3 under two different light intensities and it was found O_3, injuries were reduced under high light intensities, Menser et al., (1963b) reported that O_3 injury to the tobacco plants was reduced when they were exposed for an extended 22-hour photoperiod before fumigation. However, there was a more pronounced photosynthetic depression observed at the end of the fumigation period when leaves were exposed to of SO_2 uptake at 8°C as compared to those exposed at 18°C. While using ozone hexane mixture, Hull and Went also studied similar increased sensitivity at higher temperatures. Also, observed that plants subjected to mixture of propylene and NO_2

under artificial light conditions, they experienced critical injury at 80°F compared to 65°F or 95°F. However, further research is required to be done to understand the effects of intermediate and higher temperatures over different stages of growth.

Humidity is the next important factor of the environment that influences the physiological processes of plants under stress. Temperature and humidity are directly proportional to each other. As temperature increases, transpiration increases because the VPD between the moist leaf surface and air increases with increasing temperature (Tibbitis, 1979). An increase in the temperature causes variation in biochemical parameters which further controls the tolerance ability of plants towards pollutants. High ambient humidity results in the stomatal closure that leads to less availability of CO_2in plants (Forde et al., 1977). In *Helianthus annuus,* the net photosynthetic rate decreases due to the increase in vapor pressure difference. A similar trend was reported in the case of *Chenopodium album.* On contrary, a significant reduction in chlorophyll content was observed when there was a large vapor pressure difference.

It was also reported that, if a specific range of vapor pressure difference (0 to 10 MB) is maintained, no significant change in photosynthetic rates can be observed. Rawson and Begg (1977) reported that, when the vapor pressure deficit increases, the transpiration rate increases. However, over the range of 8 to 27MB of vapor pressure deficit (VPD), photosynthesis process was unaffected by humidity. Linear regression (r=0.96-0.99) accurately defined the relationship between the rate of transpiration and humidity. Thus, the rate of transpiration for a single leaf of any genus was largely determined by the vapor pressure deficit (VPD) between the leaf's intercellular spaces and the atmosphere. Water loss rates were higher in C3 crop plants (sunflower, wheat, and soybean) than in C4 crop plants (barnyard grass and sorghum). Low humidity also inhibits expansive growth. It is inversely linked to epicuticular wax development and morphological complexity in controlled environment conditions. It was explained in the previous literature that plants have higher cuticular conductance under humid conditions to prevent water loss (Grantz, 1990).

Precipitation is another environment parameter that usually harms the net productivity and photosynthesis of plants. This trend may vary from species to species. Excess rainfall results in the washout of the pollutants from the leaf surfaces into the soil. As a result, the plants exhibit a high chlorophyll and ascorbic acid content which results in less damage inside plants.

It implies that precipitation influences plant tolerance under stress conditions (Yu et al., 2015). The severe water stress will damage the entire photosynthesis system which may even

become the reason for the reduction of plants' productivity and resistivity towards pollutants. However, when the extent of precipitation exceeded the threshold volume, the reduction of net photosynthetic rate slowly decreases which means that when rainfall reached the threshold, the net photosynthetic rate will begin to stabilize (Banerjee et al., 2019). Prior Literature implies that Air pollution tolerance index (APTI) method necessitates refinement for improved outcomes. Additionally, several parameters such as morphological, soil type, environmental conditions, pollutant concentration, and plant type are influential. The current study focuses on two specific parameters: environmental factors and atmospheric air pollutants (SO_2 and NO_2). These parameters are prioritized due to their pronounced impact on biochemical parameters, as explained in previous literature.

CHAPTER 3 MATERIALS AND METHODS

3.1 Study area

Phagwara is a city and Municipal Corporation located in the Kapurthala district of Punjab, India. It is also positioned 40 kilometers (24 miles) away from Kapurthala and 20 kilometers (12 miles) from Jalandhar. It is located on land between the Beas and Satluj. The city of Phagwara is positioned at coordinates 31.22^0N and 75.77^0E and it stands at an elevation of 768 feet (234 meters) above sea level. It experiences a humid subtropical climate characterized by cool winters and extended hot summers. The summer season typically spans from March to June, while winters typically occur from October to February. During the summer, temperatures range from typical high temperatures of about 42^0C (107^0F) to average lows of around 28^0C (83^0F). In contrast, winter temperatures exhibit highs of 20^0C (69^0F) to lows dropping to 6^0C (43^0F). Overall, the climate is generally dry, except for a brief period during the southwest monsoon season in July, August and September. The city typically receives an average rainfall of 209mm.Phagwara is renowned for its manufacturing industries, particularly in the production of sugar, glucose, starch, fine fabric textiles, and auto parts for engines, making it a significant industrial hub in Punjab(Gazetteer of the Jullundur District, 1904; Govt of Kapurthala).

3.2 General methodology

The methodology of the proposed research is explained as follows. The biochemical analysis of plants species was carried out eight strategic locations inside the Punjab, India which served as the polluted (Phagwara, Jalandhar, Ludhiana, Amritsar, Chandigarh sector 22, 25, 53 and control site (Lovely Professional University). The latitude and longitude of all the sampling areas are given in table 3.1

Table 3.1 Latitude and Longitude of sampling sites

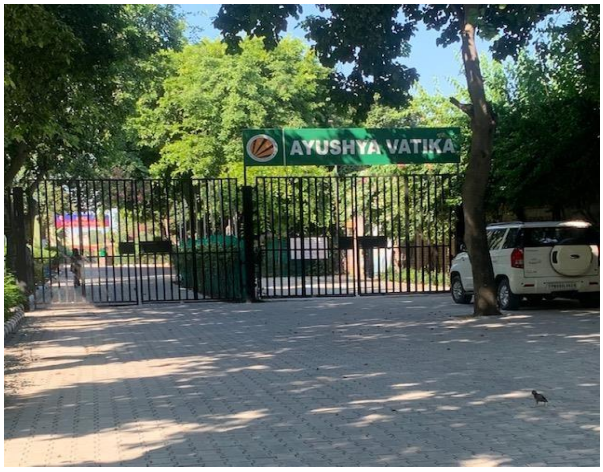
Sampling sites	**Latitude**	**Longitude**
Phagwara Industrial area(P1)	31.20^0N	75.76^0E
Phagwara Bus stand (P2)	31.21^0N	75.77^0E
Lovely Professional University (C)	31.25^0N	75.70^0E
Jalandhar (S1)	31.32^0N	75.57^0E
Ludhiana (S2)	30.90^0N	75.85^0E
Amritsar (S3)	31.63^0N	74.87^0E
Chandigarh sector 22 (S4)	30.73^0N	76.77^0E
Chandigarh sector 25 (S5)	30.75^0N	76.75^0E
Chandigarh sector 53 (S6)	30.71^0N	76.72^0E

3.2.1 Sampling of plants

The current study was conducted from August 2021 to July 2022 in Phagwara, Punjab, India. Prior to biological analysis of plants, a thorough survey of local species was made in and around the selected sampling sites. The outcome of the survey resulted in the selection of 15 plant species (Table 2.1).The use of multiple species increases the probability of selecting suitable pollution-tolerant plants with greater probability than the use of a single or few species. The plant species were sampled from two polluted areas (Industrial and Bus stand) and a control Lovely Professional University area of Phagwara, Punjab India (as shown in Figure 3.1). Ten fully matured leaves of each selected plants growing in similar ecological conditions were collected randomly in the morning hours (8:00 a.m. to 9:00 a.m). Three replicates of fully matured leaves of each species were taken. The collected leaves were packed in polythene and taken to the laboratory. Then, the leaf samples were thoroughly cleaned for biochemical parameter analysis and then kept in a refrigerator for further analysis. The fresh leaf sample were analyzed for various morphological and biochemical parameters.

(a) Phagwara Bus Stand area

(b) Phagwara Industrial area



(c) Lovely Professional University

Figure 3.1 Sampling areas in Phagwara, Punjab.

Table 3.2 Description of the plant species selected for this study

Plant species	Family	Habitat
Alstonia scholaris,	Apocynaceae	Evergreen
Cascabela thevetia	Apocynaceae	Evergreen
Ficus benghalensis	Moraceae	Evergreen
Ficus religiosa	Moraceae	Evergreen
Mangifera indica	Anacardiaceae	Evergreen
Melia azedarach	Meliaceae	Deciduous
Mentha piperita	Lamiaceae	Evergreen
Moringa oleifera,	Moringaceae	Deciduous

Plant species	Family	Habitat
Murraya koenigii	Rutaceae	Evergreen
Morus alba	Moraceae	Deciduous
Ocimum sanctum	Lamiaceae	Deciduous
Polyalthia longifolia	Annonaceae	Evergreen
Psidium guajava	Myrtaceae	Evergreen
Syzygium cumini	Myrtaceae	Evergreen
Ziziphus mauritiana	Rhamnaceae	Evergreen

3.2.2 Morphological parameters

Leaf colour: Visual observations have been made on randomly collected leaves from reference and polluted sites to record the color of the leaves.

Length and width of leaf: The length and width of leaves selected from both reference and polluted sites were measured using a ruler.

Leaf area: Leaf surface area (LSA) was calculated by tracing the leaf outline on graph paper. First of all, place a leaf on graph paper to calculate the area blocked by the leaf in cm^2 and outline its margin with pencil or pen.

The number of full squares and partial squares was marked inside the leaf outline using a pen. The areas of full squares weremultipliedby1cm^2 and the areas of partial squares were multiplied by 0.5 cm^2 and then these values were added together.

Leaf surface texture: Additionally, two dummy variables (0,1) were created for leaf roughness (1) and smooth(0) textures. Plants species were classified based on rough and smooth leaf texture (LST) with dummy variables (0, 1). Then statically analyzed in Microsoft excel software.

3.2.3 Visible effects of air pollution on plants

Some common morphological effects were observed during leaf sampling. Necrosis and chlorosis were the most common effects observed at the site of sampling. Necrosis was observed in plants as dry and brownish to black coloured spots on the tips and surfaces of leaves as shown in Figure 3.2 (a, c, d, g, h) due to collapse of mesophyll cells. Chlorosis was diagnosed as yellowing of green leaves as shown in Figure 3.2 (a, b, d, e, f, h) due to lack of chlorophyll. It may be due to vehicle emission or and industrial emissions, nutrient deficiency, soil pollution or any environmental parameters.

a b

c d

(e) (f)

(g) (h)

Figure 3.2 Examples of yellowing and dry brownish to black coloured spots on the tips and surfaces of leaves.

3.3 Selection of *Mentha piperita* and *Ocimum sanctum* growing under controlled environmental conditions

Following the APTI assessment of named plant species, significant variation in biochemical parameters of similar plants species in three different areas has been observed. These differences are presumed to be due to environmental factors which are known to strongly influence biochemical parameters as explained impervious literature. Therefore, two plants have been selected for monitoring of biochemical parameters within controlled environmental

factors to identify the patterns of effect of environmental factors on biochemical parameters. However, among the selected plant species, tree and shrubs are difficult to cultivate under controlled conditions, two herbs *Mentha piperita* and *Ocimum sanctum* have been selected for controlled conditions. They are very fast growing plants and germinate within a week and their height is optimal for controlled conditions compared to other selected plant species.

3.3.1 Environmental factors monitoring

The three environmental factors were selected in the present study namely, Temperature, Light intensity and Humidity. Monitoring of environmental factors is explained as follow; Light intensity was measured by using Lux meter, Temperature and Humidity was measured by using clock HTC-1 device (as shown in Figure 3.3).

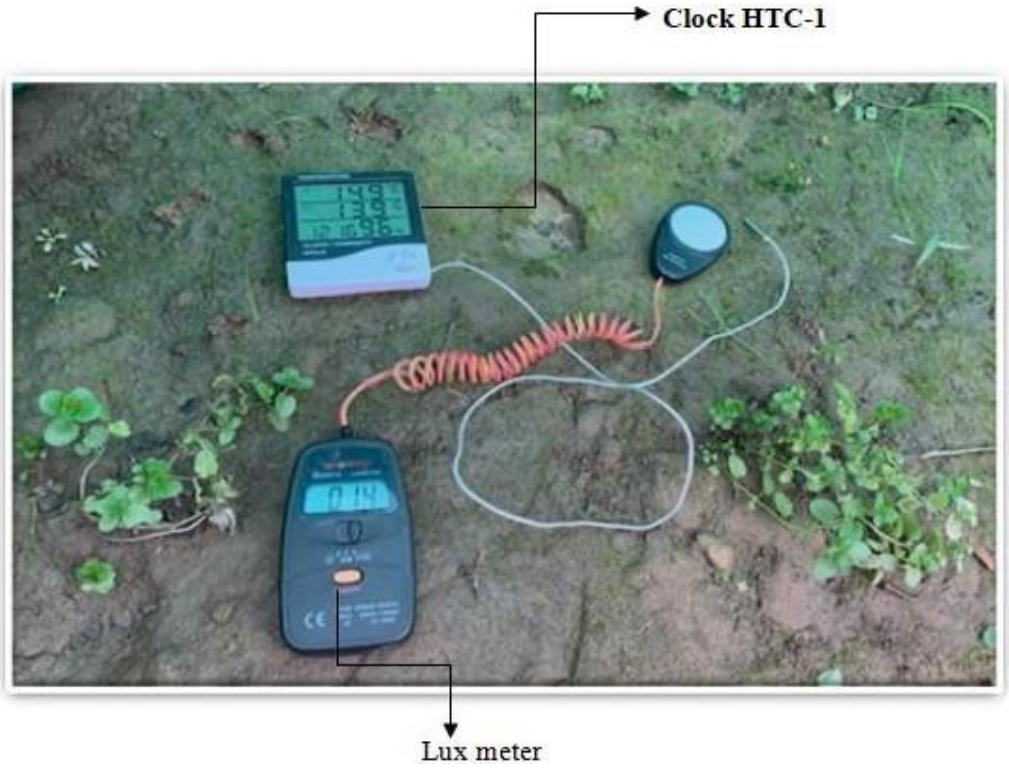


Figure 3.3 Environmental factors monitoring devices

3.3.2 Sampling of *Mentha piperita* and *Ocimum sanctum* growing under controlled environmental conditions

Mentha piperita and Ocimum sanctum were grown under shade net at different percentage (%) such as 60%, 50%, 70%, 90% and also grown under full sunlight which was considered as 100% (as shown in Figure3.4-3.9). During morning hours, 10 -15 leaves of *Mentha piperita and Ocimum sanctum* were collected in zip lock small packets from full sunlight, 60% and 50% shade net chambers. However, from 70% and 90%, 5-8 leaves were collected. Since the growth of these shade net was less. After sample collection, fresh leave were brought to the laboratory. Then, the leaf samples were thoroughly cleaned for biochemical parameter analysis and then kept in a refrigerator for further analysis. The fresh leaf sample were analyzed for various morphological and biochemical parameters.

(a) **(b)**

Figures 3.4 *Ocimum sanctum* (a) and *Mentha piperita* (b) growing under full sunlight (100%)

(c) **(d)**

Figures 3.5 *Ocimum sanctum*(c) and *Mentha piperita* (d) growing 40% light intensity shade net

(e) **(f)**

Figures 3.6 *Ocimum sanctum* (e) and *Mentha piperita* (f) growing 50% light intensity shade net

(g) **(h)**

Figures 3.7 *Ocimum sanctum* (g) and *Mentha piperita* (h) growing 70% light intensity shade net

(i) **(j)**

Figures 3.8 *Ocimum sanctum* (i) and *Mentha piperita* (j) growing 90% light intensity shade net

(k)

(l)

Figures 3.9 *Ocimum sanctum* during winter season

3.4 Selection of different regions of Punjab for sampling

Following the APTI assessment of named plant species, significant variation in biochemical parameters of similar plants species in three different areas has been observed. These differences are presumed to be due to different concentrations of atmospheric pollutants which are known to strongly influence biochemical parameters as explained in pervious literature. The six regions of Punjab were selected to study the effect of air pollutants on biochemical parameters**.** The six regions of Punjab: Jalandhar, Ludhiana, Amritsar, and Chandigarh (Sector 22, Sector 25, and Sector 26). Ludhiana is one of the most polluted cities in the state of Punjab due to rapid urbanization and heavy industrialization (Verma et al. 2022). Jalandhar is being considered as residential and commercial area. Amritsar (Golden temple) can be considered as a polluted place in Punjab due to its famous tourist place and heavy traffic load (Kaur et al. 2017). In Chandigarh, Sector 22 sector 25 and sector 53 are less polluted sites due to low traffic load and more green belts with large number of canopy trees around the roads.

Figure 3.10 Map of the study sites (Google, n.d.)

3.4.1 Collection of leaf sample

The leaf samples of 15 chosen plant species, namely Ficus *benghalensis, Ficus religiosa, Murraya koenigii, Yellow oleander, Melia azedarach, Psidium guajava, Ziziphus mauritiana, Ocimum sanctum, Mentha piperita, Syzygium cumini, Mangifera indica, Polyalthia longifolia, Morus alba, Alstonia scholaris and Moringa oleifera* were sampled from each sampled site separately. Fresh matured leaves of the plants were collected in black polythene during morning hours. Five leaves from trees and 10-15 leaves from shrubs of each plant were collected randomly. After sample collection, fresh leave were brought to the laboratory for further analysis. Various biochemical parameters has been analyzed and stored in refrigerator for further analysis.

(a) Chandigarh (Sector22

(b) Jalandhar (Civil line)

(c) Ludhiana (Punjab Agricultural University)

(d) Golden Temple

(f) Chandigarh sector53 (g) Chandigarh sector25

Figures 3.11 Sampling in six different areas of Punjab

3.4.2 Collection of air quality data at different sampling sites in Punjab

The concentration of air pollutants (SO_2 and NO_2) in the ambient air at the time of sampling was collected from CPCB online monitoring portal (as mentioned in Table 3.3).

Table 3.3 The concentrations of air pollutants in different locations of Punjab

Locations	**SO_2(µg/m^3)**	**NO_2(µg/m^3)**
Jalandhar	14	7
Amritsar	20	58
Ludhiana	14	22
Chandigarh sector 22	8	7
Chandigarh sector 25	3	42
Chandigarh sector 53	9	28
LPU	1.2	3.5

3.5 Air quality Assessment in LPU

The concentration of air pollutants (SO_2 and NO_2) in the ambient air at the time of sampling was collected from CPCB online monitoring portal.

(a)

(b)

(c)

(d)

Figures 3.12 Sampling and Analysis of Sulphur dioxide and Nitrogen dioxide in ambient air of Lovely Professional University

3.5.1 Monitoring of Sulphur dioxide and Nitrogen dioxide in LPU

The secondary data of two air pollutants ($SO_2$and NO_2) has been collected from CPCB from six different areas of Punjab, considered urban areas. It was important to compare the results from six different regions of Punjab (which can be considered as polluted sites) with control site. Thus, Lovely Professional University was selected as the control area and SO_2, NO_2were monitored with the help of high volume sampler (LATA Envirotech APM 860, Respirable Dust sampler).

3.5.2 Sampling of gaseous pollutants ($SO_2$and NO_2)

Sampling for gaseous pollutants was done as per the guidelines mentioned in NAAQS, CPCB, New Delhi. The High Volume Respirable Dust Sampler APM 400 BL (Envirotech) (Plate-3.6), which was specifically designed to capture particulate matter, was employed. To collect samples of gaseous pollutants, a gaseous sampling attachment (APM 411) was used, which was able to accommodate four borosilicate glasses impinges simultaneously, allowing simultaneous collection of samples of four different gaseous pollutants. The absorbing medium for both gaseous pollutants was placed into two impingers and set within an ice-filled tray to prevent evaporation losses caused by varying weather conditions and to enhance absorption efficiency. Subsequently, this ice tray was positioned within the APM-411 sampler attachment. The outlets of both impingers were connected in a parallel arrangement to the individual flow control valves of the gas manifold using flexible tubing, ensuring independent airflow for each impinger. Next, the flexible tubing from the rotameter was attached to the inlet of the first impinger, and the airflow was adjusted (ranging from 0.5 to 1 litre per minute) by turning the corresponding manifold pin with a screwdriver. The airflow rate was then recorded from the rotameter.

The rotameter pipe was then disconnected from the first impinge and connected to the inlet of the second impinger. The air flow rate was adjusted and noted from the rotameter for the second impinge as well. The pipe was then disconnected from the inlet of the second impinge and let free. Following this, the rotameter tubing was disconnected from the first impinger and connected to the inlet of the second impinger. The air flow rate for the second impinger was similarly adjusted and recorded from the rotameter. Subsequently, the tubing was disconnected from the inlet of the second impinger and allowed to remain free. The sampler operated for a four-hour period, with sampling time determined by noting the initial time (T1)

and final time (T2) using a time totalizer. After each sampling session, the impingers were taken out of the attachment unit, although they were not removed from the ice tray. They were immediately transferred to the laboratory for analysis, ensuring that there was no delay in preventing any loss of gases. The volume of air sampled, V (m^3), was calculated using the following equation (Eq.3.1)

$Total\ volume\ of\ air\ sampled\ (m^3) = Average\ flow\ rates\ (litres) \times 10^{-3}(m^3/litres) \times 60(minutes/hour$ .....(Eq 3.1)

3.5.2.1 Sulphur dioxide (SO_2)

Modified west and Gaeke method was used for the determination of SO_2. This method is widely used method for reliable results. Since, it is more economical and feasible, and it can detect even small changes in concentration compared to other methods.

Sulphur dioxide has been considered as a major air pollutant with significant health effects on human beings and other living organisms, it is emitted from anthropogenic sources like fossil fuel burning, smelting of metal sulphides and other industrial facilities. It is a colourless, corrosive gas characterized by a pungent irritating odour. It is analyzed by Modified West and Gaeke method. It is recognized as a major air pollutant, which has significant effects on human health and various living organisms. This gas is emitted from human-related sources, such as the burning of fossil fuels, processing of metal sulphides, and many industrial operations. It appears as a colourless, corrosive gas with pungent odour. This was analyzed using the modified West and Gaeke method.

Principle:

When sulfur dioxide present in the surrounding air is absorbed into a sodium tetra chloromercurate (TCM) solution kept in the impinger, it forms a dichlorosulfitomercurate complex that prevents oxidation by oxygen in the atmosphere. This complex remains unaffected by powerful oxidizing agents such as ozone and nitrogen oxides. Next, it is made to react with pararosaniline and formaldehyde, resulting in the formation of pararosaniline methylsulfonic acid, which exhibits a vivid pink colour. The intensity of this colour is directly related to the amount of sulphur dioxide absorbed and is measured using a spectrophotometer.

Reagents for analysis:

Absorbing reagent: Dissolve 0.1 Sodium tetrachloromercurate (TCM), 10.86 g of mercuric chloride, 0.066 g of EDTA, and 4.68 g of sodium chloride in distilled water to reach the 1-liter mark in a volumetric flask.

Sulphamic acid (0.6%)-0.6g of sulphamic acid is dissolved in 100 ml of distilled water, and this solution is prepared fresh for daily analysis.

Formaldehyde (0.2%) To make a formaldehyde solution with a concentration of 0.2%, dilute 5 ml of formaldehyde solution with distilled water to 1 liter, and prepare this solution fresh daily.

Pararosaniline stock solution - Prepare pararosaniline stock solution by dissolving 0.5 g of specially purified pararosaniline (PRA) in100ml of distilled water and kept it for 48hours before use.

Pararosaniline working solution - Take10 ml pararosaniline stock solution and mix it with 15 ml concentrated hydrochloric acid in a 250 ml volumetric flask. Then, raise the volume to the mark with distilled water.

Reagents for calibration curve:

Stock Iodine solution (0.1N) – Take 12.7gm of iodine and 40gm of potassium iodide in a 250 ml beaker and add 25 ml distilled water to it. The mixture is stirred until completely dissolved, after which it is diluted with distilled water to reach a total volume of 1 litre.

Iodine solutions (0.01N) - In a 500 ml beaker, add 50 ml of stock iodine solution and raise the volume to 500 ml with distilled water.

Starch solution-Dissolve 0.4grams of starch in a beaker of coldwater and stir until a thin paste is formed. This paste is slowly added to 200 ml of boiling water and the water is boiled until the solution becomes clear, cooled and then poured into a glass bottle.

Stock sodium thiosulfate solution (0.1N) - In a beaker, 25 g of sodium thiosulfate pentahydrate is added, followed by 0.1 g of sodium carbonate, which is dissolved using previously boiled cold distilled water. The final volume of the solution is raise to1 litre. The

final volume of the solution is raise to 1 liter. The solution is left unchanged for 24 hours before standardization.

Standardization of sodium thiosulphate solution (0.01N)

Prepare a solution by dissolving 1.5 grams of potassium iodate, previously dried at 180°C, in a 500 ml volumetric flask, and then dilute it to the mark. Next, transfer 50 ml of the iodate solution into another 500ml volumetric flask. To this flask, add 2 grams of potassium iodide and10ml of N hydrochloric acid, and seal the flask with a glass lid. Let this mixture stand for 5 minutes.

Afterward, titrate this solution against the stock thiosulfate solution until it achieves a pale yellow color. Add 5 ml of starch indicator solution and continue the titration until the blue color disappears. Calculate the normality of the stock solution using the given equation (Eq. 3.2)

$$N = \frac{W \times 10^3 \times 0.1}{V \times 35.67} \ldots\ldots (Eq. 3.2)$$

Where:

V-Volume of thiosulphate used, ml

W- weight of potassium iodate, gm

35.67- Equivalent weight of potassium iodate

Sodium thiosuphate titrant (0.01 N) - Dilute100 ml of the stock thiosulfate solution to1 liter with freshly boiled and cooled distilled water.

Standardization of sulphite solution for preparation of working sulphite

TCM solution- To prepare the solution, dissolve either 0.3 grams of sodium metabisulfite or 0.4 grams of sodium sulfite in 500 ml of freshly boiled and cooled distilled water. The concentration of the solution is determined by adding excess iodine solution and performing a reverse titration using a standard sodium thiosulfate solution. For the reverse titration, measure 50 ml of 0.01 N iodine solution and distribute it into two separate 500 ml iodine flasks labeled A and B. In flask A (blank), add 25 ml of distilled water, while in flask B

(sample), add 25 ml of the sulfite solution. Close the flasks and let the reaction occur for about 5 minutes.

Prepare the working sulfite -TCM solution by adding iodine solution to the flask. Titrate this solution against standardized 0.01 N thiosulfate in the burette until it turns pale yellow. Then, add ml of starch solution and continue titration until the blue color disappears.

Working sulfite - TCM solution: Add 2 ml of standard solution to a 100 ml volumetric using a pipette and raised it to the mark with 0.04 M TCM. Calculate the concentration of sulfur dioxide in the standard solution using the provided formula in Eq. 3.3

$$C = \frac{(V1 - V2) \times N \times K}{V} \ldots\ldots\ldots \text{(Eq.3.3)}$$

Where, C - SO_2 concentration in µg/ml

V1- volume of thiosuphate used for blank, ml

V2- volume of thiosulphate used for sample, ml

N- Normality of thiosulphate

K - 32000 (milli equivalent weight SO_2/µg)

V- Volume of standard sulphite solution, ml

Preparation of calibration curve:

With the help of pipette, transferred measured amount of working sulphite – TCM solution (like0, 0.5, 1, 2, 3 and 4 ml) into a series of 25 ml volumetric flasks. Add enough TCM solution to each flask, to increase the volume to approximately 10 ml. Then, add 1ml sulphamic acid, 2ml formaldehyde solution and 2 ml pararosaniline solution to each flask. Then all flasks are brought to the mark with distilled water and mixed well. The absorbance of each sample was measured after 30 minutes and before60 minutes. A calibration is then prepared by plotting the absorbance values.

Treatment, analysis and calculation:

To avoid any water loss due to evaporation, distilled water is added to the impinger containing the sample. In a volumetric flask, pipette out 10 ml of collected sample into a 50 ml volumetric flask. Then add 1 ml sulphamic acid, 2 ml formaldehyde solution and 2 ml of pararosaniline solution. The volume of all the flasks were raised with distilled water and mixed thoroughly. Then, all the flasks are brought to the mark with distilled water and mixed

thoroughly. A blank was prepared using the absorbing reagent that has not been exposed (in the same manner).

The absorbance of each sample was determined at 560nm in a spectrophotometer after 30minutes and before 60 minutes with the blank as reference. The concentration of sulphur dioxide in ambient air has been calculated by the provided formula in Eq 3.4:

$$SO_2\ (\mu g/\ m^3) = \frac{\mu g/SO_2 \times V_s(ml)}{V_t\ (ml) \times V_a\ (m^3)} \text{.....(Eq 3.4)}$$

Where,

SO_2 ($\mu g/\ m^3$) -SO_2 concentration from calibration curve

Vs - volume of sample (ml)

V_t - volume of aliquot taken (ml)

V_a - volume of air sampled (m^3)

3.5.2.2 Nitrogen dioxide (NO_2)

To determine the NO_2 concentration, Jacob and Hochheiser method was used. This method is most widely used for continuous monitoring in developing countries. It is simple, robust and economical method.

Nitrogen dioxide acts as a powerful absorber of ultraviolet light and serves as a major component of photochemical smog. This reddish-brown gas is recognized by its strong, irritating odour and is primarily emitted from vehicles, power plants, and industrial facilities. It is analyzed using the Jacob and Hochheiser method.

Principle:

When air is bubbled into a solution of sodium hydroxide and sodium arsenite, ambient nitrogen dioxide is trapped in the solution. Nitrite ion (NO_2^-) is formed during reaction and its concentration can be determined by reacting the nitrite ion with phosphoric acid, sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride (NEDA) and measuring the absorbance at 540 nm.

Reagents:

Absorbing reagent: Dissolve 4g of sodium hydroxide in distilled water, thenadd1gm of Sodium arsenite to it and diluted to 1 litre with distilled water.

Reagents for analysis:

Sulphanilamide solution: Dissolved 20g of sulphanilamide in 700 ml of distilled water. Subsequently, 50ml of 85% phosphoric acid is added with constant mixing and increased the volume to 1 liter with distilled water.

NEDA solution: Dissolve 0.5 g of NEDA in 500ml of distilled water.

Hydrogen peroxide solution: Diluted 0.2 ml of 30% hydrogen peroxide to 250 ml with distilled water.

Preparation of calibration curve:

Sodium Nitrite ($NaNO_2$) stock solution: Dissolve 1.5 gm desiccated sodium nitrite in distilled water and diluted to 1 litre, resulting in a solution with 1000 µg of NO_2/ml.

The quantity of $NaNO_2$ to be utilized, provided that the assay % is less than 100%, is determined using the following equation (Eq.3.5)

$$G = 1.500/A \ldots\ldots \text{(Eq. 3.5)}$$

Where,

G- Amount of $NaNO_2$, gm

1.500- Gravimetric conversion factor

A- Assay, % (should be 97 or greater)

This stock solution can be used for six weeks if stored in a refrigerator.

Sodium nitrite working standard (1.0 µg NO_2/ml)

Solution A –5ml of stock solution is pipette in to a 500mlvolumetric flask and diluted to volume with distilled water. This solution contains 10 µg NO_2/ml.

Solution B – 25 ml of solution A is pipette into a 250 ml volumetric flask and diluted to volume with distilled water. This solution contains 1 µg NO_2/ml. this solution was prepared fresh on the day of use.

Prepare calibration standards using1µg/ml working standards. Pipette out varying concentrations of calibration standards (ranging from 0 to 20 µg NO_2) into 50 ml volumetric flasks. Add sufficient absorbing reagent to each flask to bring the volume to approximately 10

ml. sequentially add 1 ml of hydrogen peroxide solution, 10 ml of sulphanilamide solution, and 1.4ml of NEDA solution into each flask, ensuring thorough mixing after each addition. Finally, fill each volumetric flask to the mark with distilled water. After a 20-minute color development period, measure the absorbance at 540 nm for each calibration standard. Use the absorbance values to prepare a calibration curve.

Treatment, analysis and calculation:

Distilled water is added to the impinger containing the sample to replace any water lost due to evaporation during sampling. Transfer 10 ml of the collected sample into a 50 ml volumetric flask.Sequentiallyadd1mlofhydrogenperoxidesolution,10 ml of sulphanilamide solution, and 1.4 ml of NEDA solution into the flask, ensuring thorough mixing after each addition. Then, fill the volumetric flask to the mark with distilled water.

Prepare a blank using the unexposed absorbing reagent following the same procedure outlined above.

After a 20-minute interval for color development, measure the absorbance of all samples at 540 nm using a spectrophotometer (Digital Spectrophotometer, Model- 305), with the blank servingas the reference. Determine the concentration of $NO_2$from the calibration curve by using the formula in Eq 3.6

$$NO_2\ (\mu g\ /m^3) = \frac{\mu g/NO_2 \times V_s\ (ml)}{V_t\ (ml) \times V_a (m^3) \times 0.82} \ldots\ldots\ (Eq\ 3.6)$$

Where, NO_2 ($\mu g/\ m^3$) - NO_2 concentration from calibration curve

V_s- Volume of sample (ml)

V_t –volume of aliquot taken (ml)

V_a-volume of air sampled (m^3)

3.6 Biochemical parameters analysis

Four biochemical parameters namely pH, relative water content, total chlorophyll and ascorbic acid were determined from leaf samples of each of the 15 plant species by applying the following methods.

3.6.1 Estimation of Relative water content (% RWC)

Approximately 8 to10 leaf segments, each measuring 1 cm^2, were carefully cut from three to four leaves of each selected species (as shown in the Figure 3.13). These leaf segments were then placed on a 4 digit precisa balance to determine their fresh weight. Subsequently, these leaf segments were immersed in water overnight, and after drying using blotting paper, their turgid weight was recorded. After dry weight measurement, the leaf sections were placed in a hot air oven at 80°C for 24 h and weighed once again to determine their dry weight. The RWC of leaf was calculated by the following formula (Eq. 3.7):

$$RWC\ (\%) = \frac{(Fresh\ weight - Dry\ weight)}{(Turgid\ weight - Dry\ weight)} \times 100 \text{ ….. (Eq. 3.7)}$$

Figure3.13 Relative water content estimation

3.6.2 Estimation of Ascorbic acid content (mg/g)

The Ascorbic acid content was estimated by volumetric method given by Sadasivam and Manickam (1996).

Principle

The 2,6 dichlorophenol indophenols dye undergoes reduction by ascorbic acid, resulting in the formation of a colourless base. Simultaneously, ascorbic acid is oxidized to dehydro ascorbic acid. Despite the dye exhibits a pink colour specifically in an acidic environment. Oxalic acid

serves as the titrating medium in this particular method.

Reagents

- Stock standard Solution: Dissolve 100mg of ascorbic acid in a 100ml solution of 4% oxalic acid within a standard conical flask (yielding concentration of 1mg/ml).

- Working standard Solution: A 10ml portion of stock solution is diluted to 100ml using a 4% oxalic acid solution. Consequently, the concentration of ascorbic acid within the resulting working standard becomes 10 mg/100 ml.

- Take 96ml of water and add 4ml oxalic acid in it (4% oxalic acid)

- Dye solution Add 42 mg of sodium bicarbonate to a small amount of distilled water. Subsequently, introduce approximately 52 mg of 2, 6- dichlorophenol indophenols dye and adjust the final volume to 200ml with distilled water.

Method

- Transfer 5ml of the working standard solution into a 100ml conical flask using a pipette.
- Add 10 ml of a 4% oxalic acid solution to the mixture and titrate it against the dye (v1 ml). The appearance of pink colour signifies the end point of this reaction. The quantity of dye used during this titration corresponds to the amount of ascorbic acid present in the titrant.
- 2g of fresh leaf sample is extracted in 4% oxalic acid solution. Final volume of the solution is made up to 100 ml with 4% Oxalic acid solution then this solution is centrifuged.
- 5 ml of the supernatant of the above solution is pipette in a flask, and 10 ml of 4% oxalic acid is added to it, then this solution is titrated against the dye (V2 ml).
- Extract 2g of a fresh leaf sample in a 4% oxalic acid solution. Adjust the final volume of the solution to 100 ml using 4% oxalic acid solution and subsequently centrifuge it. Pipette 5 ml of the resulting supernatant into a flask, add 10 ml of 4% oxalic acid, and then titrate this solution against the dye (v2 ml) (as shown in the figure 3.14).

$$Amount\ of\ AA\ \left({}^{mg}/_{100mg}\right) = \frac{0.5mg}{v1\ ml} \times \frac{v2}{5\ ml} \times \frac{100\ ml}{sample\ weight} \times 100 \text{..} \quad \text{(Eq.3.8)}$$

V1- volume of dye used against working

standard solution

V2- volume of dye used against sample extract

5ml- final volume of solution in the conical flask for titration

100 ml- final volume of solution prepared after extracting the 2g sample in 10% oxalic acid

Figure 3.14 Volumetric analysis for ascorbic estimation

3.6.3 Chlorophyll content (mg/g)

Total chlorophyll content of the leaves has been extracted without maceration using the method of Hiscox and Israelstam (1979). A 5gm of fresh leaves were immersed in a test tube containing 20 ml dimethyl sulphoxide DMSO and heated on water bath for 30 min (as shown in the Figure 3.15). The extracted liquid was scanned for optical density values at 663nm and645nm on spectrophotometer at blank solution. The quantitative estimation of TC has been calculated by using formula in Eq. 3.11

$$Chlorophyll\ a = \frac{(12 \times O.D\ at\ 663) - (2.69\ O.D\ at\ 645) \times V}{W \times 1000} \ldots\ldots \text{(Eq. 3.9)}$$

$$Chlorphyll\ b = \frac{(22.9 \times O.D\ at\ 645) - (4.68 \times O.D\ at\ 663) \times V}{W \times 1000} \ldots \text{(Eq. 3.10)}$$

$$Total\ chlorophyll = \frac{(2.12 \times O.D\ at\ 645) + (8.02\ at\ 663) \times V}{W \times 1000} \ldots\ldots \text{(Eq. 3.11)}$$

Where,

V = volume of extract in ml

W = Fresh weight of leaf in mg.

12 × O.D at 663- Optical density at 663 wavelengths multiplied by 12

2.69×O.D at 645- Optical density at 645 wavelengths multiplied by 2.69

22.9×O.D at 645- Optical density at 645 wavelengths multiplied by 22.9

4.68×O.D at 663- Optical density at 663 wavelengths multiplied by 4.68

20.12×O.D at 645- Optical density at 645 wavelengths multiplied by 20.12

8.02×O.D at 663- Optical density at 663 wavelengths multiplied by 8.02

Figure 3.15 Leaf samples on the water bath for chlorophyll estimation

3.6.4 Measurement of pH

pH quantifies the concentration of hydrogen ions in a liquid solution and is primarily influenced by the relative levels of absorbed hydrogen ions and metallic ions. This test serves as an excellent indicator for assessing the acidity and alkalinity of the leaf extract, offering valuable insights into the characteristics of air pollution.

Figure 3.16 pH meter for the estimation of leaf extract pH

The pH measurement was conducted electrochemically using a pH meter. The instrument utilizes a hydrogen sensitive electrode typically constructed with an extremely delicate and thin glass membrane.

Method

Take 1gm of freshly collected leaf sample and homogenize it in 50 ml of distilled water. Subsequently, transfer the supernatant from the resulting solution after centrifugation (referred to as the leaf wash extract) into a beaker. The pH of the leaf extract was determined using a calibrated glass electrode pH meter after filtering and homogenizing 5 g of freshly leaves in 50 mL of distilled water as shown in the figure 3.16.

These four biochemical parameters were used to calculate the APTI values of different plant species with the help of APTI method. In the present study, this (APTI) method was used to study the behaivour of plants under stress conditions. It is traditional and sustainable method. This method is simple and easy to adapt to field conditions and does not required vigorous, high quality equipment. Besides, it is cost effective, time saving and biological method. Monitoring stations incur significant costs in setting up and maintaining them. It is important to note that this financial aspect may be essentially significant in swiftly rising economies like India.

CHAPTER 4 DEVELOPMENT OF APTI INVENTORY

4.1 Background

Urban vegetation has become increasingly important because it improves the local and regional air quality, in addition to social reasons. Rapid industrialization and urbanization are responsible for the deteriorating air quality, which is now a global health concern for both the climate and human health. Air pollution has been declared a "silent killer" by the World Health Organization (WHO), with an estimated 7 million deaths yearly. India, a developing nation, has experienced rapid urbanization and industrialization, reducing ambient air quality (Haakman et al., 2020). Air pollutants like carbon monoxide (CO), nitrogen oxides (NO_x), sulphur dioxide (SO_2), Ozone (O_3), Lead (Pb) and particulate matter ($PM_{2.5}$ and PM_{10}) are discharged from various sources into the environment. These are known to be criterion pollutants. These pollutants are undoubtedly hazardous to the environment and human health, causing many diseases in humans, plants and animals (Enitan et al., 2022). Their atmospheric concentrations vary according to their sources, distribution patterns, weather patterns, and topographical characteristics of the environment (Hazarika et al., 2023). As a result, it has become essential to monitor air pollutants in ambient air to reduce air pollution in urban areas (Ghosh et al., 2021). To reduce the amount air pollutants in ambient air and promote ecological restoration, development of green belts will consider as one of the best and sustainable method.

The growth of greenbelt-identified vegetation in urban areas can improve air quality by absorbing and depositing air pollutants on leaf surfaces, reducing noise, and regulating ambient air temperatures, thereby protecting other ecosystems (Barwise and Kumar, 2020; Sapkota and Devkota, 2021, Anake et al., 2022). However, the monitoring process is expensive and the use of plants is a more efficient and cheaper way to monitor air quality in urban areas (Elawa et al., 2022).Plant resilience strategies to reduce the effects of air pollution including the screening and identification of plants that are adapted to the native environment of polluted sites (Shrestha et al.,2021).The plants growing in and around polluted areas are directly exposed to air pollutants, which accumulate and assimilate the air pollutants to reduce air pollution (Banerjee et al. 2021).

As explained in the previous literature, no abatement method (physical or chemical) can reduce pollution at its source excluding plants. It naturally purifies the ambient air by fascinating harmful gases and suspended particulate matter (SPM) (Ghosh et al., 2021).Tall trees with high foliage density are better able to absorb air pollutants. The height of the trees should be equal to or more than 20 meters, which is not common in most sites (CPCB, 2000). Roadside plantation at a distance of 10 and 150 meters from the road reduces pollutant concentration by absorption. They have different sensitivity and resistance to various air pollutants (gaseous or particulate) (Ogbonna et al., 2021). Plants exposed to polluted environments often respond by changing their morphology, physiology, and biochemistry (Verma et al., 2023).

Morphological effects are studied by the naked eye, scanning electron microscopy (SEM), light microscopy, etc. However, the changes in the biochemical and physiological parameters of plants are studied using the air pollution tolerance index (APTI) method (Dhanam et al., 2014). Different plant species vary greatly in their sensitivity to air pollutants. Higher value of APTI suggests higher tolerance of plants. Therefore, some plants exhibit tolerance to air pollution in a particular environment. An essential application of APTI is classifying plant species into receptive and tolerant groups. Sensitive plants act as bio indicators and tolerant plants act as air pollution sinks in urban and industrial areas.

Identification and classification of plants into sensitive and tolerant groups is important because the former can serve as indicators and the latter as sinks to reduce air pollution in urban and industrial habitation. Different plants behave differently under different environmental factors and as a result exhibit different tolerability (Gupta et al., 2020; Karmakar et al., 2020; Panda et al., 2018). Several changes were observed in the biochemical parameters, leading to plant morphological damage after pollutant exposure. For example, Karmakar et al., 2020 studied that pollutants cause cells to become more permeable, resulting in loss of water and dissolved nutrients and early senescence of leaves. Proper selection of plant parameters is of utmost importance to examine the level of sensitivity/tolerance of plants to air pollutants. In the present study, 15 plants species from three locations; Phagwara Bus stand, Phagwara industrial area (Polluted area) and Lovely Professional University (Control) have been selected to determine their tolerance and sensitivity with the help of APTI method.

4.2 Selection of plant species

Plant species selected for the present study were as follows

Mangifera indica commonly called Mango**,** substantial evergreen tree reaching heights between 10 to 45 meters in height. Leaves are simple, varying linear to oblong and measuring 10 to 30 centimeters long and aromatic. The inflorescence forms a large panicle while fruit is drupe.

Ficus benghalensis commonly called banyan fig or Indian banyan, commonly grown in gardens and roadside, large evergreen, 12-18 m tall, rough bark, leaves are broad ovate, ovate oblong, germinate, receptacles axillary, deep orange red, puberulous, ovoid, glabrous. *Ficus benghalensis* produces prop roots which grow downwards.

Ficus religiosa commonly called peepal or bodhi tree, commonly grown in gardens and roadside, a large evergreen, broadly ovate, rough bark, pendulous leaves, Fruits are sessile, receptacles, spherical, reddish purple, axillary, germinate. It is held a sacred by Hindus and Buddhists.

Polyalthia longifolia commonly called Ashoka, Evergreen tree and is commonly cultivated in gardens. Leaves are lanceolate, simple, glossy, tapering to a fine point, margins undulate, glabrous on both sides, stem has 37mm long woody stalk.

Alstonia scholaris commonly called devil's tree or scholar tree**,** Tall evergreen up to 20cm, bark gray, leaves in whorls of 3, petiole 1-3 cm, leaf blade narrowly obovate, leathery, apex rounded, Pedicel is long, seeds oblong, margins ciliate, Flowers greenish white 6-12mm long.

Cascabela thevetia commonly called yellow oleander, Evergreen tropical shrub or small tree, leaves are willow like, linear lanceolate, glossy green in colour, covered with waxy coating, flowers are yellow in colour, terminal clusters, and fruit is deep red black.

Melia azedarach commonly called pride of India, or chinaberry tree, tall, deciduous, Bark brownish gray, branches spreading, leaves odd pinnate, leaflets opposite, leaflet blades ovate, elliptic, flowers fragrant, Staminal tube purple, Ovary spherical, apex

shortly acuminate.

Mentha piperita commonly called Mint or Pudina, fast growing herbaceous, perennial plants grows upto 30-90cm tall, smooth stems, fibrous roots, and leaves are broad, dark green, acute apex and coarsely toothed margins, flowers are purple, flowering season lasts mid from to late summer/

Moringa oleifera commonly called Moringa or drumstick tree**,** fast growing deciduous tree, tall upto10-12m, bark is grey colour, open crown of drooping, fragile branches, leaves are tripinnate, flowers are fragrant and hermaphroditic, fruit is hanging, globular seeds.

Murraya koenigii common name curry ,small growing tree, 4-6 meters tall, aromatic leaves, leaves are small, soft surface, pinnate with 11-21 leaflets, small white flowers, large viable seed,

Morus alba commonly called mulberry or silkworm mulberry, fast growing, deciduous small to medium sized tree, 10-20 meters tall, leaves are broad, long upto 30 cm long, unlobed, cordate, fruit is long, deep purple in colour.

Ocimum sanctum commonly called Tulsi (Queen of herbs), erect branched sub shrub, 30-60 cm tall, green or purple leaves, leaves are ovate; 5cmlong, and toothed, Flowers are purplish, leaves are aromatic, long and rough surface.

Psidium guajava commonly called guava, Evergreen tree, upto13 meters tall, Bark grey, smooth, leaves are leathery, opposite, leaf blade oblong to elliptic, fruit is dark green, ovoid, 5-10cm long, flowers are axillary, solitaire, white.

Syzygium cumini commonly called jamun or Black plum, rapidly growing, evergreen tropical tree, upto 30 meters tall, bark is rough and dark grey, aromatic leaves, glossy dark green, flowers are fragrant, and fruits develop large berries.

Ziziphus mauritiana commonly called Indian Plum, or Chinese apple, spiny, evergreen shrub or small tree, upto 15 meters tall, stipular are spines and many drooping branches, fruit is variable shape and 2.5cm long, leaves are small, smooth, glossy.

The current study entailed the collection of fresh samples of each selected plant species for biochemical analysis. The significant variation in biochemical parameters of each plant species were observed and are subsequently discussed.

4.3 Materials and Methods

Fresh matured leaves samples were collected from three different locations Phagwara Bus stand, Phagwara industrial area, Lovely Professional University (Control). Leaves were analyzed for biochemical parameter such as pH, relative water content, total chlorophyll content and ascorbic acid. The detailed methodology is discussed in the chapter Materials and Methods.

4.4 Result and Discussion

The results of the analysis of four biochemical parameters of plant species from the polluted and control site has been discussed below. The data has been collected during three seasons (summer, winter and monsoon) and presented in Tables 4.1- 4.4and Figures 4.1- 4.4.

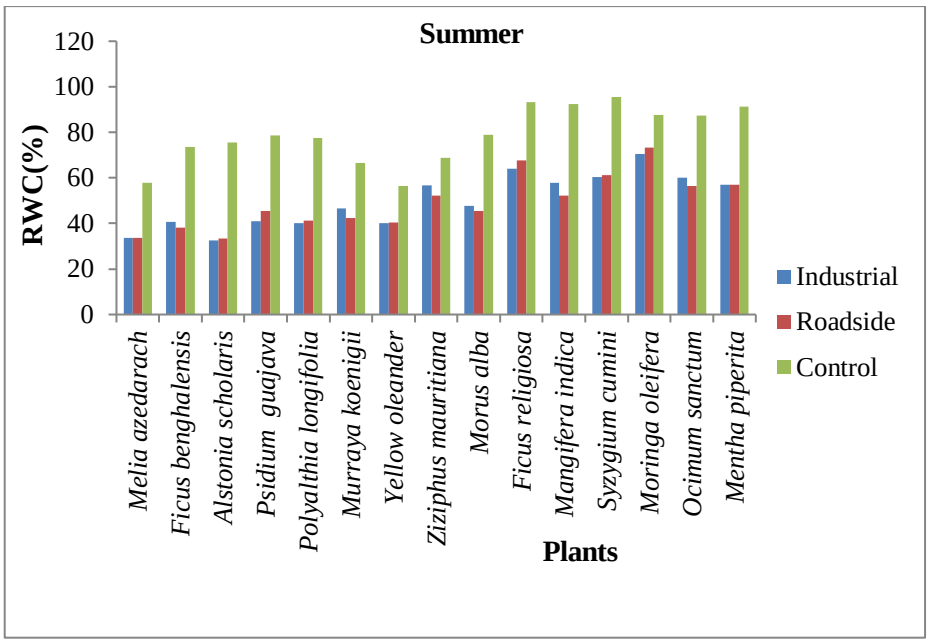
4.4.1 Relative Water Content (%)

Significant variation was observed in the relative water content (RWC) of plants sampled during three seasons. The results of the present study are shown in Table 4.1and Figures 4.1.

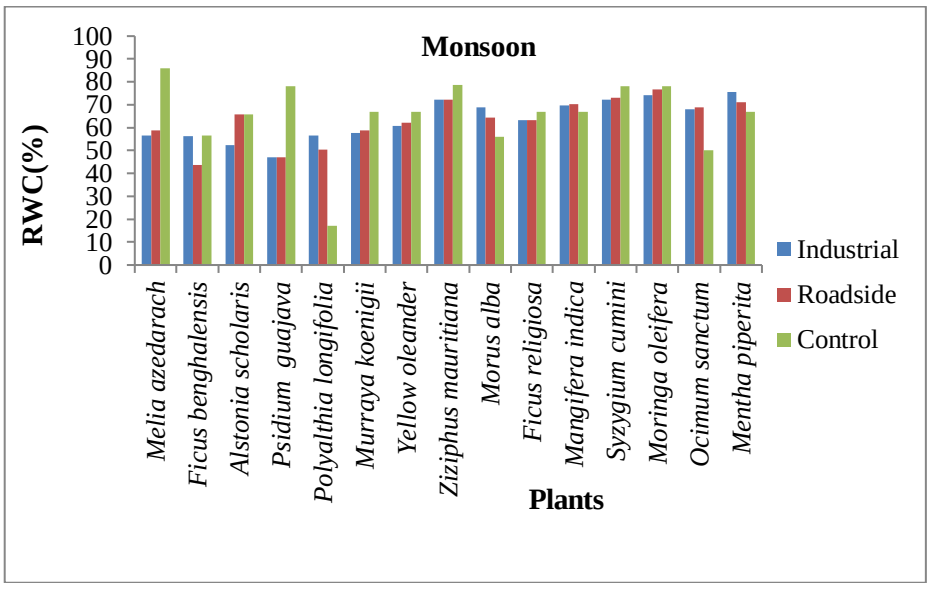
Table 4.1 Seasonal variation in leaf water content (%). Data represent mean ± Standard error (S.E) for monsoon winter and summer

Plants	**Industrial**			**Roadside**			**Control**		
	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer
Melia azedarach	56.5±9.4	42.3±0.01	33.5±8.08	58.9±10.3	42.3±0.01	33.53±8.08	86± 12.3	56±0.01	57.8±11.2
Ficus Benghalensis	56.4±4.2	41.7±1.1	48.8±11.3	43.8±3.2	38.4±0.6	38.1±9.3	56.7±4.1	67±6.5	73.5±5.5
Alstonia Scholaris	52.3±4.4	41.4±0.3	32.4±14.2	65.7±5.4	42.3±0.8	33.4±14.8	65.8±5.1	68±2.3	75.6±16.5
Psidium Guajava	47.1±10.3	47.9±1.5	40.9±11.8	47.1±10.3	43.2±0.8	45.6±14.5	78±15.4	78±5.6	78.5± 14.3
Polyalthia	56.5±8.2	42.1±2.2	40.1±5.2	50.3±2.3	48.9±8.7	41.2±1.2	77.5±10.8	60±4.6	77.5±4.5

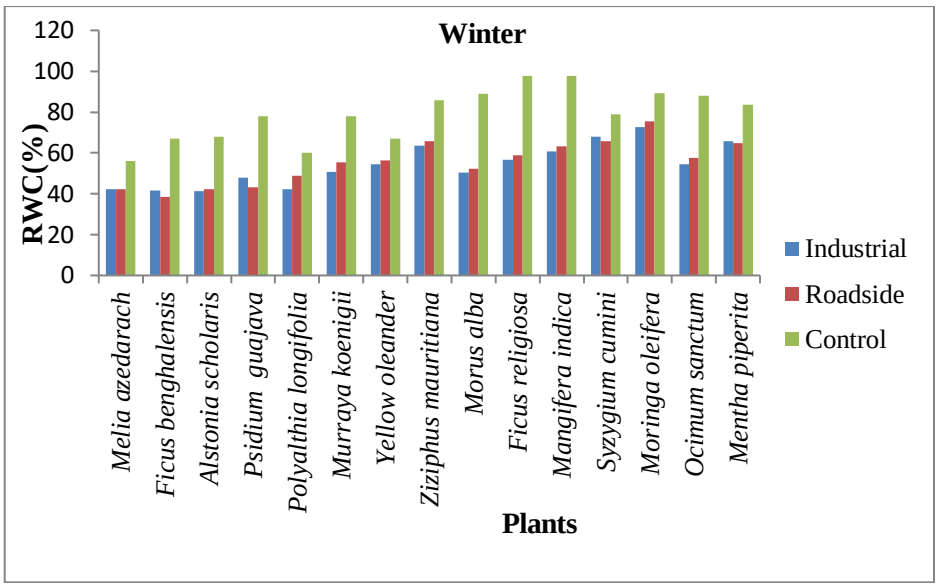
Plants	Industrial			Roadside			Control		
	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer
longifolia									
Murraya	57.8±2.9	50.8±1.5	46.7±7.4	58.9±3.2	55.4±6.5	42.3±3.4	67±3.2	78±3.8	63.5±4.5
Koenigii									
Yellow	60.8±1.8	54.3±0.6	40±5.4	62.3±1.9	56.2±1.2	40.3±5.5	66.8±3.2	67±2.3	56.4±3.4
Oleander									
Ziziphus mauritiana	72.3±2.1	63.4±0.7	56.7±4.9	72.3±2.1	65.7±1.2	52.3±3.2	78.6±4.3	85.7±3.2	68.9±5.1
Morus	68.9±3.7	50.3±0.6	47.8±10.7	64.3±2.1	52.3±1.7	45.6±9.7	56±2.3	89±3.7	78.9±13.4
Alba									
Ficus	63.4±1.2	56.8±0.6	63.9±9.18	63.4±1.2	58.7±1.2	67.8±13.2	67±3.2	97.6±5.6	93.2±4.6
Religiosa									
Mangifera	69.8±1	60.7±0.8	57.8±12.5	70.2±1.6	63.2±2.1	52.3±8.3	67±0.9	97.6±3.2	92.3±1.5
Indica									
Syzygium	72.3±1.7	67.8±0.6	60.4±11.6	73.2±	65.8±0.01	61.2±0.03	78±2.3	78.9±2.3	95.6±3.4
Cumini									
Moringa	74.3±1	72.5±1	70.4±5.3	76.8±1.5	75.6±2.3	73.4±6.5	78±3.2	89.4±3.2	87.6±6.5
Oleifera									
Ocimum	67.9±6.1	54.3±1	60.2±9.7	68.9±6.3	57.6±	56.4±2.2	50±6.5	87.9±7.6	87.3±7.1
Sanctum									
Mentha	75.6±2.4	65.7±0.2	56.9±11.4	71.2±	64.9±1.2	56.9±1.4	67±14.5	83.5±13.4	91.2±12.3
Piperita									


Summer
RWC(%)
120
100
80
60
40
20
0
Melia azedarach
Ficus benghalensis
Alstonia scholaris
Psidium guajava
Polyalthia longifolia
Murraya koenigii
Yellow oleander
Ziziphus mauritiana
Morus alba
Ficus religiosa
Mangifera indica
Syzygium cumini
Moringa oleifera
Ocimum sanctum
Mentha piperita
Plants
Industrial
Roadside
Control

(i)


Monsoon
RWC(%)
100
90
80
70
60
50
40
30
20
10
0
Melia azedarach
Ficus benghalensis
Alstonia scholaris
Psidium guajava
Polyalthia longifolia
Murraya koenigii
Yellow oleander
Ziziphus mauritiana
Morus alba
Ficus religiosa
Mangifera indica
Syzygium cumini
Moringa oleifera
Ocimum sanctum
Mentha piperita
Plants
Industrial
Roadside
Control

(ii)



(iii)

Figure 4.1 Variation in RWC measurements for (i) summer (ii) monsoon (iii) winter seasons

Melia azedarach During Monsoon season, the measurement for RWC in *Melia azedarach* at industrial site has been observed to be 56.5% and at roadside was 58.9% while at Control, it has been observed 86 %. For the summer season, the measurements for relative water content in *Melia azedarach* at industrial site and roadside has been observed to be 33.5% and 33.5% respectively while at Control, its RWC value has been observed to be 57.8%. For the winter season, the measurements for RWC *in Melia azedarach* at Control has been observed to be 56% while at industrial and roadside; its values have been observed to be 42.3% and 42.3% respectively.

Ficus religiosa During Monsoon season, the measurement for RWC in *Ficus religiosa* at Industrial site has been observed to be 56.4% and at roadside was 43.8% whereas; at Control it was observed 56.7%. For the summer season, the measurement of RWC in *Ficus religiosa* was displayed 48.8% at industrial site and 38.1% at roadside but at Control it has been observed to be 73.5%. On other side, in winter season the measurements for RWC in *Ficus religiosa* has been noted 41.7% at industrial and 38.4% at roadside and 67% has been observed at Control.

Alstonia scholaris During Monsoon season, the measurement for RWC in *Alstonia scholaris* at Industrial site has been observed to be 52.3% and at roadside was 65.7%

whereas; at Control it was observed 65.8%. During summer season, the measurement of RWC in *Alstonia scholaris* was displayed 32.4% at industrial site and 33.4% at roadside but at Control it has been observed to be 75.6%.Onother side, in winter season the measurements for RWC in *Alstonia scholaris* has been noted 41.4% at industrial and 42.3% at roadside and 68% has been observed at Control.

Polyalthia longifolia During Monsoon season, the measurement for RWC in *Polyalthia longifolia* at industrial site has been observed to be 56.5% and at roadside was 50.3% while at Control, it hasbeenobserved77.5%.For the summer season, the measurements for relative water content in *Polyalthia longifolia* at industrial site and roadside has been observed to be 40.1% and 41.2% respectively while at Control, its RWC value has been observed to be 77.5%. For the winter season, the measurements for RWC in *Polyalthia longifolia* at Control has been observed to be60% while at industrial and roadside; its values have been observed to be 42.1% and 48.9% respectively.

Psidium guajava During Monsoon season, the measurement for RWC in *Psidium guajava* at Industrial site has been observed to be 47.1% and at roadside was 47.1% whereas; at Control it was observed 78%. During summer season, the measurement of RWC in *Psidium guajava* was displayed 40.9% at industrial site and 45.6% at roadside but at Control it has been observed to be 78.5%. On other side, in winter season the measurements for RWC in *Psidium guajava* has been noted 47.9% at industrial and 43.2% at roadside and 78% has been observed at Control.

Murraya koenigii During Monsoon season, the measurement for RWC in *Murraya koenigii* at industrial site has been observed to be 57.8% and at roadside was 58.9% while at Control; it has been observed 67 %. For the summer season, the measurements for relative water content in *Murraya koenigii* at industrial site and roadside has been observed to be 46.7% and 42.3% respectively while at Control, its RWC value has been observedtobe78%. For the winter season, the measurement for RWC in Murraya koenigii at Control has been observed to be 78% while at industrial and roadside; its values have been observed to be 50.8% and 55.4% respectively.

Yellow oleander During Monsoon season, the measurement for RWC in *Yellow oleander* at industrial site has been observed to be 60.8% and at roadside was 62.3% while at Control, it has been observed 66.8%.Forthesummerseason, the measurements for relative water content in *Yellow oleander* at industrial site and roadside has been observed to be 40% and 40.3% respectively while at Control, its RWC value has been

observed to be 56.4%. For the winter season, the measurements for RWC *in Yellow oleander* at Control has been observed to be 67% while at industrial and roadside; its values have been observed to be 54.3% and 56.2% respectively.

Ziziphus mauritiana During Monsoon season, the measurement for RWC in *Ziziphus mauritiana* at industrial site has been observed to be 72.3% and at roadside was 72.3% while at Control, it has beenobserved78.6%. For the summer season, the measurements for relative water content in Ziziphus *mauritiana* at industrial site and roadside has been observed to be 56.7% and 52.3% respectively while at Control, its RWC value has been observed to be 68.9%. For the winter season, the measurements for RWC *in Ziziphus mauritiana* at Control has been observed to be 85.7% while at industrial and roadside; its values have been observed to be 63.4% and 85.7% respectively.

Morus alba During Monsoon season, the measurement for RWC in *Morus alba* at industrial site has been observed to be 68.9%and at roadside was 64.3% while at Control, it has been observed 56%. For the summer season, the measurements for relative water content in *Morus alba* at industrial site and roadside has been observed to be 47.8% and 45.6% respectively while at Control, its RWC value has been observed to be78.9%. For the winter season, the measurements for RWC *in Morus alba* at Control has been observed to be89% while at industrial and roadside; its values have been observed to be 50.3% and 52.3% respectively.

Ficus religiosa During Monsoon season, the measurement for RWC in *Ficus religiosa* at industrial site has been observed to be 63.4% and at roadside was 63.4% while at Control, it has beenobserved67%.For the summer season, the measurements for relative water content in *Ficus religiosa* at industrial site and roadside has been observed to be 63.9% and 67.8% respectively while at Control, its RWC value has been observed to be 67%. For the winter season, the measurements for RWC *in Ficus religiosa* at Control has been observed to be 97.6% while at industrial and roadside; its values have been observed to be 56.8% and 58.7% respectively.

Mangifera indica During Monsoon season, the measurement for RWC in *Mangifera indica* at industrial site has been observed to be 69.8% and at roadside was 70.2% while at Control, it has been observed 67%. For the summer season, the measurements for relative water content in *Mangifera indica* at industrial site and roadside has been observed to be 57.8% and 52.3% respectively while at Control, its RWC value has been observed to be 92.3%. For the winter season, the measurements for RWC *in Mangifera*

indica at Control has been observed to be 97.6.2% while at industrial and roadside; its values have been observed to be 60.7% and 63.2% respectively.

Syzygium cumini During Monsoon season, the measurement for RWC in *Syzygium cumini* at industrial site has been observed to be 72.3% and at roadside was 73.2% while at Control; it has been observed 78%. For the summer season, the measurements for relative water content in *Syzygium cumini* at industrial site and roadside has been observed to be 60.4% and 61.2% respectively while at Control, its RWC value has been observed to be 95.6%. For the winter season, the measurements for RWC in *Syzygium cumini* at Control has been observed to be 78.9% while at industrial and roadside; its values have been observed to be 67.8% and 65.8% respectively.

Moringa oleifera During Monsoon season, the measurement for RWC in *Moringa oleifera* at industrial site has been observed to be 74.3% and at roadside was 76.8% while at Control, it has been observed 78%. For the summer season, the measurements for relative water content in *Moringa oleifera at* industrial site and roadside has been observed to be 70.4% and 73.4% respectively while at Control, its RWC value has been observed to be 87.6%. For the winter season, the measurements for RWC *in Moringa oleifera* at Control has been observed to be89.4% while at industrial and roadside; its values have been observed to be 72.5% and 75.6% respectively.

Ocimum sanctum During Monsoon season, the measurement for RWC in *Ocimum sanctum* at industrial site has been observed to be 67.9% and at roadside was 68.9% while at Control; it has been observed 50%. For the summer season, the measurements for relative water content in *Ocimum sanctum* at industrial site and roadside has been observed to be 60.2% and 56.4 respectively while at Control, its RWC value has been observed to be 87.3%. For the winter season, the measurements for RWC *in Ocimum sanctum* at Control has been observed to be87.9% while at industrial and roadside; its values have been observed to be 54.3% and 57.6% respectively.

Mentha piperita During Monsoon season, the measurement for RWC in *Mentha piperita* at industrial site has been observed to be 75.6% and at roadside was 71.2% while at Control, it has been observed 67 %. For the summer season, the measurements for relative water content in *Mentha piperita* at industrial site and roadside has been observed to be 56.9% and 56.9% respectively while at Control, its RWC value has been observed to be 91.2%. For the winter season, the measurements for RWC *in Mentha piperita* at Control has been observed to be83.5% while at industrial and roadside; its

values have been observed to be 65.7% and 64.9% respectively.

The outcomes of the current study exhibited that higher amount of RWC in most of the plants species during the monsoon seasons followed winter and summer season. The higher relative water content of the plants during monsoon season suggests that they may not experiencing water stress. It may be because of low temperature during monsoon resulting in reduced transpiration rates in leaves. During Monsoon season pollutants are washed away to the soil and hence it may be the one of the cause of high RWC in leaves. In response to stress conditions, the plant exhibited an increase in relative water content to cope with stress conditions. Since, the high water content within a plant helps to maintain its physiological balance when exposed to stress conditions such as air pollution.

The maintenance of RWC by a plant determines its relative tolerance to pollution (Verma 2003, Singh et al., 1991, Jyothi and Jaya 2010, Krishnaveni et al., 2012; Rai, 2016; Karmakar et al., 202; Babu et al., 2013; Sharma et al., 2017).Besides, high RWC also supports drought resistance in plants. Another finding drawn from the current results is that RWC generally found higher in plant species sampled from control sites as compared to polluted site (as shown in Table 3.1 and Figure 4.1). Low water content in leaves at polluted sites may be due to air pollutants. As it results in high transpiration rates which lead to dehydration.

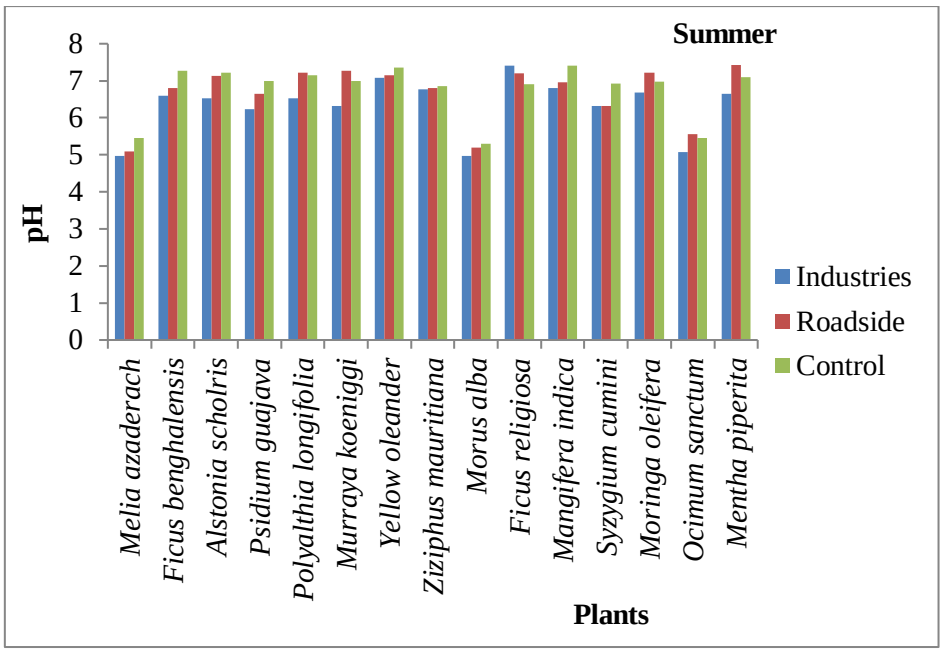
On other side, higher RWC at control site suggests that due to no or low exposure to air pollutants plants maintain their RWC in leaves. Similar studies were conducted by several researchers (Singh et al., 1991; Karmakar et al., 2021; Palit et al., 2013; Kuddus et al., 2011; Bhattacharya et al., 2012; Chouhan et al., 2021; Enete et al., 2013).

4.4.2 pH

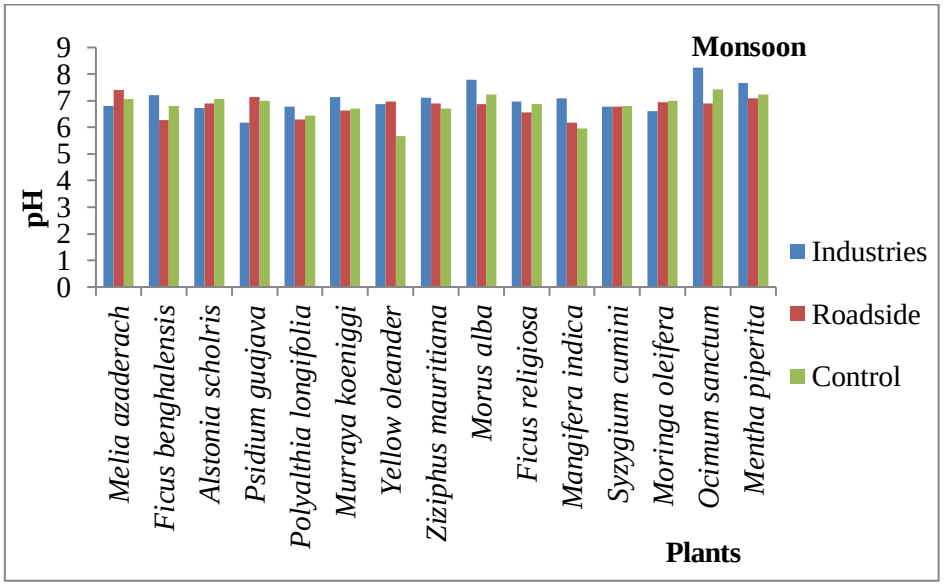
Most of the leaves samples were found to have acidic pH. It may be due to the presence of air pollutants like $SO_2$and NO_xin the ambient air. The results of the present study are shown in Table 4.2 and Figure 4.2. As a result, it was observed that plant leaf samples showed varying degree of pH value in response to air pollution.

Table 4.2 Seasonal variations in leaf extract pH. Data represent mean ±Standard error (S.E) for monsoon winter and summer.

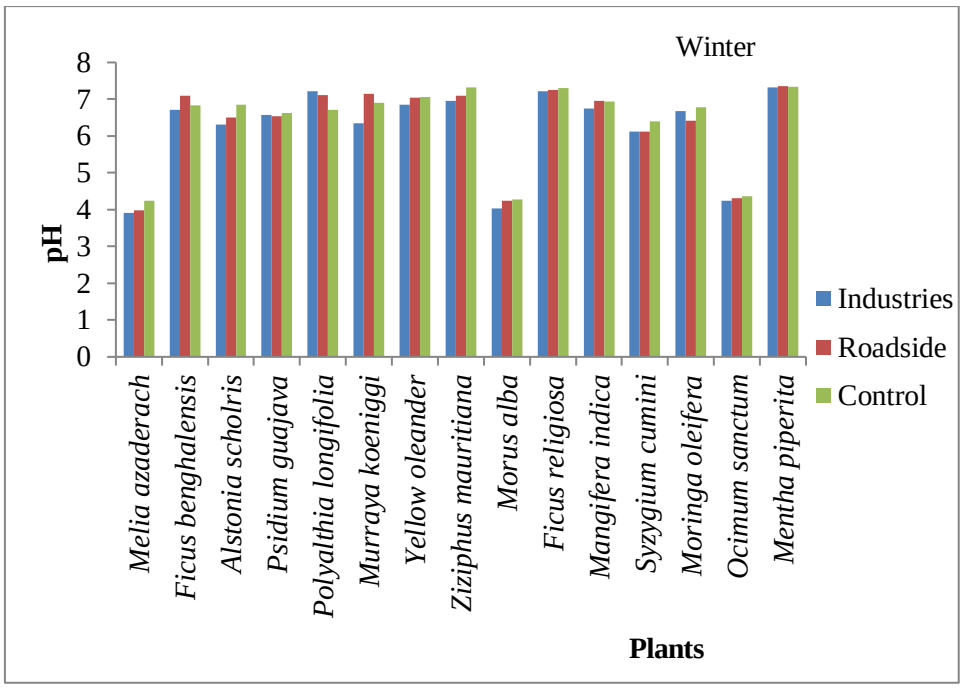
Plants species	**Industries**			**Roadside**			**Control**		
	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer
Melia azedarach	6.8±0.64	3.9±1.6	4.9±1.6	7.4± 0.51	3.9±1.6	5.1± 1.7	7± 0.49	4.2±1.7	5.4± 1.8
Ficus benghalensis	7.2±0.75	6.7±0.26	6.6±0.36	6.2± 0.43	7.1±0.18	6.8±0.12	6.8± 0.6	6.8±0.30	7.2±0.13
Alstonia scholaris	6.7±0.73	6.3±0.22	6.5±0.14	6.9± 0.75	6.5±0.17	7.1±0.13	7± 0.40	6.8±0.16	7.2±0.19
Psidium guajava	7± 0.52	6.5±0.22	6.2±0.15	7.1± 0.40	6.5±0.16	6.6±0.18	7± 0.1	6.6±0.45	7± 0.05
Polyalthia longifolia	6.7±0.67	7.2±0.15	6.5±0.19	6.3±0.58	7.1±0.03	7.2±0.11	6.4±0.17	6.7±0.14	7.1±0.12
Murraya koenigii	7.1±0.35	6.3±0.27	6.3±0.13	6.3±0.08	7.1±0.09	7.2±0.31	6.7±0.2	6.9±0.18	7±0.16
Yellow oleander	6.8±0.26	6.8±0.33	7±0.18	6.9±0.37	7±0.25	7.1±0.37	5.6±0.73	7±0.14	7.3±0.18
Ziziphus mauritiana	7.1±0.71	6.9±0.06	6.7±0.09	6.9± 0.01	7.1±0.21	6.8±0.1	6.7±0.37	7.3±0.20	6.8±0.22
Morus alba	7.7±0.65	4.0±1.6	4.9±1.6	6.8±0.57	4.2±1.7	5.2±1.7	7.2±0.50	4.2±1.7	5.3±1.7
Ficus religiosa	6.9±0.39	7.2±0.33	7.4±0.24	6.5±0.23	7.2±0.12	7.2±0.27	6.8±0.37	7.3±0.21	6.9±0.31
Mangifera indica	7.1±0.37	6.7±0.53	6.8±0.17	6.1±0.29	6.9±0.09	6.9±0.25	5.9±0.08	6.9±0.21	7.4±0.21
Syzygium Cumini	6.7±0.44	6.1±0.49	6.3±0.23	6.7±0.44	6.1±0.49	6.3±0.23	6.8±0.15	6.4±0.46	6.9±0.27
Moringa oleifera	6.6±0.1	6.6±0.12	6.6±0.16	6.9±0.54	6.4±0.25	7.2±0.28	7±0.5	6.7±0.24	6.9±0.22
Ocimum sanctum	8.2±0.81	4.2±1.7	5±1.6	6.9±0.26	4.3±1.7	5.5±1.8	7.4±0.37	4.3±1.7	5.4±1.8
Mentha piperita	7.6±0.58	7.3±0.14	6.6±0.29	7.1±0.1	7.3±0.14	7.4±0.14	7.2±0.2	7.3±0.16	7.1±0.13


Summer
pH
0
1
2
3
4
5
6
7
8
Industries
Roadside
Control
Melia azaderach
Ficus benghalensis
Alstonia scholris
Psidium guajava
Polyalthia longifolia
Murraya koeniggi
Yellow oleander
Ziziphus mauritiana
Morus alba
Ficus religiosa
Mangifera indica
Syzygium cumini
Moringa oleifera
Ocimum sanctum
Mentha piperita
Plants

(i)


Monsoon
pH
0
1
2
3
4
5
6
7
8
9
Industries
Roadside
Control
Melia azaderach
Ficus benghalensis
Alstonia scholris
Psidium guajava
Polyalthia longifolia
Murraya koeniggi
Yellow oleander
Ziziphus mauritiana
Morus alba
Ficus religiosa
Mangifera indica
Syzygium cumini
Moringa oleifera
Ocimum sanctum
Mentha piperita
Plants

(ii)



(iii)

Figure 4.2 VariationinpH measurements for (i) summer,(ii) monsoon and (iii) winter seasons

Melia azedarach During Monsoon season, the measurement for pH in *Melia azedarach* at industrial site has been observed to be 6.8 and at roadside was 7.4 while at Control, it has been observed 7. For the summer season, the measurements for pH in *Melia azedarach* at industrial site and roadside has been observed to be 4.9 and 5.1 respectively while at Control, its pH value has been observed to be 5.4. For the winter season, the measurements for pH in *Melia azedarach* at Control has been observed to be 4.2 while at industrial and roadside; its values have been observed to be 3.9 and 3.9 respectively.

Ficus religiosa During Monsoon season, the measurement for pH in *Ficus religiosa* at Industrial site has been observed to be 7.2 and at roadside was 6.2 whereas; at Control it was observed 6.8. For the summer season, the measurement of pH in *Ficus religiosa* was displayed6.6 at industrial site and 6.8 at roadside but at Control it has been observed to be 7.2.

On other side, in winter season the measurements for pH in *Ficus religiosa* has been noted 6.7 at industrial and 7.1 at roadside and 6.8 has been observed at Control.

Alstonia scholaris During Monsoon season, the measurement for pH in *Alstonia scholaris* at Industrial site has been observed to be 6.7 and at roadside was 6.9 whereas; at Control it was observed7.During summer season, the measurement of pH in *Alstonia scholaris* was displayed 6.5 at industrial site and 7.1 at roadside but at Control it has been observed to be 7.2. On other side, in winter season the measurements for pH in *Alstonia scholaris* has been noted 6.3 at industrial and 6.5 at roadside and 6.8 has been observed at Control.

Polyalthia longifolia During Monsoon season, the measurement for pH in *Polyalthia longifolia* at industrial site has been observed to be 6.7 and at roadside was 6.3 while at Control, it has been observed 6.4. For the summer season, the measurements for pH in *Polyalthia longifolia* at industrial site and roadside has been observed to be 6.5 and 7.2 respectively while at Control, its pH value has been observed to be 7.1. For the winter season, the measurements for pH in *Polyalthia longifolia* at Control has been observed to be 6.7 while at industrial and roadside; its values have been observed to be 7.2 and 7.1 respectively.

Psidium guajava During Monsoon season, the measurement for pH in *Psidium guajava* at Industrial site has been observed to be 7 and at roadside was 7.1 whereas; at Control it was observed7. During summer season, the measurement of pH in *Psidium guajava* was displayed 6.2 at industrial siteand6.6at roadside but at Control it has been observed to be7. On other side, in winter season the measurements for pH in *Psidium guajava* has been noted 6.5 at industrial and 6.5at roadside and 6.6 has been observed at Control.

Murraya koenigii During Monsoon season, the measurement for pH in *Murraya koenigii* at industrial site has been observed to be 7.1 and at roadside was 6.3 while at Control, it has been observed 6.7. For the summer season, the measurement for pH in Mu*rraya koenigii* at industrial site and roadside has been observed to be 6.3 and 7.2 respectively while at Control, its pH value has been observedtobe7. For the winter season, the measurement for pH in *Murraya koenigii* at Control has been observed to be 6.9 while at industrial and roadside; its values have been observed to be 6.3 and 7.1respectively.

Yellow oleander During Monsoon season, the measurement for pH in *Yellow oleander* at industrial site has been observed to be 6.8 and at roadside was 6.9 while at Control, it

has been observed 5.6. For the summer season, the measurement for pH in Yellow oleander at industrial site and roadside has been observedtobe7 and7.1respectively while at Control, its pH value has been observed to be 7.3. For the winter season, the measurement for pH *in Yellow oleander* at Control has been observed to be7 while at industrial and roadside; its values have been observed to be 6.8 and 7 respectively.

Ziziphus mauritiana During Monsoon season, the measurement for pH in *Ziziphus mauritiana* at industrial site has been observed to be 7.1 and at roadside was 6.9 while at Control, it has been observed 6.7. For the summer season, the measurements for pH in Ziziphus *mauritiana* at industrial site and roadside has been observed to be 6.7 and 6.8 respectively while at Control, its pH value has been observed to be 6.8. For the winter season, the measurements for pH *in Ziziphus mauritiana* at Control has been observedtobe7.3 while at industrial and roadside; its values have been observed to be 6.9 and 7.1 respectively.

Morus alba During Monsoon season, the measurement for pH in *Morus alba* at industrial site has been observed to be 7.7 and at roadside was6.8 while at Control, it has been observed 7.2. For the summer season, the measurements for pH in *Morus alba* at industrial site and roadside has been observed to be 4.9 and 5.2 respectively while at Control, its pH value has been observed to be 5.3. For the winter season, the measurements for pH *in Morus alba* at Control has been observed to be 4.2 while at industrial and roadside; its values have been observed to be 4 and 4.2 respectively.

Ficus religiosa During Monsoon season, the measurement for pH in *Ficus religiosa* at industrial site has been observed to be 6.9 and at roadside was 6.5while at Control, it has been observed 6.8. For the summer season, the measurements for pH in *Ficus religiosa* at industrial site and roadside has been observed to be 7.4 and 7.2 respectively while at Control, its pH value has been observed to be 6.9. For the winter season, the measurements for pH *in Ficus religiosa* at Control hasbeenobservedtobe7.3while at industrial and roadside; its values have been observed to be
7.2 and 7.2 respectively.

Mangifera indica During Monsoon season, the measurement for pH in *Mangifera indica* at industrial site has been observed to be 7.1 and at roadside was 6.1 while at Control, it has been observed 5.9. For the summer season, the measurements for pH in *Mangifera indica* at industrial site and roadside has been observed to be 6.8 and 6.9 respectively while at Control, its pH value has been observed to be 7.4. For the winter

season, the measurements for pH in *Mangifera indica* at Control has been observed to be 6.9 while at industrial and roadside; its values have been observed to be 6.7 and 6.9 respectively.

Syzygium cumini During Monsoon season, the measurement for pH in *Syzygium cumini* at industrial site has been observed to be 6.7 and at roadside was 6.7 while at Control, it has been observed 6.8. For the summer season, the measurement for pH in Syzygium cumini at industrial site and roadside has been observed to be 6.3 and 6.3 respectively while at Control, its pH value has been observed to be 6.9. For the winter season, the measurement for pH in Syzygium cumini at Control has been observed to be 6.4 while at industrial and roadside; its values have been observed to be 6.1 and 6.1 respectively.

Moringa oleifera During Monsoon season, the measurement for pH in *Moringa oleifera* at industrial site has been observed to be 6.6 and at roadside was 6.9 while at Control, it has been observed 7. For the summer season, the measurements for pH in *Moringa oleifera at* industrial site and roadside has been observed to be 6.6 and 7.2 respectively while at Control, its pH value has been observedtobe6.9. For the winter season, the measurements for pH *in Moringa oleifera* at Control has been observed to be 6.7 while at industrial and roadside; its values have been observed to be 6.6 and 6.4 respectively.

Ocimum sanctum During Monsoon season, the measurement for pH in *Ocimum sanctum* at industrial site has been observed to be 8.2 and at roadside was 6.9 while at Control; it has been observed 7.4. For the summer season, the measurement for pH in Ocimum sanctum at industrial site and roadside has been observed to be 5 and 5.5 respectively while at Control, its pH value has beenobservedtobe5.4. For the winter season, the measurements for pH *in Ocimum sanctum* at Control has been observed to be 4.3 while at industrial and roadside; its values have been observed to be 4.2 and 4.3 respectively.

Mentha piperita During Monsoon season, the measurement for pH in *Mentha piperita* at industrial site has been observed to be 7.6 and at roadside was 7.1 while at Control, it has been observed 7.2. For the summer season, the measurements for pH in *Mentha piperita* at industrial site and roadside has been observed to be 6.6 and 7.4 respectively while at Control, its pH value has been observed to be 7.1. For the winter season, the measurements for pH in *Mentha piperita* at Control has been observed to be 7.3 while at industrial and roadside; its values have been observed to be 7.3 and 7.3 respectively.

The results of the current study exhibited low pH in most of the plants species during the winter season followed by summer season and monsoon season. Generally, the drop in the pH indicates the sensitivity of the plant species to air pollution (Singh et al., 1991). However, in the current study most of the plants were found in the acidic range. This can be possible when air pollutants, mainly gaseous types disperse and react with water inside the cell, forming acid radicals (Karmakar et al., 2020). For instance, SO_2passes through stomata; it combines with water and produce bisulphate, sulphites and their ionic species, resulting in proton formation, which affected the cell's pH (Karmakar et al., 2020). Kousar et al., 2014; Khanoranga and Khalid, 2019 also studied that it changes the pH to an acidic level inside the plant due to SO_2. Thus, it plays a most important function in the directive of SO_2 sensitivity in plants. Another reason could be high relative water content, as high RWC also reduces the acidity of cell sap and helps tolerate drought stress. Similar conclusions have drawn by Palit et al., 2013; Rai., 2016.

Severe decline in pH was observed in plants like *Melia azedarach*, pH was low during winter season followed by summer season and monsoon season (range from 3 to 5) at polluted and control site as. *Morus alba* and *Ocimum sanctum* also showed low pH (range from 4 to 5) during the winter season followed by summer season and monsoon season at both polluted and control site. It was observed that the pH value at the polluted site was lower than that at the control site during the study period.

The reduction of pH in plants from polluted areas reflects the sensitivity and closure of the stomata to pollutants. Our present finding regarding reduction of pH in polluted sites has exhibited it's counteract mechanism to fight with air pollution and it is consistent with previous literature. *Melia azedarach, Morus alba* and *Ocimum sanctum* exhibited low pH and can be considered as sensitive while *Mentha piperita* and *Ficus religiosa* exhibited higher pH and can be considered as tolerant to air pollution. Hence, *Ficus religiosa* and *Mentha piperita* can be planted at the polluted site and serve as sink in the study area.

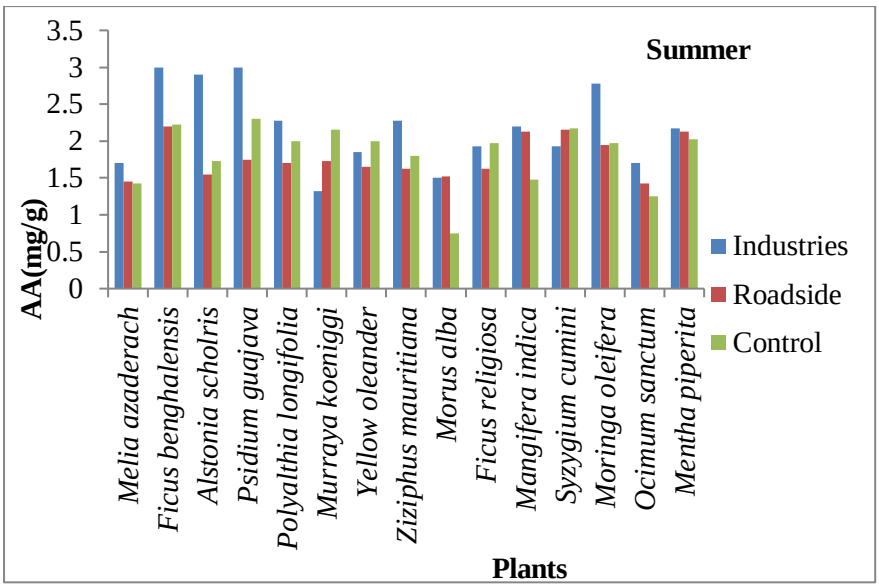
4.4.3 Ascorbic acid content (mg/g)

Ascorbic acid serves as an antioxidant in plants, activating resistance mechanisms particularly in plants growing in polluted environments. The ascorbic acid content in the studied plant species during the rainy, winter, and summer seasons is detailed in Table

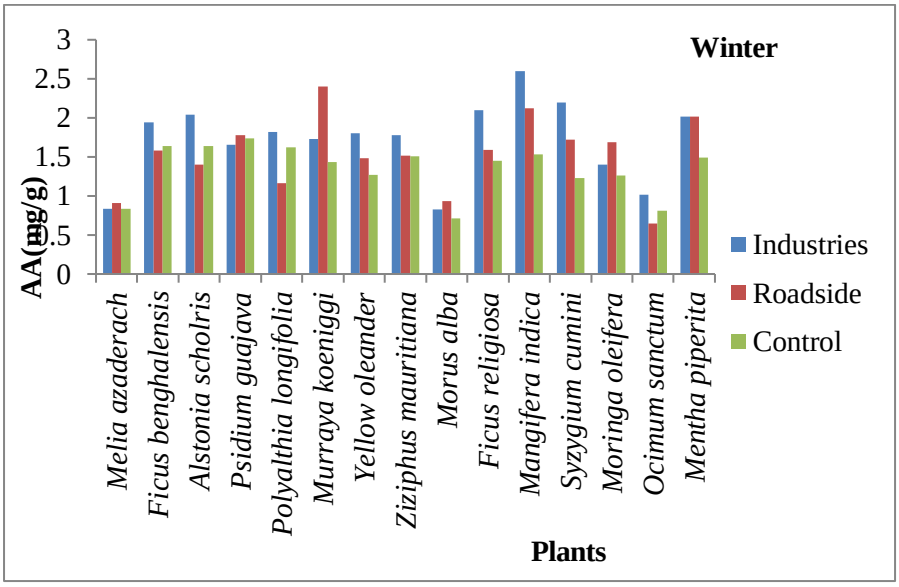
4.3 and depicted in Figure 4.4. Consequently, it was noted that leaf samples from plants exhibited varying levels of ascorbic acid in response to air pollution.

Table 4.3 Seasonal variations in ascorbic acid content (mg/g). Data represent mean ±Standard error (S.E) for monsoon winter and summer.

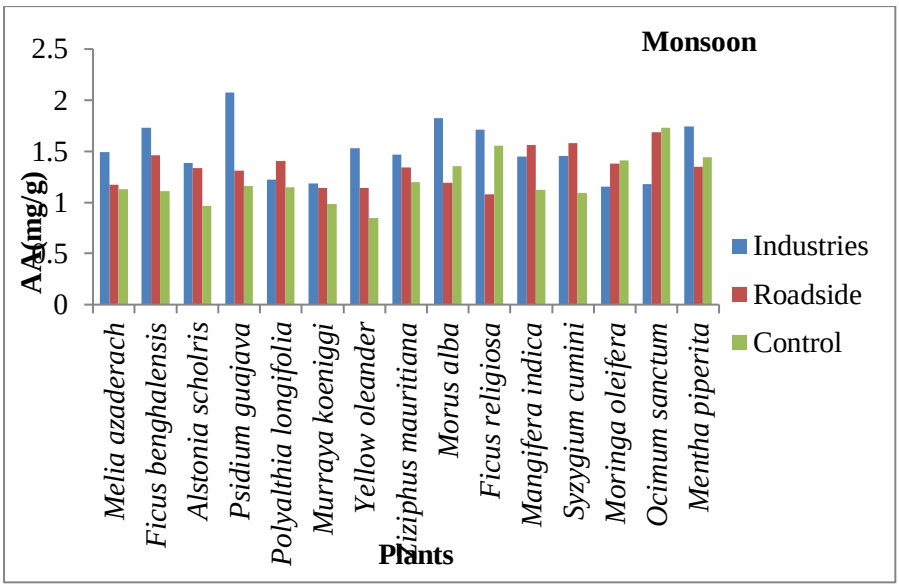
Plants species	**Industrial**			**Roadside**			**Control**		
	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer
Melia azedarach	1.4±0.87	0.8±0.37	1.7±0.65	1.1±0.50	0.9±0.39	1.4±0.52	1.1±0.46	0.8±0.36	1.4±0.48
Ficus benghalensis	1.7±0.72	1.9±0.15	3±0.07	1.4±1.46	1.5±0.25	2.2±0.22	1.1±1.11	1.6±0.09	2.2±0.17
Alstonia Scholaris	1.3±0.81	2±0.43	2.9±0.14	1.3±55	1.3±0.29	1.5±0.35	0.9±0.52	1.6±0.26	1.7±0.21
Psidium Guajava	2±0.29	1.6±	3±0.5	1.3±0.11	1.7±	1.7±0.19	1.1±	1.7±	2.3±0.12
Polyalthia longifolia	1.2±0.11	1.8±0.45	2.2±0.36	1.4±0.57	1.1±0.08	1.7±0.27	1.1±0.49	1.6±0.42	2±0.20
Murraya Koenigii	1.1±0.15	1.7±0.41	1.3±0.1	1.1±0.43	2.4±1.1	1.7±0.22	0.9±0.41	1.4±0.29	2.1±0.23
Yellow oleander	1.5±0.60	1.8±0.38	1.8±0.25	1.1±0.43	1.4±0.21	1.6±0.11	0.8±0.33	1.2±0.14	2.2±0.05
Ziziphus mauritiana	1.4±0.46	1.7±0.22	2.2±0.13	1.3±0.32	1.5±0.16	1.6±0.37	1.1±0.48	1.5±0.20	1.8±0.10
Morus Alba	1.8±0.5	0.8±0.34	1.5±0.57	1.1±0.16	0.9±0.38	1.5±0.63	1.3±0.54	0.7±0.34	0.7±0.23
Ficus Religiosa	1.7±0.63	2.1±0.44	1.9±0.07	1±0.16	1.5±0.20	1.6±0.21	1.5±0.36	1.4±0.20	1.9±0.27
Mangifera indica	1.4±0.50	2.5±0.63	2.2±0.31	1.5±0.32	2.1±0.36	2.1±0.14	1.1±0.49	1.5±0.20	1.4±0.14
Syzygium Cumini	1.4±0.67	2.2±0.44	1.9±0.33	1.5±0.31	1.7±0.20	2.1±0.08	1±0.45	1.2±0.14	2.1±0.11
Moringa oleifera	1.1±0.44	1.4±0.35	2.7±0.12	1.3±0.51	1.6±0.27	1.9±0.33	1.4±0.45	1.2±0.31	1.9±0.12
Ocimum sanctum	1.1±0.16	1±0.42	1.7±.0.63	1.6±0.40	0.6±0.27	1.4±0.55	1.7±0.20	0.8±0.34	1.2±0.42
Mentha piperita	1.7±0.44	2±0.39	2.1±0.38	1.3±0.32	2±0.41	2.1±0.27	1.4±0.32	1.4±0.20	2±0.075


Summer
AA(mg/g)
3.5
3
2.5
2
1.5
1
0.5
0
Industries
Roadside
Control
Melia azaderach
Ficus benghalensis
Alstonia scholris
Psidium guajava
Polyalthia longifolia
Murraya koeniggi
Yellow oleander
Ziziphus mauritiana
Morus alba
Ficus religiosa
Mangifera indica
Syzygium cumini
Moringa oleifera
Ocimum sanctum
Mentha piperita
Plants

(i)


Winter
AA(mg/g)
3
2.5
2
1.5
1
0.5
0
Industries
Roadside
Control
Melia azaderach
Ficus benghalensis
Alstonia scholris
Psidium guajava
Polyalthia longifolia
Murraya koeniggi
Yellow oleander
Ziziphus mauritiana
Morus alba
Ficus religiosa
Mangifera indica
Syzygium cumini
Moringa oleifera
Ocimum sanctum
Mentha piperita
Plants

(ii)



(iii)

Figure 4.3 Variation in AA (mg/g) measurements for (i) summer, (ii) winter and (iii) monsoon seasons

Melia azedarach During Monsoon season, the measurement for AA in *Melia azedarach* at industrial site has been observed to be 1.4mg/g and at roadside was 1.1mg/g while at Control; it has been observed 1.1 mg/g. For the summer season, the measurements for AA in *Melia azedarach* at industrial site and roadside has been observed to be 1.7 mg/g and 1.4mg/g respectively while at Control, its AA value has been observed to be 1.4mg/g. For the winter season, the measurements for AA in *Melia azedarach* at Control has been observedtobe0.8mg/g while at industrial and roadside; its values have been observed to be 0.8mg/g and 0.9mg/g respectively.

Ficus religiosa During Monsoon season, the measurement for AA in *Ficus religiosa* at Industrial site has been observed to be 1.7mg/g and at roadside was 1.4mg/g whereas; at Control it was observed 1.1mg/g. For the summer season, the measurement of AA in *Ficus religiosa* was displayed 3mg/g at industrial site and 2.2mg/g at roadside but at Control it has been observed to be2.2mg/g. On other side, in winter season the measurements for AA in *Ficus religiosa* has been noted 1.9mg/g at industrial and 1.5mg/g at roadside and 1.6mg/g has been observed at Control.

Alstonia scholaris During Monsoon season, the measurement for AA in *Alstonia scholaris* at Industrial site has been observed to be 1.3mg/g and at roadside was 1.3mg/g whereas; at Control it was observed 0.9mg/g. During summer season, the measurement of AA in *Alstonia scholaris* was displayed 2.9mg/g at industrial site and 1.5mg/g at roadside but at Control it has been observed to be 1.7mg/g. On other side, in winter season the measurements for AA in *Alstonia scholaris* has been noted 1.6mg/g at industrial and 1.7mg/g at roadside and 1.7mg/g has been observed at Control.

Polyalthia longifolia During Monsoon season, the measurement for AA in *Polyalthia longifolia* at industrial site has been observedtobe1.2mg/g and at roadside was 1.4mg/g while at Control; it has been observed 1.1mg/g. For the summer season, the measurements for AA in *Polyalthia longifolia* at industrial site and roadside has been observed to be 2.2mg/g and 1.7mg/g respectively while at Control, its AA value has beenobservedtobe2mg/g. For the winter season, the measurements for AA in *Polyalthia longifolia* at Control has been observed to be 1.6mg/g while at industrial and roadside; its values have been observed to be 1.8mg/g and 1.1mg/g respectively.

Psidium guajava During Monsoon season, the measurement for AA in *Psidium guajava* at Industrial site has been observed to be 2mg/g and at roadside was 1.3mg/g whereas; at Control it was observed 1.1mg/g. During summer season, the measurement of AA in *Psidium guajava* was displayed 3mg/g at industrial site and 1.7mg/g roadside but at Control it has been observed to be2.3mg/g. On other side, in winter season the measurements for AA in *Psidium guajava* has been noted 1.6mg/g at industrial and 1.7mg/g at roadside and 1.7mg/g has been observed at Control.

Murraya koenigii During Monsoon season, the measurement for AA in *Murraya koenigii* at industrial site has been observed to be 1.1mg/g and at roadside was 1.1mg/g while at Control; it has been observed 0.9mg/g. For the summer season, the measurements for AA in *Murraya koenigii* at industrial site and roadside has been observed to be1.3mg/g and 1.7mg/g respectively while at Control, its AA value has been observed to be 2.1mg/g. For the winter season, the measurement for AA in Murraya koenigii at Control has been observed to be 1.4mg/g while at industrial and roadside; its values have been observed to be 1.7mg/g and 2.4mg/g respectively.

Yellow oleander During Monsoon season, the measurement for AA in *Yellow oleander* at industrial site has been observed to be 1.5mg/g and at roadside was1.1mg/g while at

Control; it has been observed 0.8mg/g. For the summer season, the measurements for AA in *Yellow oleander* at industrial site and roadside has been observed to be 1.8mg/g and 1.6mg/g respectively while at Control, its AA value has been observed to be 2.2mg/g. For the winter season, the measurement for AA *in Yellow oleander* at Control has been observed to be 1.2mg/g while at industrial and roadside; its values have been observed to be 1.8mg/g and 1.4mg/g respectively.

Ziziphus mauritiana During Monsoon season, the measurement for AA in *Ziziphus mauritiana* at industrial site has been observed to be 1.4mg/g and at roadside was 1.3mg/g while at Control; it has been observed 1.1mg/g. For the summer season, the measurements for AA in Ziziphus *mauritiana* at industrial site and roadside has been observed to be 2.2mg/g and 1.6mg/g respectively while at Control, its AA value has been observed to be 1.8mg/g. For the winter season, the measurements for AA *in Ziziphus mauritiana* at Control has been observed to be1.5mg/g while at industrial and roadside; its values have been observed to be 1.7mg/g and1.5mg/g respectively.

Morus alba During Monsoon season, the measurement for AA in *Morus alba* at industrial site has been observed to be 1.8mg/g and at roadside was 1.1mg/g while at Control, it has been observed 1.3mg/g. For the summer season, the measurements for AA in *Morus alba* at industrial site and roadside has been observed to be 1.5mg/g and 1.5mg/g respectively while at Control, its AA value has been observed to be 0.7mg/g. For the winter season, the measurements for AA in *Morus alba* at Control has been observed to be 0.7mg/g while at industrial and roadside; its values have been observed to be 0.8mg/g and 0.9mg/g respectively.

Ficus religiosa During Monsoon season, the measurement for AA in *Ficus religiosa* at industrial site has been observed to be 1.7mg/g and at roadside was 1mg/g while at Control; it has been observed 1.5mg/g. For the summer season, the measurements for AA in *Ficus religiosa* at industrial site and roadside has been observed to be 1.9mg/g and 1.6mg/g respectively while at Control, its AA value has been observed to be 1.9mg/g. For the winter season, the measurements for AA in *Ficus religiosa* at Control has been observed to be 1.4mg/g while at industrial and roadside; its values have been observed to be 2.1mg/g and 1.5mg/g respectively.

Mangifera indica During Monsoon season, the measurement for AA in *Mangifera indica* at industrial site has been observed to be 1.4mg/g and at roadside was 1.5mg/g while at Control; it hasbeenobserved1.1mg/g. For the summer season, the

measurements for AA in *Mangifera indica* at industrial site and roadside has been observed to be 2.2mg/g and 2.1mg/g respectively while at Control, its AA value has been observed to be 1.4mg/g. For the winter season, the measurements for AA in *Mangifera indica* at Control has been observed to be 1.5mg/g while at industrial and roadside; its values have been observed to be 2.5mg/g and 2.1mg/g respectively.

Syzygium cumini During Monsoon season, the measurement for AA in *Syzygium cumini* at industrial site has been observed to be 1.4mg/g and at roadside was 1.5mg/g while at Control; it has been observed 1mg/g. For the summer season, the measurements for AA in *Syzygium cumini* at industrial site and roadside has been observed to be 1.9mg/g and 2.1mg/g respectively while at Control, its AA value has been observed to be 2.1mg/g. For the winter season, the measurements for AA in *Syzygium cumini* at Control has been observed to be 1.2mg/g while at industrial and roadside; its values have been observed to be 2.2mg/g and 1.7mg/g respectively.

Moringa oleifera During Monsoon season, the measurement for AA in *Moringa oleifera* at industrial site has been observed to be 1.1mg/g and at roadside was 1.3mg/g while at Control; it has been observed 1.4mg/g. For the summer season, the measurements for AA in *Moringa oleifera at* industrial site and roadside has been observed to be 2.7mg/g and 1.9mg/g respectively while at Control, its AA value has been observed to be 1.9mg/g. For the winter season, the measurements for AA *in Moringa oleifera* at Control has been observed to be 1.2mg/g while at industrial and roadside; its values have been observed to be 1.4mg/g and 1.6mg/g respectively.

Ocimum sanctum During Monsoon season, the measurement for AA in *Ocimum sanctum* at industrial site has been observed to be 1.1mg/g and at roadside was 1.6mg/g while at Control; it has been observed 1.7mg/g. For the summer season, the measurements for AA in *Ocimum sanctum* at industrial site and roadside has been observed to be1.7mg/g and 1.4mg/g respectively while at Control, its AA value has been observed to be 1.2mg/g. For the winter season, the measurements for AA in *Ocimum sanctum* at Control has been observed to be 0.8mg/g while at industrial and roadside; its values have been observed to be 1mg/g and 0.6mg/g respectively.

Mentha piperita During Monsoon season, the measurement for AA in *Mentha piperita* at industrial site has been observed to be 1.7mg/g and at roadside was 1.3mg/g while at Control; it hasbeenobserved1.4mg/g. For the summer season, the measurements for AA in *Mentha piperita* at industrial site and roadside has been observed to be 2.1mg/g and

2.1mg/g respectively while at Control, its AA value has been observed to be 2mg/g. For the winter season, the measurements for AA in *Mentha piperita* at Control has been observed to be 1.4mg/g while at industrial and roadside; its values have been observed to be 2mg/g and 2mg/g respectively.

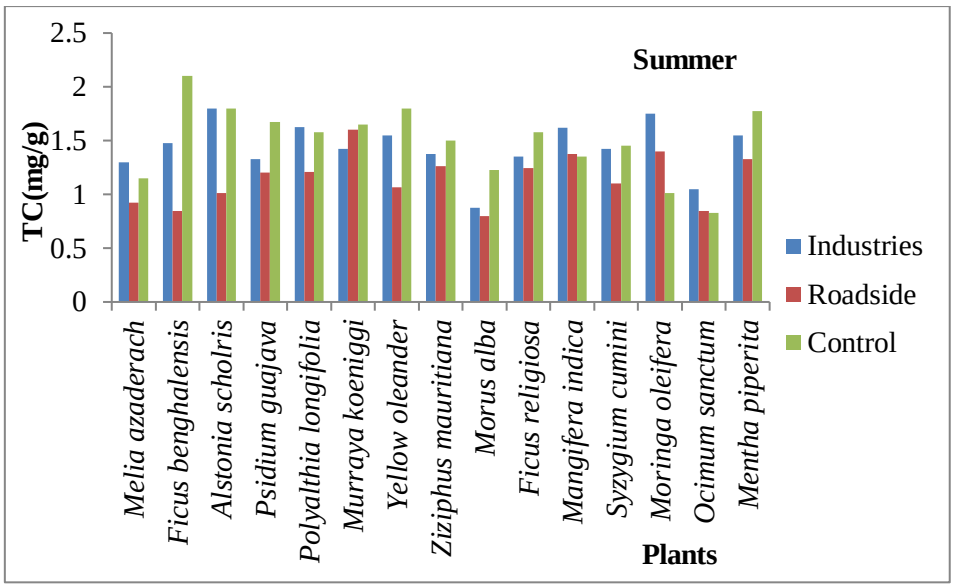
The outcomes of the current study exhibited that higher amount of AA (mg/g) in most of the plants species during the winter season followed by summer season and monsoon season. Similar findings were reported by Das and Prasad, (2010). As discussed above, the higher water content in plants had observed during winter season and this might be considered as a reason. Since, the amount of ascorbic acid content increases to protect the thylakoid membrane from oxidative damage during water stress condition. Das et al., 2018 also found similar findings in their study. Plants with higher ascorbic acid content are generally considered to be more resistant to air pollution (Rao and Dubey,1990; Karmakar et al., 2020, Yadav and Pandey, 2020; Ghafari et al., 2021;Elawa etal., 2021).Pandeya et al.,2015;Banerjeeet al., 2018stated that increasing levels of ascorbic acid in plants is one of the defense strategies of antioxidants against reactive oxygen species. This can be considered a reason for the results of the current study showing higher ascorbic acid plants in winter. It might be a defense strategy for plants to deal with low temperatures. As also explained in the literature high and low temperatures influenced reactive oxygen species (ROS). In the present study, *Mangifera indica*, *Alstonia scholaris, Psidium guajava*, *Moringa oleifera* and *Ficus benghalensis* were found to have the highest AA value and can be considered tolerant to air pollution. They can also be suggested to be grown in the study area.

4.4.4 Total chlorophyll content (mg/g)

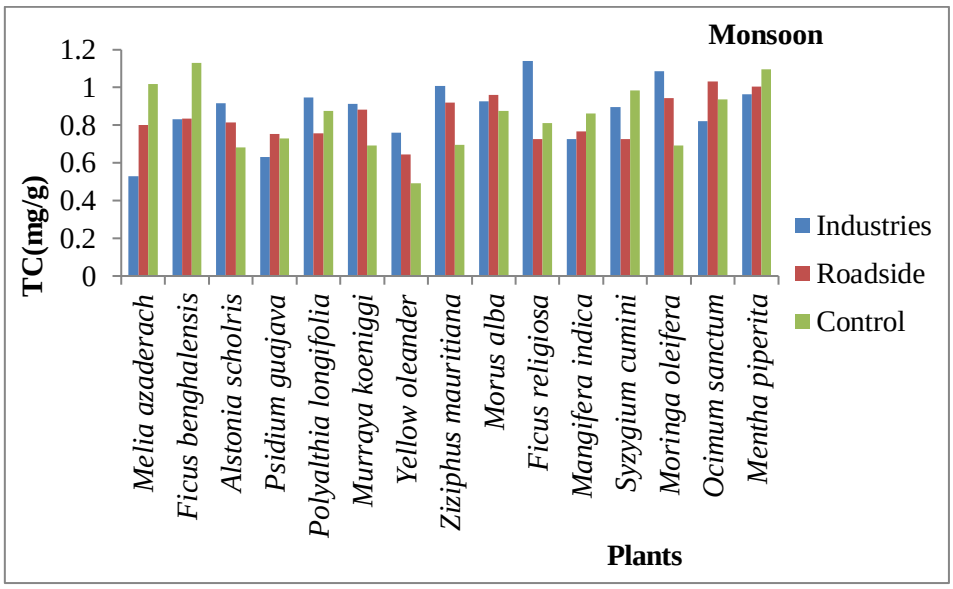
The measurement of total chlorophyll content (TC) is considered a highly useful method for evaluating the influence of air pollutants on plants. The results are shown in Table 4.4 and Figure 4.4. The result indicated that plant leaf samples showed varying degree of TC (mg/g) value in response to air pollution.

Table 4.4 Seasonal variations in Total chlorophyll content (mg/g). Data represent mean ±Standard error (S.E) for monsoon winter and summer.

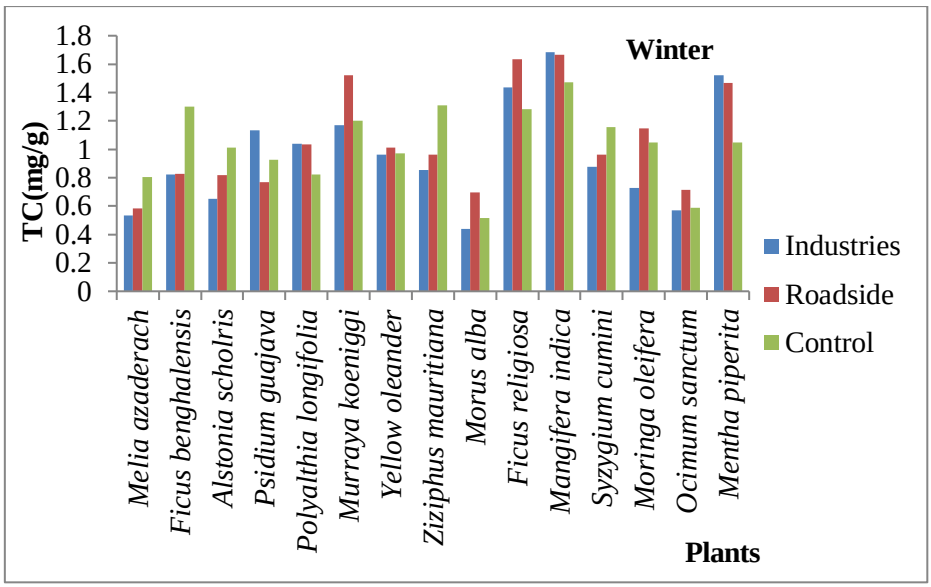
Plants Species	**Industrial**			**Roadside**			**Control**		
	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer
Melia azedarach	0.5±0.30	0.5±0.19	1.3±0.44	0.8±0.26	0.5±0.13	0.9±0.32	1±0.40	0.8±0.19	1.1±0.41
Ficus benghalensis	0.8±0.53	0.8±	1.4±0.18	0.8±0.28	0.8±	0.8±0.27	1.1±0.49	1.3±	2.1±0.1
Alstonia Scholaris	0.9±0.59	0.6±0.07	1.8±0.35	0.8±0.19	0.8±0.04	1±0.10	0.6±0.15	1±0.10	1.8±0.12
Psidium guajava	0.6±0.19	1.1±0.12	1.3±0.16	0.7±0.37	0.7±0.10	1.2±0.10	0.7±0.21	0.9±0.05	1.6±0.075
Polyalthia longifolia	0.9±0.58	1±0.11	1.6±0.16	0.7±0.12	1±0.08	1.2±0.20	0.8±0.12	0.8±0.13	1.5±0.07
Murraya Koenigii	0.9±0.55	1.1±0.15	1.4±0.18	0.8±0.32	1.5±0.18	1.6±0.15	0.6±0.18	1.2±0.11	1.6±0.21
Yellow oleander	0.7±0.67	0.9±0.18	1.5±0.20	0.6±0.42	1±0.11	1±0.05	0.4±0.25	0.9±0.02	1.8±0.17
Ziziphus mauritiana	1±0.44	0.8±0.10	1.3±0.18	0.9±0.24	0.9±0.11	1.2±0.24	0.6±0.14	1.3±0.086	1.5±0.16
Morus Alba	0.9±0.46	0.4±0.20	0.8±0.29	0.9±0.07	0.6±0.16	0.7±0.26	0.8±0.11	0.5±0.11	1.2±0.44
Ficus Religiosa	1.1±0.32	1.4±0.20	1.3±0.10	0.7±0.31	1.6±0.18	1.2±0.16	0.8±0.31	1.2±0.11	1.5±0.07
Mangifera indica	0.7±0.14	1.6±0.19	1.6±0.24	0.7±0.24	1.6±0.17	1.3±0.13	0.8±0.18	1.4±0.10	1.3±0.05
Syzygium Cumini	0.8±0.47	0.8±0.08	1.4±0.19	0.7±0.40	0.9±0.07	1.1±0.38	0.9±0.32	1.1±0.08	1.4±0.08
Moringa oleifera	1±0.35	0.7±0.11	1.7±0.19	0.9±0.18	1.1±0.11	1.4±0.10	0.6±0.26	1±0.02	1±0.02
Ocimum sanctum	0.8±0.20	0.5±0.16	1±0.39	1±0.18	0.7±0.16	0.8±0.30	0.9±0.20	0.5±0.13	0.8±0.27
Mentha piperita	0.9±0.39	1.5±0.20	1.5±0.17	1±0.05	1.4±0.18	1.3±0.13	1±0.10	1±0.09	1.7±0.23


Summer
TC(mg/g)
2.5
2
1.5
1
0.5
0
Industries
Roadside
Control
Melia azaderach
Ficus benghalensis
Alstonia scholris
Psidium guajava
Polyalthia longifolia
Murraya koeniggi
Yellow oleander
Ziziphus mauritiana
Morus alba
Ficus religiosa
Mangifera indica
Syzygium cumini
Moringa oleifera
Ocimum sanctum
Mentha piperita
Plants

(i)


Monsoon
TC(mg/g)
1.2
1
0.8
0.6
0.4
0.2
0
Industries
Roadside
Control
Melia azaderach
Ficus benghalensis
Alstonia scholris
Psidium guajava
Polyalthia longifolia
Murraya koeniggi
Yellow oleander
Ziziphus mauritiana
Morus alba
Ficus religiosa
Mangifera indica
Syzygium cumini
Moringa oleifera
Ocimum sanctum
Mentha piperita
Plants

(ii)



(iii)

Figure 4.4 Variation in TC (mg/g) measurements for (i) summer, (ii) winter and (iii) monsoon seasons

Melia azedarach During Monsoon season, the measurement for TC in *Melia azedarach* at industrial site has been observed to be 0.5mg/g and at roadside was 0.8mg/g while at Control; it has been observed1 mg/g. For the summer season, the measurements for TC in *Melia azedarach* at industrial site and roadside has been observed to be 1.3 mg/g and 0.9mg/g respectively while at Control, its TC value has been observed to be 1.1mg/g. For the winter season, the measurements for TC in *Melia azedarach* at Control has been observed to be 0.8mg/g while at industrial and roadside; its values have been observed to be 0.5mg/g and 0.5mg/g respectively.

Ficus religiosa During Monsoon season, the measurement for TC in *Ficus religiosa* at Industrial site has been observed to be 0.8mg/g and at roadside was 0.8mg/g whereas; at Control it was observed 1.1mg/g. For the summer season, the measurement of TC in *Ficus religiosa* was displayed 1.4mg/g at industrial site and 0.8mg/g at roadside but at Control it has been observed to be2.1mg/g. On other side, in winter season the measurements for TC in *Ficus religiosa* has been noted 0.8mg/g at industrial and 0.8mg/g at roadside and 1.3mg/g has been observed at Control.

Alstonia scholaris During Monsoon season, the measurement for TC in *Alstonia scholaris* at Industrial site has been observed to be 0.9mg/g and at roadside was 0.8mg/g whereas; at Control it was observed 0.6mg/g. During summer season, the

measurement of TC in *Alstonia scholaris* was displayed1.8mg/gat industrial site and 1mg/g at roadside but at Control it has been observed tobe1.8mg/g. On other side, in winter season the measurements for TC in *Alstonia scholaris* has been noted 0.6mg/g at industrial and 0.8mg/g at roadside and 1mg/g has been observed at Control.

Polyalthia longifolia During Monsoon season, the measurement for TC in *Polyalthia longifolia* at industrial site has been observedtobe0.9mg/g and at roadside was 0.7mg/g while at Control; it has been observed 0.8mg/g. For the summer season, the measurements for TC in *Polyalthia longifolia* at industrial site and roadside has been observed to be 1.6mg/g and 1.2mg/g respectively while at Control, its TC value has been observed to be 1.5mg/g. For the winter season, the measurements for TC in *Polyalthia longifolia* at Control has been observed to be 0.8mg/g while at industrial and roadside; its values have been observed to be 1mg/g and 1mg/g respectively.

Psidium guajava During Monsoon season, the measurement for TC in *Psidium guajava* at Industrial site has been observed to be 0.6mg/g and at roadside was 0.7mg/g whereas; at Control it was observed 0.7mg/g. During summer season, the measurement of TC in *Psidium guajava* was displayed 1.3mg/g at industrial site and 1.2mg/g roadside but at Control it has been observed to be 1.6mg/g. On other side, in winter season the measurements for TC in *Psidium guajava* has been noted 1.1mg/g at industrial and 0.7mg/g at roadside and 0.9mg/g has been observed at Control.

Murraya koenigii During Monsoon season, the measurement for TC in *Murraya koenigii* at industrial site has been observed to be 0.9mg/g and at roadside was 0.8mg/g while at Control; it has been observed 0.6mg/g. For the summer season, the measurements for TC in *Murraya koenigii* at industrial site and roadside has been observed to be 1.4mg/g and 1.6mg/g respectively while at Control, its TC value has been observed to be 1.6mg/g. For the winter season, the measurements for TC in *Murraya koenigii* at Control has been observed to be 1.2mg/g while at industrial and roadside; its values have been observed to be 1.1mg/g and 1.5mg/g respectively.

Yellow oleander During Monsoon season, the measurement for TC in *Yellow oleander* at industrial site has been observed to be 0.7mg/g and at roadside was 0.6mg/g while at Control; it has beenobserved0.4mg/g. For the summer season, the measurements for TC in *Yellow oleander* at industrial site and roadside has been observed to be 1.5mg/g and 1mg/g respectively while at Control, its TC value has been observed to be 1.8mg/g. For the winter season, the measurement for TC *in Yellow oleander* at Control has been

observed to be 0.9mg/g while at industrial and roadside; its values have been observed to be 0.9mg/g and 1mg/g respectively.

Ziziphus mauritiana During Monsoon season, the measurement for TC in *Ziziphus mauritiana* at industrial site has been observedtobe1mg/g and at roadside was 0.9mg/g while at Control; it has beenobserved0.6mg/g. For the summer season, the measurements for TC in *Ziziphus mauritiana* at industrial site and roadside has been observed to be 1.3mg/g and1.2mg/g respectively while at Control, its TC value has been observed to be 1.5mg/g. For the winter season, the measurements for TC *in Ziziphus mauritiana* at Control has been observed to be1.3mg/g while at industrial and roadside; its values have been observed to be 0.8mg/g and 0.9mg/g respectively.

Morus alba During Monsoon season, the measurement for TC in *Morus alba* at industrial site has been observed to be 0.9mg/g and at roadside was 0.9mg/g while at Control, it has been observed 0.8mg/g. For the summer season, the measurements for TC in *Morus alba* at industrial site and roadside has been observed to be 0.8mg/g and 0.8mg/g respectively while at Control, its TC value has been observed to be 1.2mg/g. For the winter season, the measurements for TC in *Morus alba* at Control has been observed to be 0.5mg/g while at industrial and roadside; its values have been observed to be 0.4mg/g and 0.6mg/g respectively.

Ficus religiosa During Monsoon season, the measurement for TC in *Ficus religiosa* at industrial site has been observed to be 1.1mg/g and at roadside was 0.7mg/g while at Control; it has been observed 0.8mg/g. For the summer season, the measurements for TC in *Ficus religiosa* at industrial site and roadside has been observed to be 1.3mg/g and 1.2mg/g respectively while at Control, its TC value has been observed to be 1.5mg/g. For the winter season, the measurements for TC in *Ficus religiosa* at Control has been observed to be 1.2mg/g while at industrial and roadside; its values have been observed to be 1.4mg/g and 1.2mg/g respectively.

Mangifera indica During Monsoon season, the measurement for TC in *Mangifera indica* at industrial site has been observed to be 0.7mg/g and at roadside was0.7mg/g while at Control; it has been observed 0.8mg/g. For the summer season, the measurements for TC in *Mangifera indica* at industrial site and roadside has been observed to be 1.6mg/g and 1.3mg/g respectively while at Control, its TC value has been observed to be 1.3mg/g. For the winter season, the measurements for TC in *Mangifera indica* at Control has been observed to be 1.4mg/g while at industrial and

roadside; its values have been observed to be 1.6mg/g and 1.6mg/g respectively.

Syzygium cumini During Monsoon season, the measurement for TC in *Syzygium cumini* at industrial site has been observed to be 0.8mg/g and at roadside was 0.7mg/g while at Control; it has been observed 0.9mg/g. For the summer season, the measurements for TC in *Syzygium cumini* at industrial site and roadside has been observed to be 1.4mg/g and 1.1mg/g respectively while at Control, its TC value has been observed to be 1.4mg/g. For the winter season, the measurements for TC in *Syzygium cumini* at Control has been observed to be 1.1mg/g while at industrial and roadside; its values have been observed to be 0.8mg/g and 0.9mg/g respectively.

Moringa oleifera During Monsoon season, the measurement for TC in *Moringa oleifera* at industrial site has been observedtobe1mg/g and at roadside was 0.9mg/g while at Control; it has been observed0.6mg/g. For the summer season, the measurements for TC in *Moringa oleifera at* industrial site and roadside has been observed to be 1.7mg/g and 1.4mg/g respectively while at Control, its TC valuehasbeenobservedtobe1mg/g. For the winter season, the measurements for TC *in Moringa oleifera* at Control has been observed to be 1mg/g while at industrial and roadside; its values have been observed to be 0.7 /g and 1.1mg/g respectively.

Ocimum sanctum During Monsoon season, the measurement for TC in *Ocimum sanctum* at industrial site has been observed to be 0.8mg/g and at roadside was 1mg/g while at Control; it has been observed0.9mg/g. For the summer season, the measurements for TC in *Ocimum sanctum* at industrial site and roadside has been observed to be 1mg/g and 0.8mg/g respectively while at Control, its TC value has been observed to be 0.8mg/g. For the winter season, the measurements for TC in *Ocimum sanctum* at Control has been observed to be 0.5mg/g while at industrial and roadside; its values have been observed to be 0.5mg/g and 0.7mg/g respectively.

Mentha piperita During Monsoon season, the measurement for TC in *Mentha piperita* at industrial site has been observedtobe0.9mg/g and at roadside was 1mg/g while at Control; it has been observed 1mg/g. For the summer season, the measurements for TC in *Mentha piperita* at industrial site and roadside has been observed to be 1.5mg/g and 1.3mg/g respectively while at Control, its TC value has been observed to be 1.7mg/g. For the winter season, the measurements for TC in *Mentha piperita* at Control has been observed to be 1mg/g while at industrial and roadside; its values have been observed to be 1.5mg/g and 1.4mg/g respectively.

The results of the current study showed that higher amount of TC (mg/g) in all the plants species during the summer season followed by winter season and monsoon season. It may be the adaption of plants to high temperature during summer. As high temperature, influenced the stomatal openings and plants experience photosynthetic stress. Similar study conducted by Amini et al., (2009) and found highest TC in the leaves of sampled plants and stated that increase in TC is indicative of tolerance of pollution by plants. Plants with higher chlorophyll content are generally considered as air pollutant tolerant. The amount of chlorophyll in plants varies, depending on the age of the leaf, the level of pollution, and biotic/abiotic factors (Das et al., 2018; Joshi and Swami, 2007). In the present study, *Alstonia scholaris, Polyalthia longifolia, Mangifera indica, Moringa oleifera, Mentha piperita* were found to have the highest chlorophyll content and can be considered as tolerant plant species.

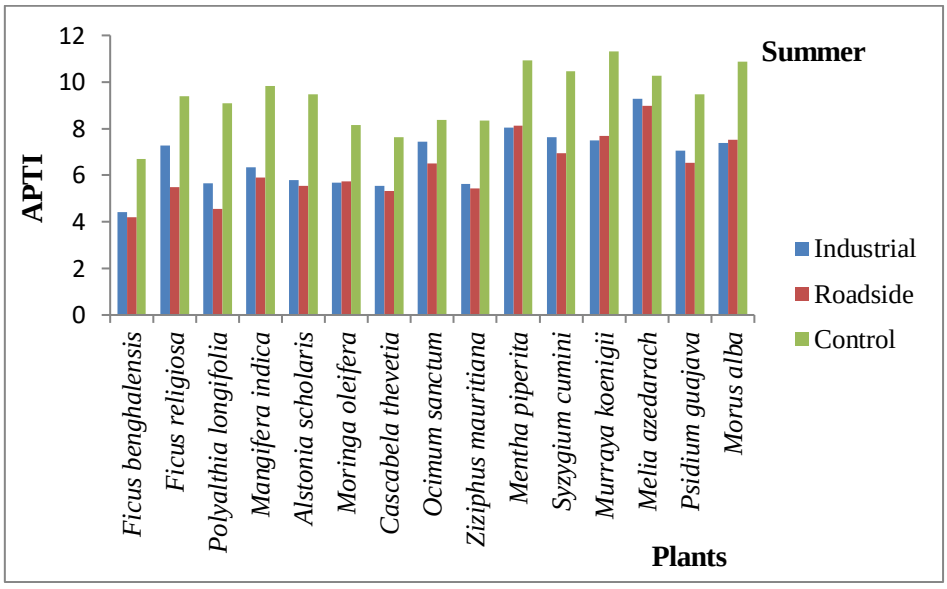
4.4.5 APTI

The APTI values were calculated for each plant species. Depending upon the APTI values, the level of sensitivity of each sampled plant to air pollutants has been examined. Plants species with higher APTI can be serves as tolerant and used as sink for sampling site where as lower APTI exhibited plants are considered as sensitive and act as bio indicator to air pollution. In the present study, the results of APTI values calculated for selected plant species studied during three seasons are shown in Table 4.5 and Figure 4.5.

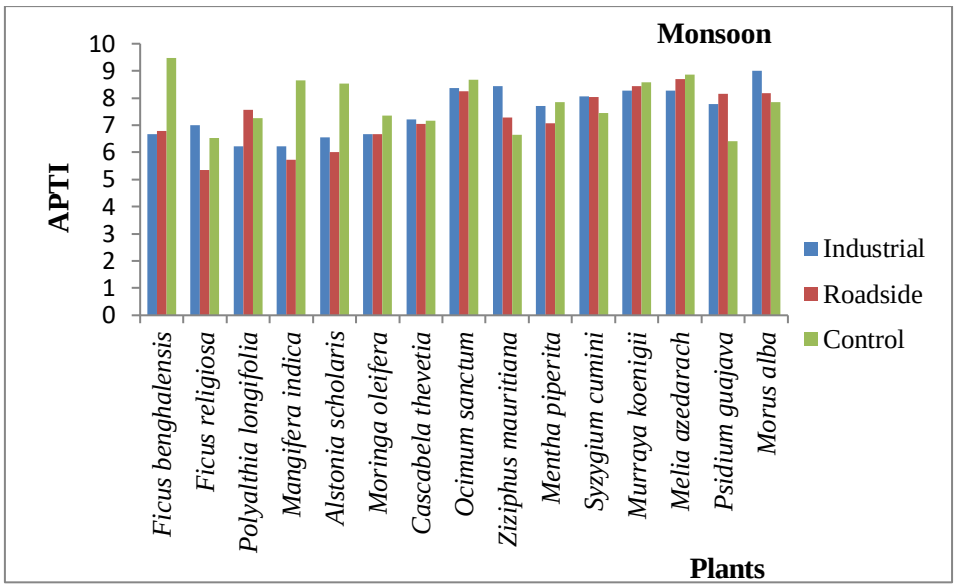
Table 4.5 Seasonal variations in APTI Data represent mean ±Standard error (S.E) for monsoon winter and summer.

Plants species	**Industrial**			**Roadside**			**Control**		
	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer
Melia azedarach	6.67±2.5	4.58±3.0	4.40±3.7	6.79±1.8	4.62±3.3	4.19±3.7	9.48±1.68	6±4.59	6.69±5.48
Ficus benghalensis	7±0.99	5.59±1.75	7.28±0.75	5.36±2.8	5.02±1.3	5.48±0.70	6.53±3.01	7.99±0.53	9.39±0.11
Alstonia scholaris	6.21±1.4	5.52±3.5	5.64±0.13	7.57±2.7	5.17±0.81	4.55±0.36	7.26±1.01	8.04±0.53	9.09±0.22
Psidium guajava	6.23±1	6.00±1	6.34±1.09	5.72±0.80	5.54±0.60	5.88±0.53	8.64±1.31	9.07±1.50	9.82±0.09
Polyalthia longifolia	6.56±1.2	5.68±0.97	5.79±1.6	6.01±1.91	5.78±1.55	5.54±1.277	8.54±0.25	7.2±0.87	9.47±0.26

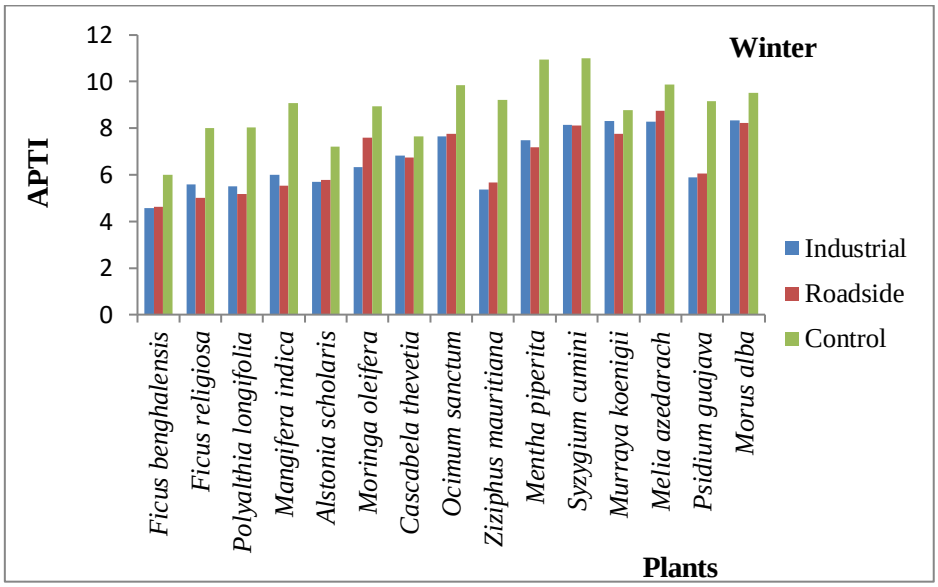
Murraya koenigii	6.66±1.30	6.33±1.37	5.67±1.09	6.67±2.99	7.60±0.83	5.72±0.45	7.35±2.44	8.93±0.73	8.15±0.19
Yellow oleander	7.20±0.64	6.81±2.0	5.53±1.22	7.05±2.74	6.74±0.46	5.32±0.06	7.16±3.40	7.64±0.28	7.64±0.25
Ziziphus mauritiana	8.36±1.31	7.64±0.73	7.43±0.68	8.24±4.06	7.77±0.62	6.51±0.85	8.66±1.39	9.86±0.74	8.38±1.21
Morus alba	8.43±2.3	5.38±2.9	5.63±3.8	7.27±2.29	5.66±2.45	5.44±3.71	6.64±0.93	9.22±4.87	8.34±4.94
Ficus religiosa	7.7±0.23	7.48±1.47	8.04±0.49	7.06±0.25	7.19±1.20	8.12±2.22	7.84±0.29	10.95±0.24	10.91±0.30
Mangifera indica	8.07±3.31	8.14±1.61	7.62±1.72	8.04±3.24	8.10±1.86	6.95±1.08	7.43±0.17	11.0±3	10.44±0.27
Syzygium cumini	8.28±1.48	8.29±1.91	7.50±1.15	8.43±3.27	7.77±2.20	7.67±1.0	8.57±2.63	8.79±0.67	11.30±0.10
Moringa oleifera	8.26±1.45	8.27±1.8	9.28±0.36	8.69±2.91	8.76±0.42	8.97±1.10	8.86±2.50	9.86±0.76	10.26±0.30
Ocimum sanctum	7.78±1.12	5.9±4.18	7.04±3.99	8.15±0.41	6.06±3.37	6.52±3.69	6.41±0.37	9.17±4.28	9.47±4.15
Mentha piperita	9.0±0.70	8.33±1.2	7.39±1.21	8.17±2.02	8.23±0.49	7.51±0.27	7.84±1.45	9.51±0.57	10.88±0.14



(i)



(ii)



(iii)

Figure 4.5 APTI measurements for (i) summer, (ii) monsoon and (iii) winter seasons

Melia azedarach During Monsoon season, the measurement for APTI in Melia azedarach at industrial site has been observed to be 6.67 and at roadside was 6.79 while at Control, it has been observed 9.48. For the summer season, the measurements for APTI in Melia azedarach at industrial site and roadside has been observed to be 4.40 and 4.19 respectively while at Control, its APTI value has been observed to be 6.69. For the winter season, the measurements for APTI in Melia azedarach at Control has been

observed to be 6 while at industrial and roadside; its values have been observed to be 4.58 and 4.62 respectively.

Ficus religiosa During Monsoon season, the measurement for APTI in *Ficus religiosa* at Industrial site has been observed to be 7 and at roadside was 5.36 whereas; at Control it was observed 6.53. For the summer season, the measurement of APTI in *Ficus religiosa* was displayed 7.28 at industrial site and 5.48 at roadside but at Control it has been observed to be 9.39. On other side, in winter season the measurements for APTI in *Ficus religiosa* has been noted 5.59 at industrial and 5.02 at roadside and 7.99 has been observed at Control.

Alstonia scholaris During Monsoon season, the measurement for APTI in *Alstonia scholaris* at Industrial site has been observed to be 6.21 and at roadside was 7.57 whereas; at Control it was observed 7.26. During summer season, the measurement of APTI in *Alstonia scholaris* was displayed5.64 at industrial site and 4.55 at roadside but at Control it has been observed to be 9.09. On other side, in winter season the measurements for APTI in *Alstonia scholaris* has been noted 5.52 at industrial and 5.17 at roadside and 8.04 has been observed at Control.

Polyalthia longifolia During Monsoon season, the measurement for APTI in *Polyalthia longifolia* at industrial site has been observed to be 6.56 and at roadside was 6.01 while at Control, it has been observed 8.54. For the summer season, the measurements for APTI in *Polyalthia longifolia* at industrial site and roadside has been observed to be 5.79 and 5.54 respectively while at Control, its APTI value has beenobservedtobe9.47. For the winter season, the measurements for APTI in *Polyalthia longifolia* at Control has been observed to be 7.2 while at industrial and roadside; its values have been observed to be 5.68 and 5.78 respectively.

Psidium guajava During Monsoon season, the measurement for APTI in *Psidium guajava* at Industrial site has been observed to be 6.23 and at roadside was 5.72 whereas; at Control it was observed 8.64. During summer season, the measurement of APTI in *Psidium guajava* was displayed 6.34 at industrial site and 5.88 at roadside but at Control it has been observed to be 9.82. On other side, in winter season the measurements for APTI in *Psidium guajava* has been noted 6.00 at industrial and 5.54 at roadside and 9.07 has been observed at Control.

Murraya koenigii During Monsoon season, the measurement for APTI in *Murraya koenigii* at industrial site has been observed to be6.66 and at roadside was 6.67 while at Control, it has been observed 7.35. For the summer season, the measurements for APTI in *Murraya koenigii* at industrial site and roadside has been observed to be 5.67 and 5.72 respectively while at Control, its APTI value has been observed to be 8.15. For the winter season, the measurement for APTI in *Murraya koenigii* at Control has been observed to be 8.93 while at industrial and roadside; its values have been observed to be 6.33 and 7.60 respectively.

Yellow oleander During Monsoon season, the measurement for APTI in *Yellow oleander* at industrial site has been observed to be7.20 and at roadside were7.05 while at Control it has been observed 7.16. For the summer season, the measurements for APTI in *Yellow oleander* at industrial site and roadside has been observed to be 5.53 and 5.32 respectively while at Control, its APTI value has been observedtobe7.64. For the winter season, the measurement for APTI *in Yellow oleander* at Control has been observed to be 7.64 while at industrial and roadside; its values have been observed to be 6.81 and 6.74 respectively.

Ziziphus mauritiana During Monsoon season, the measurement for APTI in *Ziziphus mauritiana* at industrial site has been observed to be 8.36 and at roadside was 8.24 while at Control, it has been observed8.666.For the summer season, the measurements for APTI in *Ziziphus mauritiana* at industrial site and roadside has been observed to be 7.43 and 6.51 respectively while at Control, its APTI value has been observed to be 8.38. For the winter season, the measurements for APTI *in Ziziphus mauritiana* at Control has been observed to be 9.86 while at industrial and roadside; its values have been observed to be 7.64 and 7.77 respectively.

Morus alba During Monsoon season, the measurement for APTI in *Morus alba* at industrial site hasbeenobservedtobe8.43andatroadsidewas7.27whileatControl,it has been observed 6.64. For the summer season, the measurements for APTI in *Morus alba* at industrial site and roadside has been observed to be 5.63 and 5.44 respectively while at Control, its APTI value has been observed to be 8.34. For the winter season, the measurements for APTI *in Morus alba* at Control has been observed to be 9.22 while at industrial and roadside; its values have been observed to be 5.38 and 5.66 respectively.

Ficus religiosa During Monsoon season, the measurement for APTI in *Ficus religiosa* at industrial site has been observed to be7.7and at roadside was 7.06 while at Control, it has been observed 7.84. For the summer season, the measurements for APTI in *Ficus religiosa* at industrial site and roadside has been observed to be 8.04 and 8.12 respectively while at Control, its APTI value has been observed to be 10.91. For the winter season, the measurements for APTI *in Ficus religiosa* at Control has been observed to be 10.95 while at industrial and roadside; its values have been observed to be 7.48 and 7.19 respectively.

Mangifera indica During Monsoon season, the measurement for APTI in *Mangifera indica* at industrial site has been observed to be8.07 and at roadside was 8.04 while at Control, it has been observed 7.43. For the summer season, the measurements for APTI in *Mangifera indica* at industrial site and roadside has been observed to be 7.62 and 6.95 respectively while at Control, its APTI value has been observed to be10.44. For the winter season, the measurements for APTI in *Mangifera indica* at Control has been observed to be 11 while at industrial and roadside; its values have been observed to be 8.14 and 8.10 respectively.

Syzygium cumini During Monsoon season, the measurement for APTI in *Syzygium cumini* at industrial site has been observed to be8.28 and at roadside was 8.43 while at Control, it has been observed 8.57. For the summer season, the measurements for APTI in *Syzygium cumini* at industrial site and roadside has been observed to be 7.50 and 7.67 respectively while at Control, its APTI value has been observed to be 11.3. For the winter season, the measurements for APTI in *Syzygium cumini* at Control has been observed to be 8.79 while at industrial and roadside; its values have been observed to be 8.29 and 7.77 respectively.

Moringa oleifera During Monsoon season, the measurement for APTI in *Moringa oleifera* at industrial site has been observed to be8.26 and at roadside was 8.69 while at Control, it has been observed 8.86. For the summer season, the measurements for APTI in *Moringa oleifera at* industrial site and roadside has been observed to be 9.28 and 8.97 respectively while at Control, its APTI value has been observed to be 10.26. For the winter season, the measurement for APTI in *Moringa oleifera* at Control has been observed to be 9.86 while at industrial and roadside; its values have been observed to be 8.27 and 8.69 respectively.

Ocimum sanctum During Monsoon season, the measurement for APTI in *Ocimum sanctum* at industrial site has been observed to be7.78 and at roadside was 8.15 while at Control; it has been observed 6.41. For the summer season, the measurements for APTI in *Ocimum sanctum* at industrial site and roadside has been observed to be 7.04 and 6.52respectively while at Control, its APTI value has been observed to be 9.47. For the winter season, the measurements for APTI *in Ocimum sanctum* at Control has been observed to be 9.17 while at industrial and roadside; its values have been observed to be 5.9 and 6.0 respectively.

Mentha piperita During Monsoon season, the measurement for APTI in *Mentha piperita* at industrial site has been observed to be 9.0 and at roadside was 8.17 while at Control, it has been observed 7.84. For the summer season, the measurements for APTI in *Mentha piperita* at industrial site and roadside has been observed to be 7.39 and 7.51 respectively while at Control, its APTI value has been observed to be 10.8. For the winter season, the measurements for APTI in *Mentha piperita* at Control has been observed to be 9.51 while at industrial and roadside; its values have been observed to be 8.33and 8.23 respectively. All the plant species in the present study indicated higher APTI during summer followed by winter season and monsoon season. This could be linked to differences in air pollution levels and temperatures during the three different seasons or other factors underlying the parameters affecting the APTI.

The results of the current study showed higher APTI in all the plants species during the monsoon season followed by summer and winter at both the polluted sites and at control site higher APTI was found in summer followed by Monsoon and winter. During monsoon generally pollutants wash away from the leaves and plants absorbs low amount of pollutants. Due to which less variation in biochemical parameters of plants has been observed resulting in higher APTI during Monsoon. Based on the APTI values, Plants species such as, *Syzygium cumini*, *Ficus religiosa*, *Mentha piperita*, *Ziziphus mauritiana*, *Mangifera indica*, *Moringa oleifera* and *Morus alba* were found to be more tolerant as compared to other plants species. Different plants species show considerable variation in the order of tolerance. Similar findings were observed by Singh et al, 1991, Das and Prasad, 2010, Karmakaret al., 2020; Roy et al., 2020; Sahu et al., 2020; Bandaraet al., 2021, Elawa et al., 2021; Ghafari et al., 2021.

4.5 Statistical analysis of bio-indicators responses

Pearson's correlation coefficient analysis of the four biochemical parameters with APTI is evaluated in Table4.6. It was used to determine the relationship between biochemical parameters and APTI. Significant correlation has been determined between biochemical parameters and APTI. A significant positive correlation at $p<0.005$level was observed between APTI and RWC ($R^2 = 0.95$).The strong and positive correlation of APTI with relative water, indicating that the amount of RWC in plant leaves affects the APTI value. Further, APTI showed positive correlation with pH ($R^2 = 0.64$) followed by TC ($R^2 = 0.60$) and lowest positive correlation was observed in AA ($R^2 = 0.54$). This implies that relative water content is the most significant factor when considering the plant's tolerance potential in the study location. Similar results were also observed by Das and Das (2018) and Elawa et al., (2021); Yadav and Pandey, (2020).

Table 4.6 Correlation between biochemical parameters

	RWC	pH	AA	TC	APTI
RWC	1				
pH	0.630627	1			
AA	0.441341	0.433006	1		
TC	0.555623	0.441876	0.615002	1	
APTI	0.956881	0.648187	0.541246	0.609887	1

Analysis of individual parameter may lack comprehensive insight into the alterations induced by pollution. Hence, a more dependable approach involves determining the tolerance thresholds of various plant species thriving in polluted environment, encompassing a broader spectrum of influential parameters.

4.6 Environmental factors

The findings for each plant species displayed significant variations in their biochemical parameters. Multiple factors were identified as influential in determining plant tolerance. The observed significant differences across summer winter and monsoon seasons suggest that environmental factors such as temperatures, humidity, light intensity etc likely play a predominant role in the variation of biochemical parameter and assessment of plant tolerance index (Detailed experimental investigations are discussed in chapter 5).

4.7 Pollutants factors

In the current findings, it was found that the control area exhibited higher APTI values than the polluted areas. All the studied biochemical parameters; TC, AA, RWC and pH were found higher in control areas than in polluted areas. It is plausible that air pollutants are responsible for the decline in biochemical parameters observed in polluted areas. This observation is in line with previous literature suggesting that TC and AA are predominantly affected by air pollution and environmental stress (Detailed experimental investigations are discussed in chapter 6).

4.8 Morphological factors

The previous literature has highlighted the sensitivity of various morphological parameters including size, shape, leaf shape, leaf surface texture, leaf dimensions, leaf vein patterns, petiole length etc(Taylor, 2014; Rai et al., 2010; Shakeel et al., 2023). Hence, these morphological parameters may serve as contributing factors to the variation observed in biochemical parameters. Consequently, the further study focuses on investigating the relationship between two specific morphological parameters (namely leaf surface area and leaf surface texture) and APTI along with the biochemical parameters stress (A detailed experimental study is discussed below)

There are various parameters or limitations that may account for the variation in the present study results. These are morphological, anatomical, genetic parameters, data volume, pollutants type, plant habitat, plant exposure, plant type, environmental factors, pollutant factors, morphological parameters etc. In the present morphology study was conducted and significant outcomes are discussed below. Furthermore, according to previous literature environmental factors and pollutant factors played significant role in the variation of plant tolerance behaivour to air pollutants.

4.8.1 Air Pollution Tolerance Index (APTI)

The tolerance index is one of the most helpful methods to assess the tolerance of plants to air pollution by considering biochemical and physiological leaf trait. Using APTI value, the tolerance level of different plants can be compared and classified as tolerant and sensitive. Additionally, it is time saving and economical to implement in real world settings without acquiring expensive environmental monitoring setups. The APTI is

calculated by measuring four biochemical parameters of leaf such as AA, TC, RWC and pH. The mean levels of biochemical parameters and APTI of all plants are presented in Table 4.7 and Table 4.8. Additionally, the annual mean values with the standard deviations of biochemical parameters and APTI of sampling sites have been presented below in figure 4.6 and figure 4.7respectively. According to the study reported by Singh et al., 1991, the limits of tolerance to air pollution are different for trees, shrubs and herbs.

Table 4.7 Calculated biochemical parameters and APTI values of plant species sampled in industrial areas are shown

Plants	**RWC**	**pH**	**AA**	**TC**	**APTI**	**LSA**	**LST**
Ficus benghalensis	94.3	6.8	2.2	1	11.14	161	Rough
Alstonia scholaris	92.3	6.4	2.1	1.1	10.80	74	Smooth
Psidium guajava	91.2	6.3	2.2	1	10.72	52	Rough
Morus Alba	86.6	5.2	1.3	0.7	9.42	44	Rough
Ficus religiosa	96.5	7.2	1.9	1.3	11.26	70	Rough
Mangifera indica	98.6	6.8	2.1	1.4	11.58	63	Rough
Syzygium cumini	91.9	6.3	1.9	1	10.57	52	Smooth
Polyalthia Longifolia	92.7	6.8	1.8	1.2	10.71	45	Smooth
Melia Azedarach	83	4.9	1.2	0.7	8.97	12	Smooth
Cascabela thevetia	92.9	6.9	1.7	1.1	10.65	8	Smooth
Ocimum sanctum	95.3	5.5	1.2	0.7	10.27	6	Smooth
Murraya	96.5	6.5	1.4	1.1	10.71	2.5	Smooth

Plants	RWC	pH	AA	TC	APTI	LSA	LST
Koenigii							
Ziziphus Mauritiana	96.2	6.9	1.8	1	11.0	12	Smooth
Moringa oleifera	91.2	6.6	1.7	1.1	10.42	3	Smooth
Mentha Piperita	92.3	7.1	2	1.3	10.91	4	Rough

These threshold limits of APTI values demarcating tolerant, moderately tolerant, intermediate and sensitive species were determined after finding the mean APTI. This is due to the tendency of different types of plants growing in different environments to exhibit different responses to pollution stress (Singh et al., 1991). Similarly, significant variation was observed in the biochemical parameters and APTI values of 15 plant species in the study sites (Table 4.7 and Table 4.8). This may be because different plants species vary greatly in their susceptibility to air pollutants. A different tolerance value of the same plant has been observed at different polluted sites. For example *Ziziphus mauritiana* in an industrial area has an APTI value of 11, while similar plant species on a roadside have an APTI value of 10.47. The highest APTI values were observed in *Mangifera indica* (11.58) and *Ficus religiosa* (11.26) and at an industrial site. The same species *Mangifera indica* (11.17) has higher APTI at a roadside, followed by *Ficus benghalensis* (11.14) and *Ficus religiosa* (11). High APTI values indicate that a plant is tolerant to air pollution and may also act as a filter to reduce air pollution.

Table 4.8 Calculated biochemical parameters and APTI values of plant species sampled in roadside areas are shown.

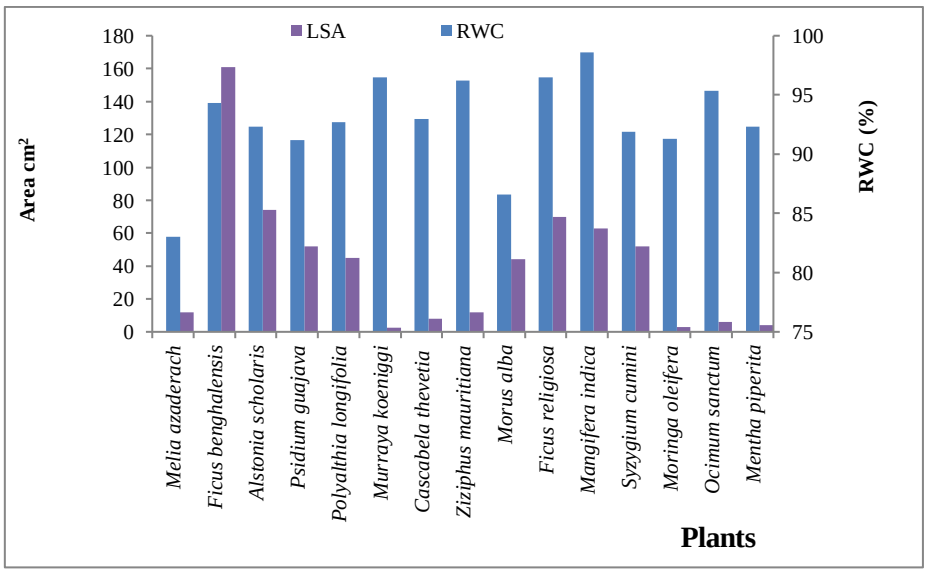
Plants	RWC	pH	AA	TC	APTI	LSA	LST
Ficus benghalensis	98.7	6.7	1.7	0.8	11.14	161	Rough
Alstonia scholaris	90.4	6.8	1.4	0.8	10.10	74	Smooth
Psidium guajava	89.5	6.7	1.6	0.9	10.16	52	Rough
Morus	77.8	5.2	1.1	0.7	8.42	44	Rough

Plants	RWC	pH	AA	TC	APTI	LSA	LST
alba							
Ficus religiosa	98.7	7	1.4	1.2	11	70	Rough
Mangifera indica	96.5	6.7	1.9	1.3	11.17	63	Rough
Syzygium cumini	91.2	6.6	1.8	1.1	10.5	52	Smooth
Polyalthia longifolia	93.2	6.9	1.4	1	10.42	45	Smooth
Melia azedarach	90.6	5.2	1.1	0.7	9.70	12	Smooth
Cascabela thevetia	91.2	7	1.4	0.9	10.22	8	Smooth
Ocimum Sanctum	95.9	5.3	1.1	0.8	10.26	6	Smooth
Murraya koenigii	94.3	7	1.8	1.3	10.92	2.5	Smooth
Ziziphus mauritiana	92.9	6.9	1.5	1	10.47	12	Smooth
Moringa oleifera	91.2	6.8	1.6	1.1	10.38	3	Smooth
Mentha piperita	94.2	7.3	1.8	1.3	10.96	4	Rough

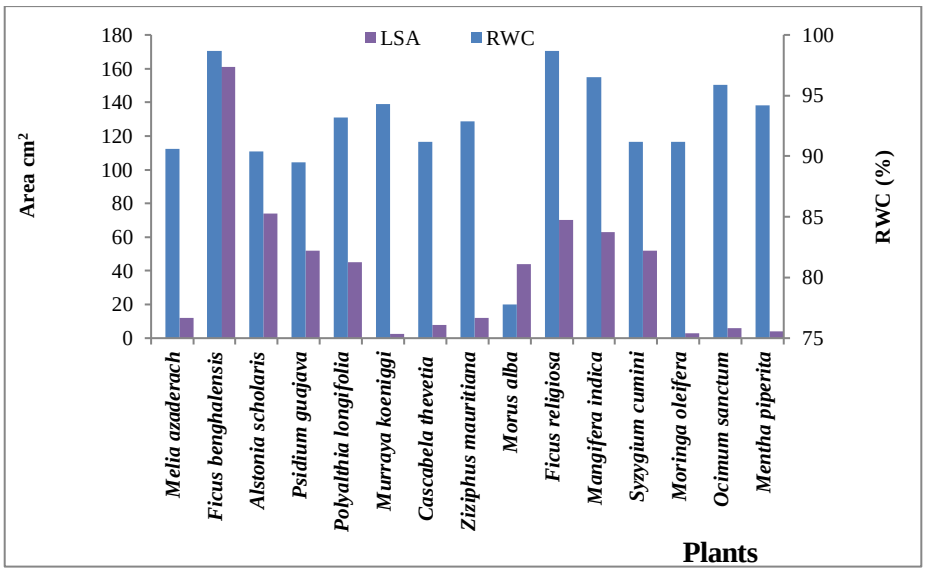
Conversely, plants with low APTI values were considered sensitive and could be suitable bio indicators. Likewise, *Morus alba* and *Melia azedarach* has low APTI and have been identified as sensitive at both the polluted sites. This may be because of the fact APTI of plants may vary from place to place due to geographic and climatic variations. Also, it may be due to differences in air quality, temperature, humidity etc. Similar findings were also

drawn by Shrestha et al., 2021.Based on tolerance towards air pollution, *Ficus religiosa* and *Mangifera indica* were most tolerant at industrial plant species while *Ficus benghalensis* was intermediate. Whereas on roadside, *Mangifera indica* and *Ficus benghalensis* were most tolerant while *Ficus religiosa* was intermediate tolerant. The order of tolerance was *Mangifera indica* > *Ficus religiosa* = *Ficus benghalensis at* both the polluted sites. Among selected plant species, *Mangifera indica* and *Ficus religiosa* were found to be tolerant in both the study areas. This may be possible due to higher RWC in leaf samples which may provide greater resistance in plants and cause higher APTI. A similar conclusion was drawn by Watson and Bai (2021),where by two species (*Ficus elastica* and *Canna indica*) that were sampled from Kerala highways depicted higher APTI values. According to Pradhan et al. (2016), two plant species (*Polyalthia longifolia and Tectona grandis*) collected from national Highway 6 (NH-6) passing through Sambalpur city, Odisha, India were found to have high APTI scores (Pradhan et al., 2016). Another reason could be the texture of their leaf surface. In the present study, plants with rough leaf surface were identified as highly tolerant.

The rough leaf surface of the plant reduces the absorption of air pollutants and the plant uses this as a defense mechanism to limit the level of exposure to air-borne pollution (Rai et al., 2010; Lendzian & Baur, 2020). Furthermore, plants exhibiting higher APTI values were generally found to have larger surface area. Similar findings have been reported by Banerjee et al., (2022) that large leaf surface areas in the polluted area were evidenced for absorption and accumulation of pollution (Banerjee et al., 2022).



(a) industrial

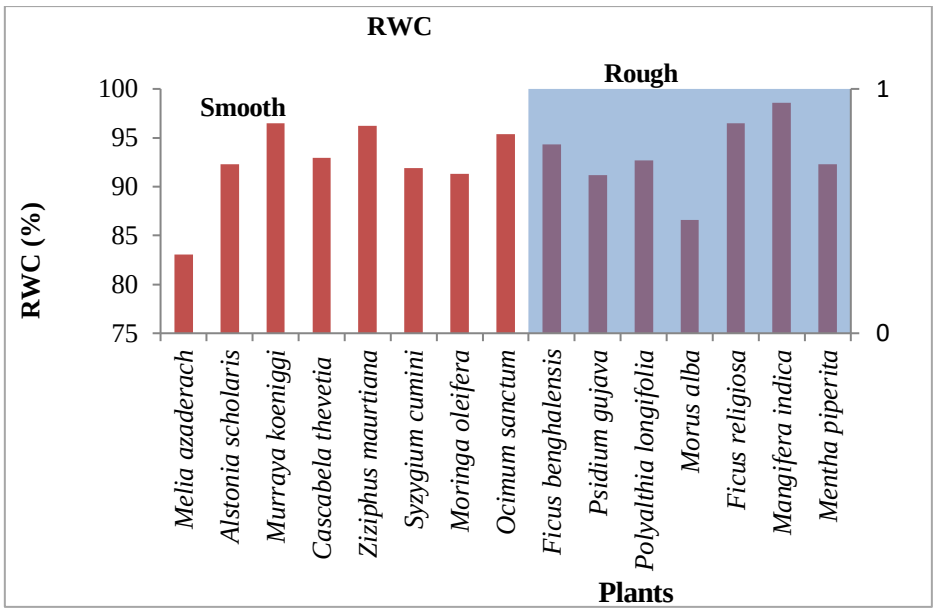


b (roadside)

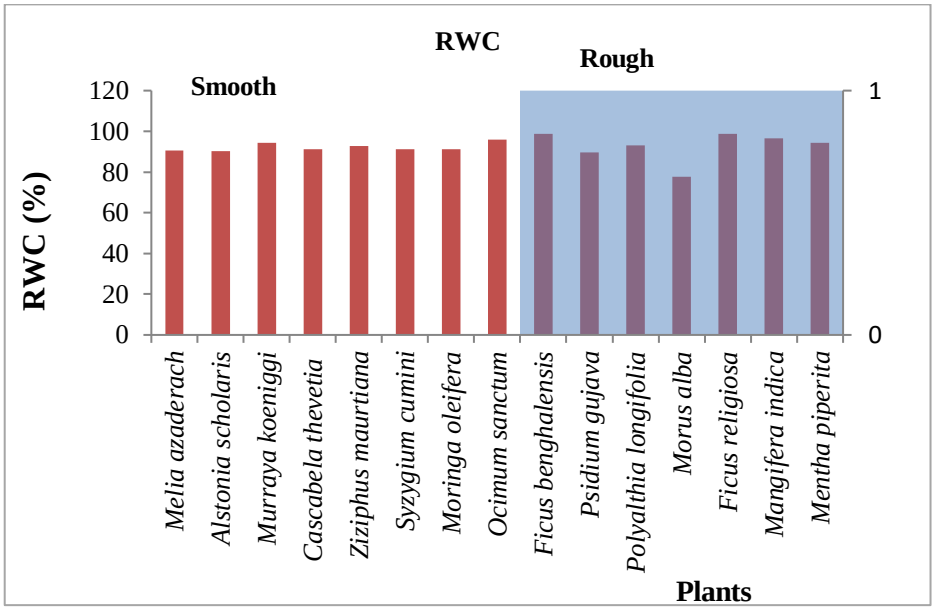
Figure 4.6 Change in RWC of industrial and roadside plant species, respectively (grouped by LSA)

The values of RWC (%) have showed variation within plant species, as shown in Figure 4.6 (a and b). In the present study, RWC was found to be highest in *Mangifera indica* (98.6%) followed by *Ficus religiosa* (96.5%) and lowest in *Melia azedarach* (83%) at the industrial site. Similarly, at the roadside, *Ficus benghalensis* (98.7%), followed by *Ficus religiosa* (98.7%) have higher RWC values and lower RWC was found in *Morus alba* (77.8%). Relative water content is higher in most plant species regardless of surface area at both polluted sites. However, as shown in Figure 4.7 (a and b), plants at roadside had high RWC than industrial site. High relative water content (RWC) is beneficial for maintaining physiological functions amid pollutant stress. In both polluted plant species, RWC was higher, which may be a result of dust accumulation by leaves which reduced transpiration due to closure of stomata (Dhanam et al.,2014;Pandey et al., 2015;Karmakar et al., 2020 Shakeel et al., 2022). Important plant physiological processes like respiration, transpiration, and growth directly correlate to leaf water status (Dhankhar et al., 2015; Koc et al., 2022; Koc & Nzokou, 2023).

Under difficult conditions, when the relative content is high, the plants are known to tolerate air pollution (Jyothi & Jaya, 2010;Singh et al., 1991;Palit et al., 2013 Key et al., 2022 Isinkaralar et al., 2022; Cesur et al., 2022).In the current study, it was found that LST and LSA did not show any specific trend with RWC.



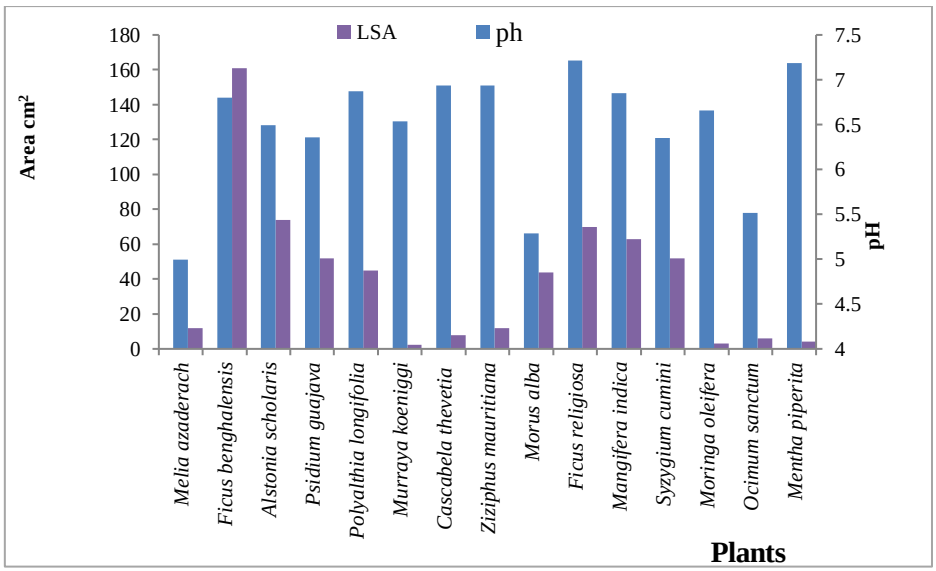
a industrial



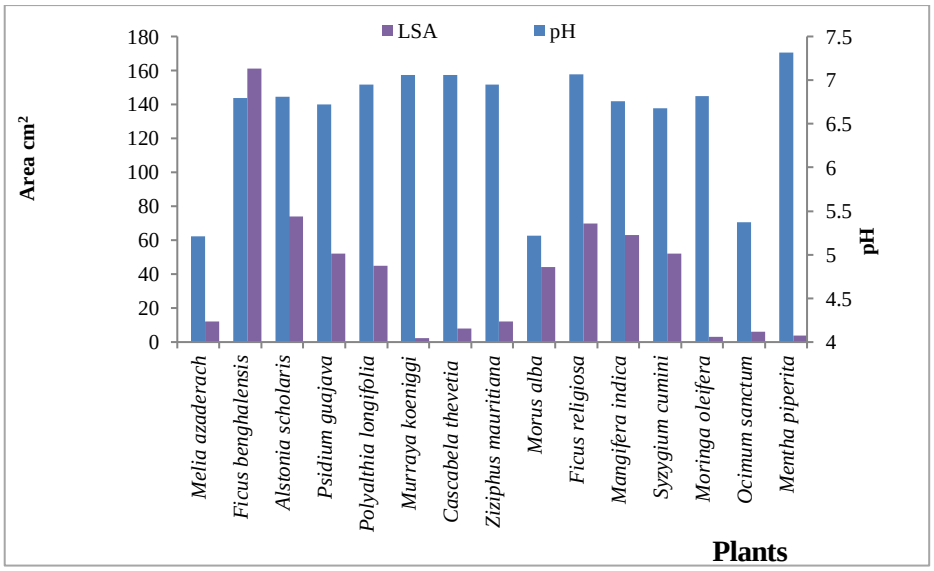
b roadside

Figure 4.7 Change in RWC of industrial and roadside plants species respectively (grouped by smooth and rough)

Significant variation was observed in the pH values, as shown in Figure 4.8 (a and b).This may result from the sensitivity of the stomata to air pollution (Verma & Singh, 2006). Almost all the samples collected and studied from the industrial site exhibited acidic pH.



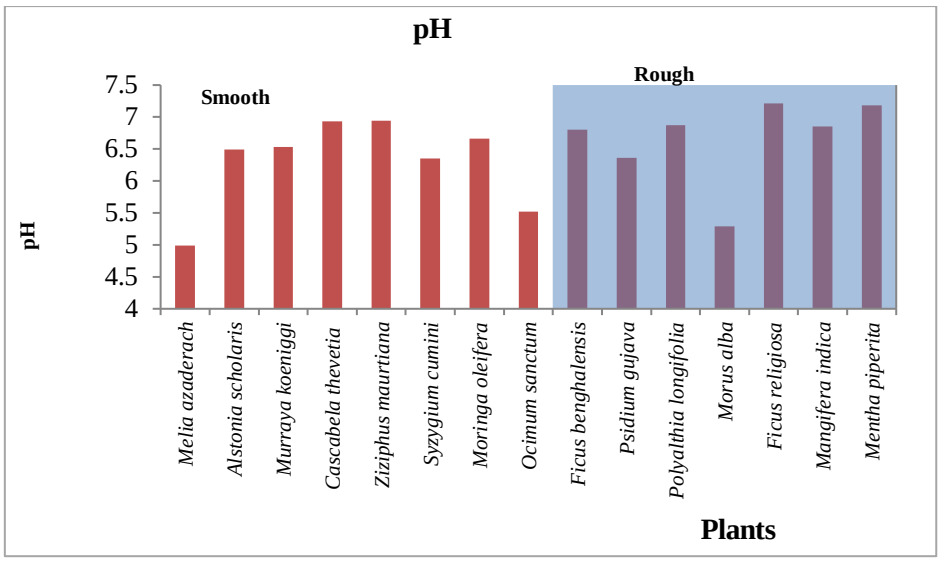
(a) industrial



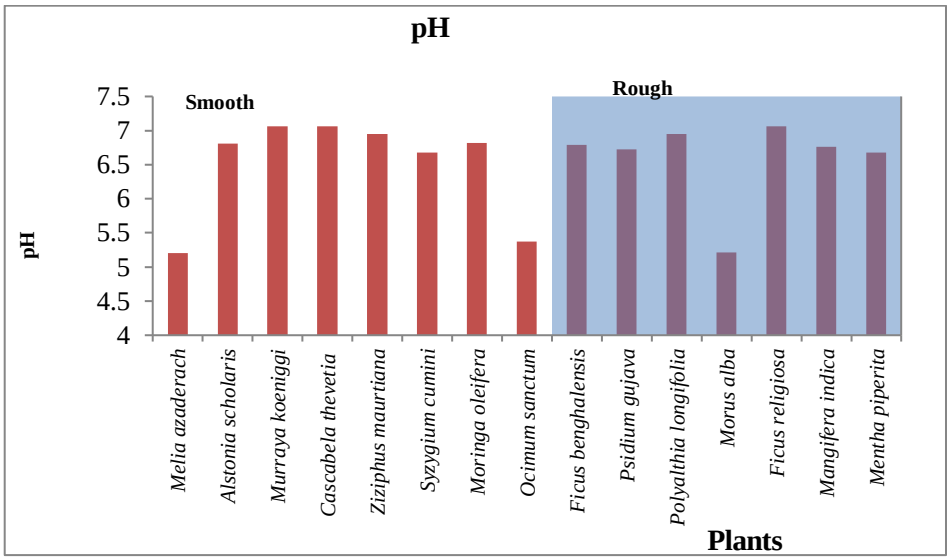
(b) roadside

Figure 4.8 Change in pH of industrial and roadside plant species, respectively (grouped by LSA)

This may be due to the presence of air pollutants like SO_2 and NO_X in the ambient air. When gaseous air pollutants like SO_2, NO_2 and CO_2 diffuse into the cell sap and convert into acidic radicals (Joshi & Swami, 2007), this may be the reason for the acidic pH in the current study. Plants species exhibited acidic pH; *Melia azedarach* (4.9), *Morus alba* (5.2), *Ocimum sanctum* (5.5), *Syzygium cumini* (6.3), *Psidium guajava* (6.3) and *Alstonia scholaris* (6.4).Similarly, the pH of roadside plants was found to be between 5to 7. The plant species *Ficus religiosa* (7) and *Mentha piperita* (7.3), *Cascabela thevetia* (7) and *Murraya koenigii* (7) were found to have pH values 7.



(a) industrial

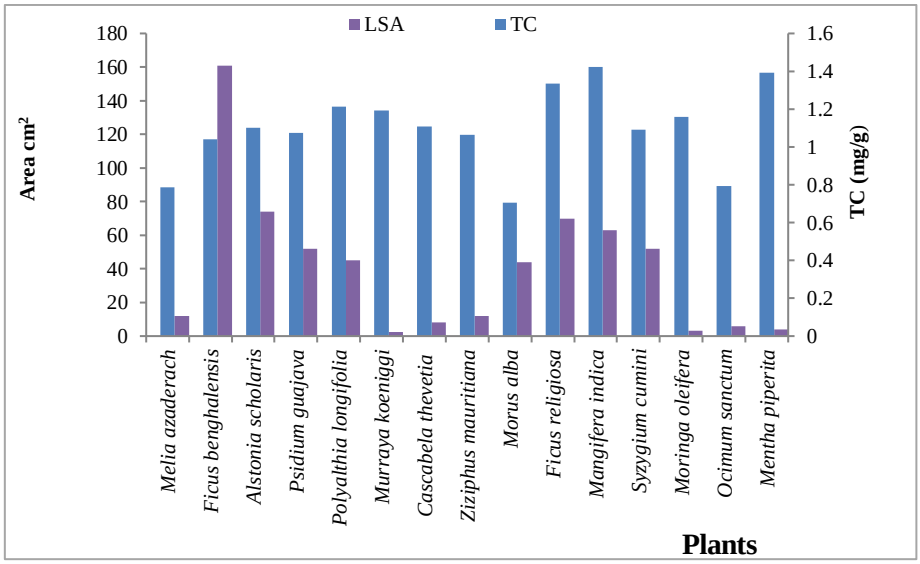


(b) roadside

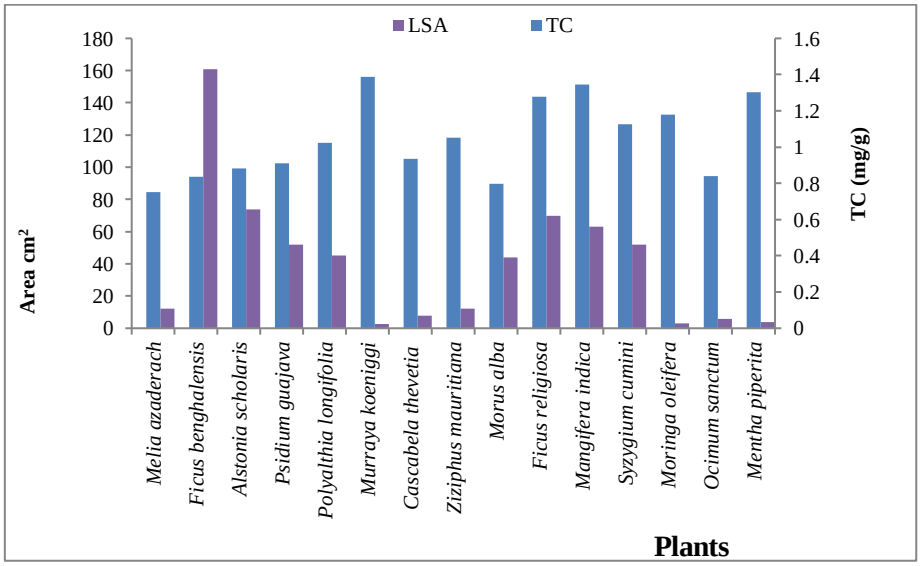
Figure 4.9 Change in pH of industrial and roadside plants species respectively (grouped by rough and smooth)

This means these plants are more tolerant of air pollution than those having low pH. Generally, leaves with a lower pH value are more affected by air pollution, though leaves with a higher pH value are more resistant (Govindaraju et al., 2012). In the present study in Figure 4.9 (a and b), it is observed that plants show almost similar pH irrespective of rough/smooth and small/large leaf surfaces at both the studied sites. Furthermore, it has been observed that LSA and LST do not have a significant relationship with pH. It may be because many other parameters overshadow the

variation of pH in the leaf, like pollutant dosage, environmental parameters, soil conditions, etc.



(a) industrial

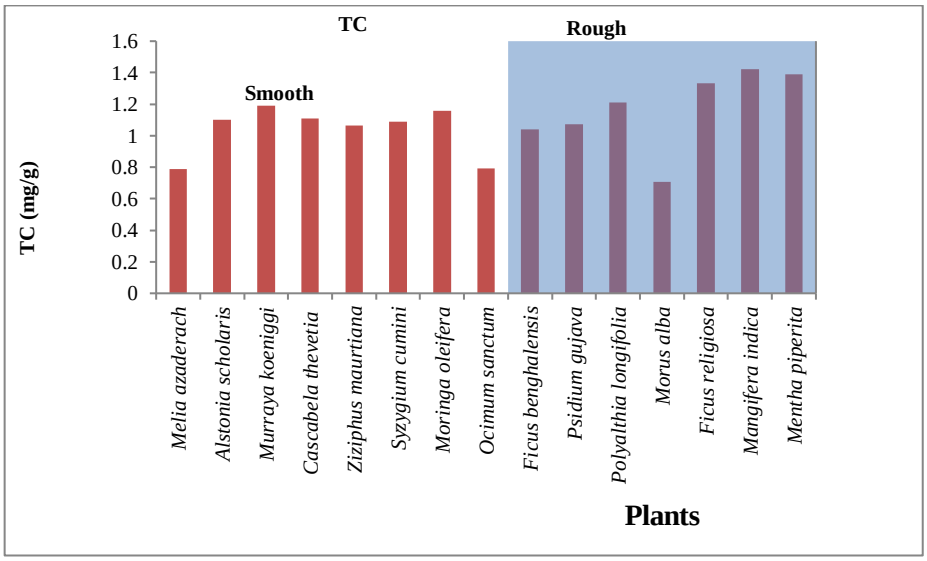


(b) roadside

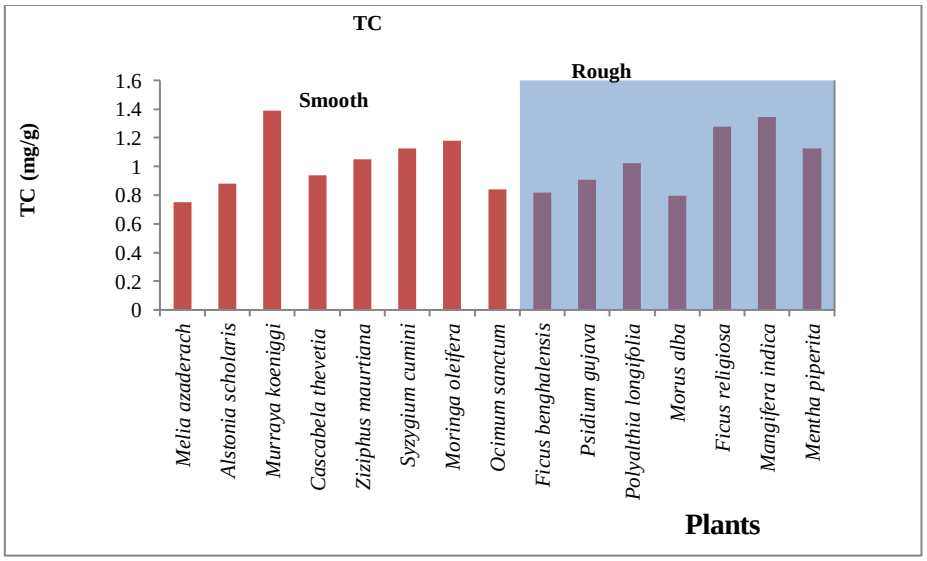
Figure 4.10 Change in TC of industrial and roadside plant species, respectively (grouped by LSA)

The findings in the sampling areas (roadside and industrial) showed varying amounts of TC in plants as *Mangifera indica* (1.3-1.4mg/g), *Syzygium cumini*(1-1.1mg/g), *Ocimum sanctum* (0.7-0.8mg/g), *Ficus religiosa* (1.2-1.3mg/g), *Mentha piperita* (1.1-1.3mg/g), *Psidium guajava* (1-0.9mg/g), *Melia azedarach* (0.75-0.78mg/g), *Ficus benghalensis*(0.8-1mg/g), *Polyalthia longifolia* (1-1.2mg/g), *Ziziphus mauritiana*(1.0-

1.0mg/g) and *Murraya koenigii* (1.1-1.3mg/g). Measurement of chlorophyll is considered a very important tool to evaluate the effect of air pollutants on plants. Plant growth is directly proportional to the chlorophyll concentration of plants Increase in pollutant levels reduces chlorophyll synthesis and increases chlorophyll degradation (Karmakar et al., 2020). Samples of plant leaves collected from industries had higher chlorophyll content compared than samples collected from roadside areas Figure 4.10 (a and b).



(**a**) industrial

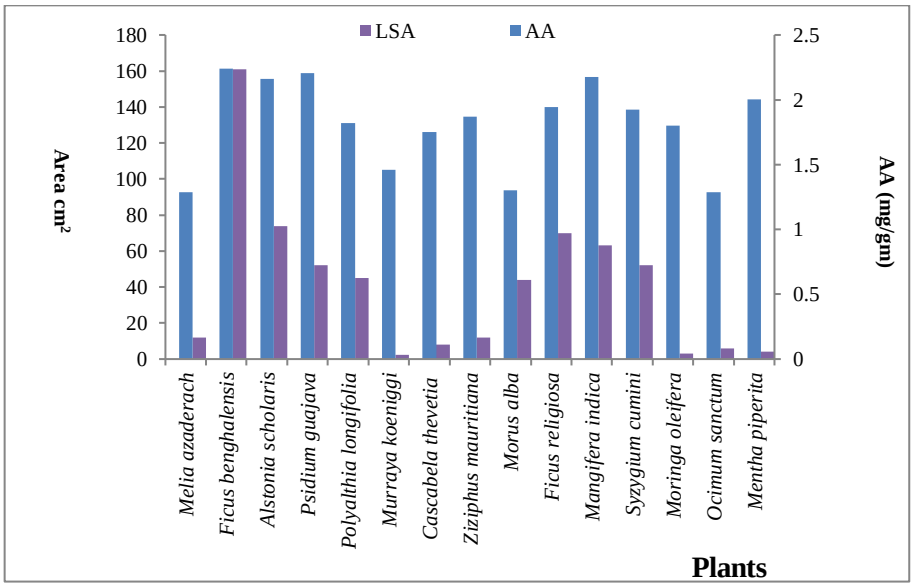


(b) roadside

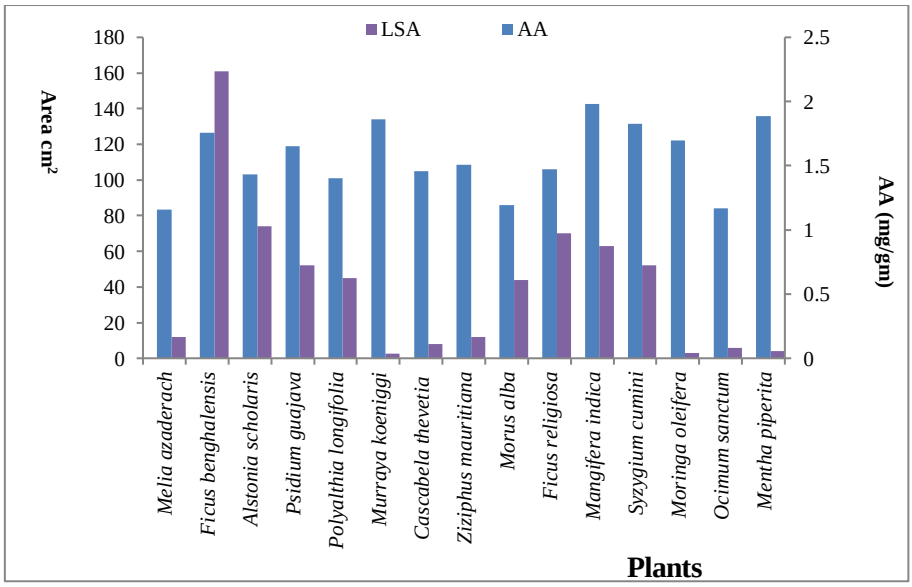
Figure 4.11 Change in TC of industrial and roadside plant species, respectively (Grouped by smooth and rough)

Plants with high chlorophyll content under field conditions are generally considered

tolerant to pollutants. In addition, it is observed that TC does not show any significant trend with LST in Figure 4.11 (a and b)



(a**)** industrial



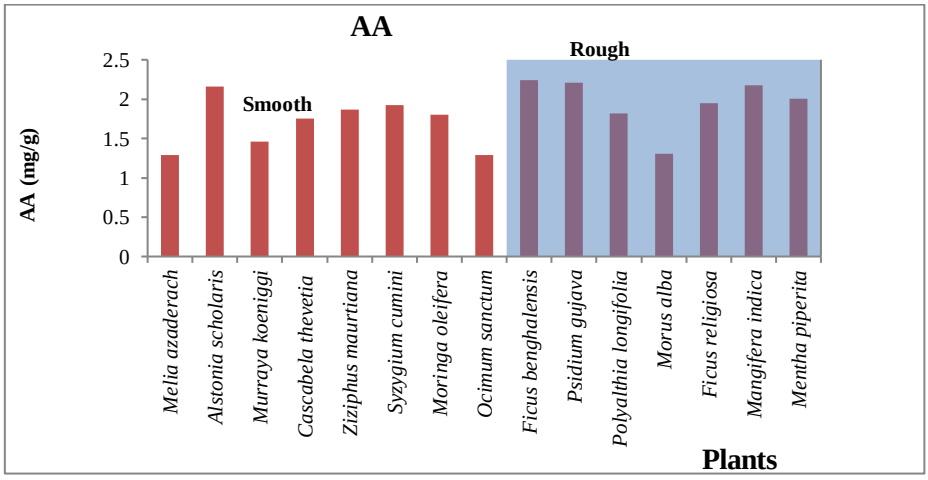
(b) roadside

Figure 4.12 Change in AA of industrial and roadside plant species, respectively (grouped by LSA)

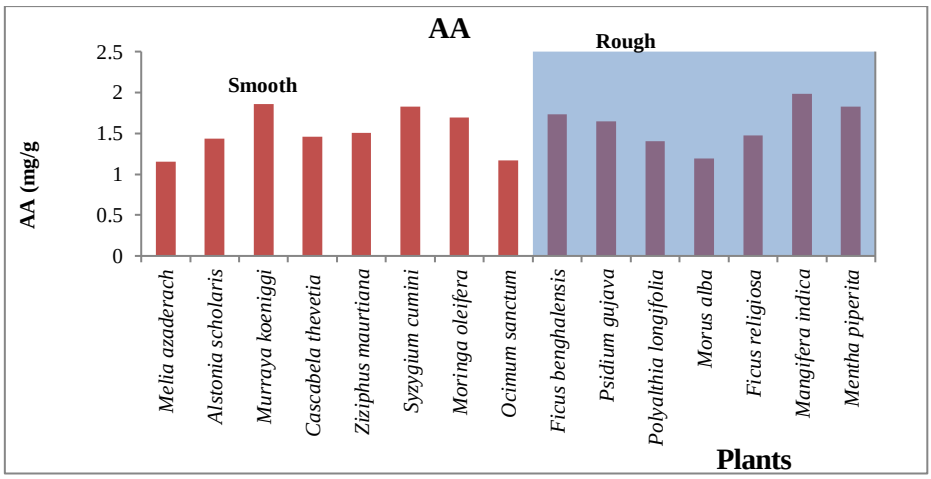
Plant species exhibited variation in the absorption of AA content as shown in Figure 4.12 (a and b). A higher amount of AA content has been observed in *Ficus benghalensis* (2.1), followed by *Psidium guajava* (2.2), *Mangifera indica (2.1)*, *Alstonia scholaris* (2.1), and *Mentha piperita* (2) at an industrial site. Similarly, plants at the roadside

exhibited different pattern of higher AA content. The higher AA has been studied in *Mangifera indica* (1.9), followed by *Murraya koenigii* (1.8), *Mentha piperita* (1.8), and *Ficus benghalensis* (1.7). This may be a defense mechanism of antioxidants against reactive oxygen species (ROS) produced by the photosynthetic system (Pandey et al., 2015). The second reason may be due to high rate of production of ROS like SO_3^-, HSO_3^-, OH^-, O_2^- etc. which generate sulphite by absorbing SO_2 and photo oxidation of SO_3^- to SO_4^- (Karmakar et al., 2020). It functions as a powerful antioxidant in plants by inducing their defense mechanism against the production of ROS, which is influenced by absorbed pollutants under various environmental stress conditions (Anake et al., 2022).

The increase in AA content results in the advanced ROS production rate, including SO^{3-}, HSO^{3-}, OH^-, etc.(Karmakar et al.,2020). As a result, plant with rough surfaces at industrial sites has been shown to contain more AA than plants with smooth surfaces. This may be a defense mechanism of plants against stress. But, on the other hand, no noteworthy significance was found between AA and LST of roadside plants as shown in Figure 4.13 (a and b).



(a) industrial



(b) roadside

Figure 4.13 Change in AA of industrial and roadside plant species, respectively (grouped by smooth and rough)

4.8.2 Pearson correlation matrix

Pearson's correlation analysis has been used to establish the influence of morphological parameters (LSA and LST) in terms of positive and negative correlations on the biochemical parameters of plants. The results are shown in Tables 4.9 and 4.10. Additionally, the comparison of Pearson's correlation coefficient is shown in Figure 4.16 below. Among the four biochemical parameters, it was observed that AA has significant correlation with LSA (Table 4.9). A pH and RWC has a comparatively weak correlation with LSA ($r = 0.19$ and $r = 0.16$ respectively), but TC ($r= 0.07$) has the lowest correlation with LSA at a significance level of $p < 0.05$ for industrial plants. Similarly, LST has a weak correlation with RWC ($r = -0.09$), AA ($r = 0.19$) and TC ($r = 0.32$) exhibit a weak positive correlation followed by pH ($r = 0.26$) at $p < 0.05$ significance level for the industrial plants.

Table 4.9 Pearson's correlation coefficient analysis of two morphological parameters (LSA and LST) and four biochemical parameters of selected plants of industrial area (*Marked correlations between morphological and biochemical parameters are significant at $p < 0.05$)

	LSA	LST	RWC	pH	AA	TC
LSA	1					
LST	0.50*	1				
RWC	0.16	0.093	1			
pH	0.19	0.26	0.67	1		
AA	0.59*	-0.40	0.66	0.71	1	
TC	0.07	0.32	0.78	0.87	0.66	1

In roadside plants, LST and LSA have a weak correlation with RWC ($r = -0.03$ and $r = 0.24$ respectively) but TC ($r = 0.11$, $r = 0.22$) followed by pH ($r = 0.14$, $r = 0.12$) and AA ($r = 0.19$, $r= 0.16$) have found weaker correlation with LST and LSA respectively (Table 4.10).

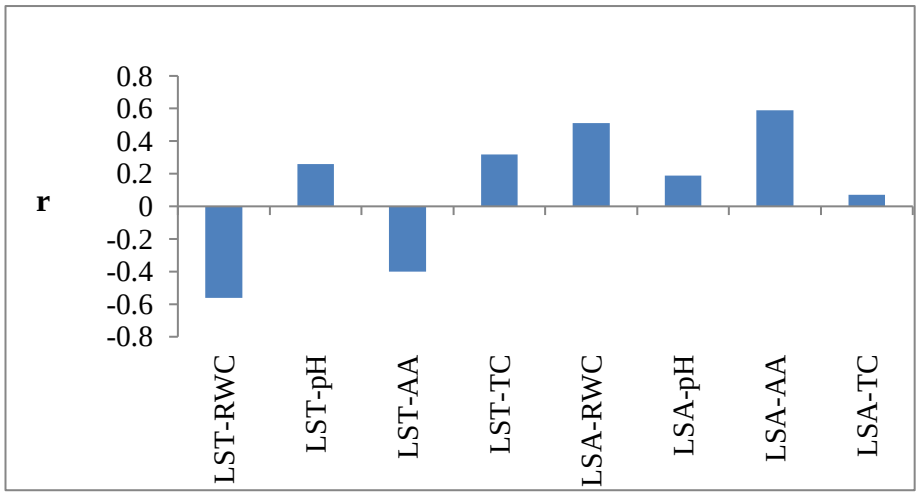
Table 4.10 Pearson's correlation coefficient analysis of two morphological parameters (LSA and LST) and four biochemical parameters of selected plants of roadside area (*Marked correlations between morphological and biochemical parameters are significant at $p < 0.05$)

	LSA	LST	RWC	pH	AA	TC
LSA	1					
LST	0.50*	1				
RWC	0.24	-0.03	1			
pH	0.12	0.14	0.86	1		
AA	0.16	0.19	0.75	0.71	1	
TC	0.22	0.11	0.67	0.65	0.74	1

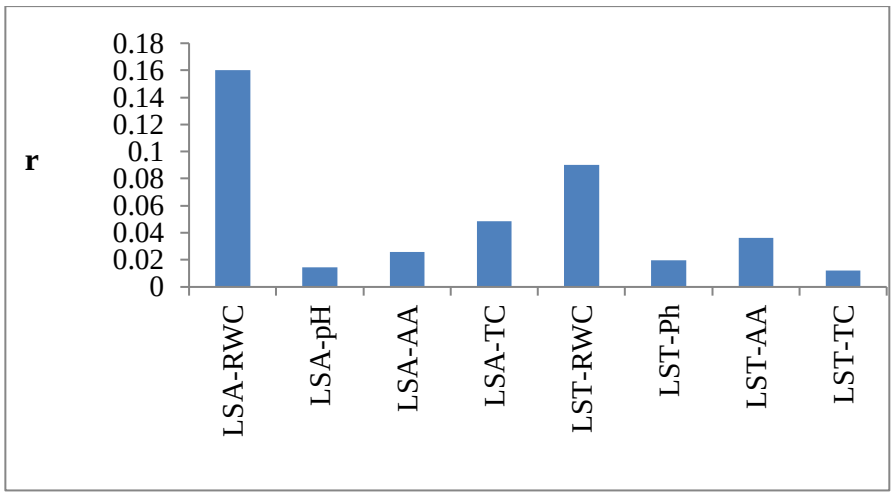
It is observed that the morphological parameters considered in the present study are not very significant in changing the biochemical parameters of the plants. However, AA showed significant positive correlation, among four biochemical parameters. Multiple parameter analysis increases the likelihood of identifying air pollutant tolerant plant species compared to single parameter analysis. Plants usually modify themselves to overcome stress conditions (both morphologically and physiologically).The overall

development and growth of plants are influenced by a number of environmental factors, including soil, water and air. Also, according to previous literature, many parameters influence the tolerance behavior of plants, such as stomatal anatomy and density, petiole size, leaf texture, leaf size, etc. The ability of plants to reduce air pollution also depends on these parameters (Katiyar & Dubey, 2000; Tekin et al., 2022; Cobanoglu et al., 2023; Cetin et al., 2023). As a result, biochemical parameters values of two different polluted sites with similar plants species displayed variation in their tolerance indices. This means that some other parameters including pollution source, landscape, topography, wind velocity and other meteorological conditions are also participating in plant tolerance. For example, Alobaidy and Rabee (2018) investigated that some plants have smaller petiole length and leaf surface, less interaction with any pollutants and environmental factors and act as tolerant species in harsh environment.

This may be because the smaller leaf area absorbs smaller amounts of air pollutants and which do not harm the plant. Also, this can indicate the role of morphological parameter in tolerance and may be necessary for evaluating the effect of air pollution on plants. The choice of problem adopted in the present work provides insight into the effect of morphological parameters on plant biochemical parameters. In the current study, morphological parameters reflected more progressive variation in biochemical parameters regardless of leaf surfaces or leaf textures. The variability of plant biochemical parameters and APTI determination may not be the only way to classify plant species as air pollution tolerant or sensitive (Karmakar et al., 2020).



(a) industrial



(b) roadside

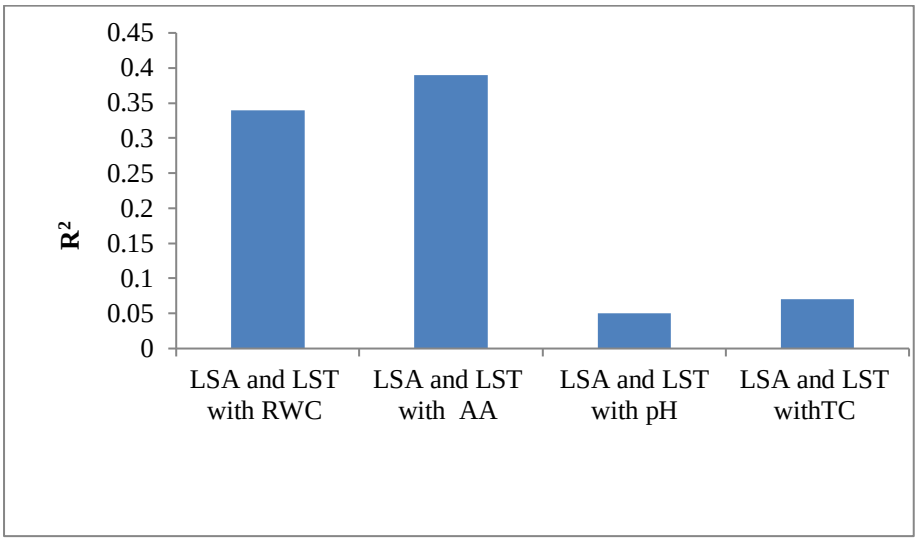
Figure 4.16 Comparison of Pearson's correlation coefficients between Morphological parameters (leaf surface area and leaf surface texture) and Biochemical parameters

Many other parameters participate in changing the tolerance of plants to air pollution. This needs to be considered while calculating plant tolerance index for effective screening and identification of plants for effective mitigation measures against air pollution.

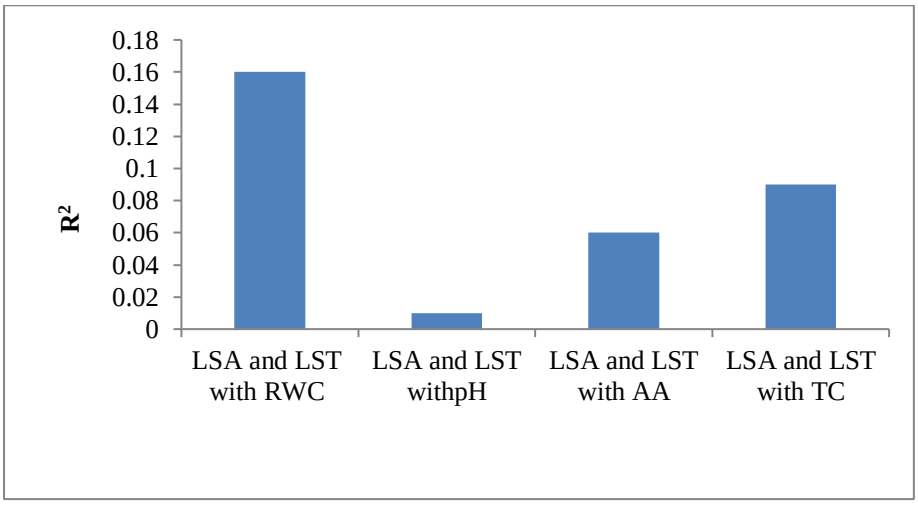
4.8.3 OLS regression

Regression coefficient analysis was used to describe the relationship (negative and positive) between independent variables (LSA and LST) and the dependent variables (biochemical parameters). Comparison of multiple regression coefficients (R^2) is shown in Figure 4.17, in which it is observed that LST and LSA have larger coefficient with AA followed by RWC, TC and pH at industrial side. While, at roadside LST and LSA are showing higher coefficient value with RWC, followed by TC, AA and pH. Regression coefficients indicate positive and negative relation between independent and dependent variables. In industrial area, the regression coefficients showed positive relationship between LSA and LST with RWC and AA ($R^2 = 0.34$) and ($R^2 = 0.39$) at p value (0.08) and (0.05) respectively. A smaller p value indicates that the independent variables are statistically significant in predicting the dependent variable (RWC and AA). For pH and TC regression coefficient ($R^2 = 0.05$) and ($R^2 = 0.07$) at p value 0.733 and 0.628 respectively which showed relationship is not significant.

Some parameters (RWC, AA) show moderate predictive power while others (pH, TC) do not. Additionally, multicollinearity may affect the results. On other side, in roadside the regression coefficients showed no significant relationship between LSA and LST with RWC and pH ($R^2 = 0.16$) and ($R^2 = 0.01$) at p value (0.34) and (0.92) respectively. For AA and TC regression coefficient ($R^2 = 0.06$) and ($R^2 = 0.09$) at p value 0.662 and 0.56 respectively. A larger p value indicates that the independent variables are not statistically significant in predicting the dependent variable (RWC and pH). As a result, it was indicated that the all the predicting parameters (RWC, pH, AA and TC) are not statistically significant at the roadside.



(a) industrial



(b) roadside

Figure 4.17 Comparison of Multiple regression coefficients between Morphological parameters (leaf surface area and leaf surface texture) and Biochemical parameters

Additional variables may be needed to better understand the predictive power of the parameters. Regression results align with the Pearson correlation coefficient, shows the relationship between variables are in the expected direction. Additional parameters may be required to establish causal relationships.

4.9 Justification for the further studies

The selected morphological parameters Leaf surface texture (LST) and Leaf surface area (LSA) did not exhibit significant correlation with biochemical parameters. However no definitive or statistically significant conclusions could be drawn. Since, environmental factors have been recognized in prior literature as potential drivers of variability in biochemical parameters. Another experiment (Experiment 2) was conducted to investigate the influence of environmental factors on plant tolerance. Two plant species have been selected from the initial selected plants in the previous experiment 1.These plants were grown in controlled environmental factors to assess the effect of environmental factors namely temperature, humidity and light intensity on their biochemical parameters.However, in a separate experiment (Experiment 3) the effect of air pollutants on biochemical parameters of plants was also investigated. Plants samples were collected from distinct areas of Punjab. The study also examined the variations in biochemical parameters of the plants in response to different concentrations of pollutants (SO_2 and NO_2) present in ambient air.

CHAPTER 5 EFFECTS OF ENVIRONMENTAL FACTORS ON BIOCHEMICAL PARAMETERS

5.1 Background

The response of plants to different environmental conditions varies depending on the plant's ability. Plants continually encounter a variety of environmental conditions that prompt adjustments in their metabolic processes to uphold a steady-state balance between energy production and consumption. These environmental factors have the potential to adversely impact plant metabolism, growth, and development, ultimately leading to mortality with prolonged exposure. These factors are various abiotic stresses, including drought, salinity, extreme temperatures (both high and low), and anaerobic conditions, all of which limit plant growth and productivity (Lawlor, 2002). The plant's responses to these stresses are intricate, often resulting in disruptive effects on metabolic pathways, cellular homeostasis, and physiological and biochemical processes. Temperature stress affects photosynthesis and increases photorespiration consequently influencing homeostasis of plant cells. Both high and low temperature is considered to be major abiotic stress for restricting plant productivity and leads to substantial yield loss. When plant exposed to high temperature or excess light, as a result, plant produces reactive oxygen species (ROS) that is considered highly toxic to damage the carbohydrates, proteins, lipids, and results in oxidative stress (Awasthi et al., 2015). Besides, high ROS causing damage to cells and some plants have antioxidant enzyme system to eliminate ROS and improves resistance against abiotic stresses (Gill and Tuteja, 2010).Bhullar and Jenner (1983) reported chlorophyll degradation under high temperature. Almeselmani et al., (2006) also found in their study that decreases in chlorophyll content due to high temperature. However, Gao et al., (2019) found in their study that among the main environmental factor, solar radiations is the most important that controls photosynthesis which provides the plant survival, growth and adaptations.

Light intensity has long been considered the most important factor influencing various aspects of plant growth, morphology, anatomy and physiology (Gao et al., 2019). It has also been studied from previous literature that light intensity primarily targets photosynthesis by damaging chlorophyll. It indirectly affects the productivity of plants as it depends strongly on photosynthesis. However, leaves are able to adapt to high or low light intensity depending on the plants growth environment (Taiz and Zeiger, 2002). It may be due to the presence of chloroplasts regulate the amount of light absorption thereby preventing damage to the

photosynthetic system from excessive light absorption. Steinger et al., (2003) found in their study that low levels of light intensity may increase the specific leaf area and plant height while high light intensity reduced the specific leaf area, increase leaf thickness.

Plants develop adaptations and plasticity to deal with different light regimes. Most of the plants have modified physiological, morphological and biochemical changes in response to different light intensities (Zervoudakis, 2012). Awasthi et al. (2015) highlighted that plants are subject to diverse environmental conditions, and temperature stress found a significant factor shaping plant structure and function. This stress encompasses both low and high temperatures and is recognized as a major abiotic stressor for crop plants.Humidity is the one of the important factor of the environment that influences the response of plants under stress. Two major physiological activities of plants that are directly controlled by humidity are transpiration (water loss) and stomatal opening. Temperature and humidity are directly proportional to each other. As temperature increases, transpiration increases because the vapor pressure difference between the moist leaf surface and air increases with increasing temperature (Ford and Thorne, 1974).Humidity indirectly affects the photosynthesis and water content. It affects plants via its effect on transpiration. Humidity directly affects the opening of stomata. Stomata close when the disparity between the vapor pressure of the air and the vapor pressure of the cells lining the sub-stomatal chamber of the leaf surpasses a critical threshold (Bunce, 1982). As explained by the previous literature, the effect of environmental parameters on photosynthesis, transpiration, morphology, physiology.

Among various environmental factors, light is the critical environmental factor influencing plant survival, growth, reproduction, and distribution, affects numerous physiological and morphological processes in plants (Keller et al., 2005; Kumar et al., 2011). Temperature and Humidity are another major environmental factor that plays a fundamental role in biological systems, as chemical reaction rates are intricately linked to tissue temperature (Moore et al., 2021; Tibbitis, 1979; Amin et al., 2023 respectively). Among all the environmental factors these are the most influential and thus have been chosen for the present study to investigate their effect on biochemical parameters.

5.2 Materials and Methods

Fresh matured leaves were sampled of *Ocimum sanctum* and *Mentha piperita* grown under different light intensity. Relative water content was determined by Singh (1977) , Total chlorophyll method by Hiscox and and Israelstam (1979), pH was measured with help of pH meter and Ascorbic acid was determined by volumetric method given by Sadasivam and

Manickam (1996). Sampling has been done twice in a month.

5.3 Results

Significant variation has been shown in the biochemical parameters of *Mentha piperita* growing under different environmental factors (temperature, light and humidity) exposure. The results are presented in Tables 5.1- 5.10 and Figures 5.1-5.8.

5.3.1 *Mentha piperita* exposed to 100% sunlight

As shown in the Table 5.1, considerable variations were observed in the biochemical parameters of *Mentha piperita* exposed to 100% exposure. Relative water content in leaves was increasing during the initial months January to July and decline in August to October and then again increased in December. Also, the temperature was initially increasing January to July and then decreased from August to December.

Humidity and light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 58% to 98.2% ;97lm/m^2 to 307.8lm/m^2;15^{0}C to 46^{o}C; 55 to 95.3 respectively. High RWC (91.2% and 94.4%) was found in the month of October at temperature 19.9^{0}C and 21^{0}C; humidity 91.1 and 86.9; light intensity 192lm/$m^2$and 225.8lm/m^2. Highest RWC (93.4% and 98.2%) was found in the month of September at temperature 22.8^{0}C and 26.7^{0}C; humidity 85.9 and 82.9; light intensity 217.5lm/m^2 and 257.6lm/m^2.pH of the *Mentha piperita* throughout the year has been observed in the range of 6-7.9.Highest pH (7.9) was found in the month of September at temperature 22.8^{0}C and 26.7^{0}C, humidity 85.9 and 82.9; light intensity 217.5lm/m^2 and 257.6lm/m^2 respectively. The chlorophyll content in leaves varied ranged from 0.6-2.7.Highest chlorophyll (2.7mg/g and 2.6 mg/g) was found in the month of September at temperature 22.8^{0}C and 26.7^{0}C, humidity 85.9 and 82.9; light intensity 217.5lm/m^2 and 257.6lm/m^2.

The AA content in leaves of *Mentha piperita* ranged from 1.7mg/g to 3.2mg/g. High AA (3mg/g) was found in March at temperature 17^{0}C; humidity 90.3; light intensity 307.8lm/m^2. Highest AA (3.2mg/g) content in leaves was found in the month of February at temperature 18.1^{0}C and 19.1^{0}C; humidity72.4 and 79.6; light intensity 279.2lm/m^2 and 267.1lm/m^2. The increasing orders for RWC (seasonally): monsoon < summer < winter. For pH, the order is monsoon > summer > winter. For AA, the order is summer > winter > monsoon. For TC, the order is monsoon > summer > winter.

Table 5.1 *Mentha piperita* exposed to 100%

Date	RWC	pH	AA	TC	Temperature (ºC)	Humidity	Light Intensity (lm/m^2)
1/01/22 - 15/01/22	59.2	7.2	2.1	0.7	15.7	95.3	193.9
16/01/22- 31/01/22	58.8	7.2	2	0.7	17.1	87.2	228.9
1/02/22 - 15/02/22	64.6	6.8	3.2	0.6	18.1	79.6	279.2
16/02/22 - 28/02/22	64.6	6.8	3.2	0.6	19.1	72.4	267.1
1/03/22 -15/03/22	68	7.5	3	1.5	17	90.3	307.8
16/03/22 - 31/03/22	68	7	2.9	1.5	18.1	90.8	242.5
1/04/22 - 15/04/22	68	7.8	2	1.4	22.1	74	241.8
16/04/22 - 31/04/22	68	7.8	2	1.4	27.5	72.4	241.8
1/04/22 - 15/04/22	78.6	7.8	2.5	1.2	30.7	77.9	252.4
16/04/22 - 31/04/22	82.3	7.8	2.8	1.6	32.9	73.6	269.2
1/06/22 - 15/06/22	78.6	7.8	2.8	2.1	39.2	59.9	265.6
16/06/22 - 30/06/22	80.2	7.5	2.1	2.5	41.02	55.6	249.6
1/07/22 - 15/07/22	83.4	7.8	2.9	2.1	43.6	62.6	237.7
16/07/22 - 31/07/22	86.4	7.6	2.1	2.1	46.02	54.3	245.7
1/08/22 - 15/08/22	91.2	7.8	1.9	2.5	35.7	69.2	241.8
16/08/22 - 31/08/22	93.4	7.9	1.9	2.2	32.7	76.6	279.2
1/09/22 - 15/09/22	93.4	7.9	2.9	2.6	26.7	82.9	257.6
16/09/22 - 30/09/22	98.2	7.9	1.9	2.7	22.8	85.9	217.5
1/10/22 - 15/10/22	91.2	7.2	2.9	2.5	21	86.9	225.8
16/10/22 - 31/10/22	94.2	7.2	2.9	2.5	19.9	91.1	192
1/11/22 - 15/11/22	63.2	7.6	2.0	0.8	19.3	91.6	216.8
16/11/22 - 30/11/22	64.5	7.6	2.2	0.8	18	87	212.1
1/12/22 - 15/12/22	73.4	7.1	1.7	0.7	15	70	205.8
16/12/22 - 31/12/22	72.1	7.3	1.7	0.7	17.6	85.8	211.2

The Lowest RWC (59.2% and 58.8%) was found in the month of January at temperature 15.7^0C and 17.1^0C; humidity 95.3 and 87.2; light intensity 193.9lm/m^2 and 228.9lm/m^2. Lowest pH (6.8) has been observed in the month of February at temperature 18.1^0C and 19.1^0C; humidity 79.6 and 72.4; light intensity 279.2lm/m^2 and 267.1lm/m^2 respectively. The Low TC (0.68mg/g) has been found in the month of February at temperature 18.1^0C and

19.1^{0}C; humidity 79.6 and 72.4; light intensity 279.2lm/m^2 and 267.1lm/m^2 respectively. Low AA (1.75mg/g) was observed in the month of December at temperature 15^{0}C and 17.6^{0}C; humidity 70 and 85.8; light intensity 205.8lm/m^2 and 211.2lm/m^2.The decreasing order for RWC (seasonally): winter < summer < monsoon. For pH, the order is winter < summer < monsoon. For AA, the order is winter < summer < monsoon. For TC, the order is winter < summer = monsoon.

5.3.2 *Mentha piperita* exposed to 60% sunlight

As shown, in the Table 5.2, a considerable variation was observed in the biochemical parameters of *Mentha piperita* exposed to 60% exposure. Initially, temperature was increasing then decreasing. Humidity and light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 58% to 95.4%;123lm/m^2 to 184.2lm/m^2;15 ^{0}C to 42.8^{o}C; 53.9 to 95.2. High RWC (89.4%) was observed in the month of September at temperature 26.4^{0}C and 25.4^{0}C; humidity 86.7 and 89.9; light intensity 154.5lm/m^2 and 130.5lm/m^2. Highest RWC (97.5%) was found in the month of October at temperature 21.3^{0}C and 19.5^{0}C; humidity 85.7 and 87.2; light intensity135.4lm/m^2 and 115.2lm/m^2.pH of the *Mentha piperita* throughout the year has been observed in the range of 6.9-8. High pH (8) was found in the month of November and December at temperature 17.6^{0}C and 16.3^{0}C; humidity 91.6 and 82.6; light intensity 130lm/m^{2}and lm/m^2 126.7 respectively. Also, it has been observed highest pH has been found at comparatively low temperatures and high humidity. The chlorophyll content in leaves varied ranged from 0.48-2.2. Furthermore, high chlorophyll (2.1mg/g and 2mg/g) was found in the month of September (2.1mg/g and 2mg/g) at temperature 26.4^{0}C and 25.4^{0}C; humidity 86.7 and 89.9; light intensity 154.5lm/m^{2}and 130.5lm/m^2. The highest Chlorophyll (2 mg/g) was found in the month of October at temperature 21.3^{0}C; humidity 85.7; light intensity 135.4lm/m^2. The AA content in leaves of *Mentha piperita* ranged from 1.5mg/g to 5.5mg/g. Highest AA (5.5mg/g and 5.1mg/g) content in leaves were found in the month of February at temperature 18.4^{0}C and 18.5^{0}C; Humidity 68.1 and 72.4; light intensity 167.5lm/m^2 and 160.2lm/m^2. The increasing orders for RWC (seasonally): monsoon= winter > summer. For pH, the order is winter > summer > monsoon. For AA, the order is winter > summer = monsoon. For TC, the order is summer > monsoon > winter.

Table 5.2 *Mentha piperita* exposed to 60% exposure

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
1/01/22 - 15/01/22	81.7	7.5	1.24	0.62	15.7	95.2	116.3
16/01/22- 31/01/22	81.5	7.5	1.5	0.65	18.2	81	137.3
1/02/22 - 15/02/22	79.5	7.5	5.5	0.65	18.4	68.1	167.5
16/02/22 -28/02/22	79.5	7.2	5.1	0.65	18.5	72.4	160.2
1/03/22 -15/03/22	89.5	7.2	3.1	1.2	16.9	84	184.6
16/03/22 -31/03/22	70.1	7.1	2.5	1.2	17.9	86.7	145.5
1/04/22 - 15/04/22	69.5	7.7	1.6	0.98	22.4	70	145
16/04/22 -31/04/22	70.2	7.7	1.6	0.98	26.9	70.2	145
1/04/22 - 15/04/22	67.0	7.1	2.1	1.4	30.7	64.6	151.2
16/04/22 -31/04/22	67.7	7.5	2.5	1.7	32.5	64.3	161.5
1/06/22 - 15/06/22	67.0	6.9	2.5	1.9	37.9	64	159.3
16/06/22 -30/06/22	67.1	7	2.5	1.5	40.6	59.5	149.7
1/07/22 - 15/07/22	63.4	7.5	2.6	1.9	42.8	53.9	142.6
16/07/22 -31/07/22	68.4	7.3	2.1	1.8	40.1	62.4	147.4
1/08/22 - 15/08/22	75.5	7.1	2.1	1.5	35.3	70.8	145
16/08/22 -31/08/22	88.8	7.5	1.9	1.9	32.6	80.2	167.5
1/09/22 - 15/09/22	89.4	7	1.9	2.1	26.4	86.7	154.5
16/0/22 - 30/09/22	71.2	7.6	1.7	2.2	25.4	89.9	130.5
1/10/22 - 15/10/22	95.4	7.1	2.1	2	21.3	85.7	135.4
16/10/22 -31/10/22	89.9	7.1	2.9	1.8	19.5	87.2	115.2
1/11/22 - 15/11/22	60.9	8	1.96	0.52	18.7	91.4	130
16/11/22 -30/11/22	58.1	8	1.95	0.55	17.6	91.6	127.32
1/12/22 - 15/12/22	70.1	7.9	1.56	0.48	17.5	81.4	123.4
16/12/22 -31/12/22	70.8	8	1.95	0.55	16.3	82.6	126.7

Lowest RWC (58.1% and 60.9%) was observed in the month of November at temperature 17.6^{0}C and 18.7^{0}C; humidity 91.6 and 91.4; light intensity 127.3lm/m^2 and 130lm/m^2. Lowest pH (6.9 and 7) has been observed in the month of June at temperature 37.9^{0}C and 40.6^{0}C; humidity 64 and 59.5; light intensity 159.3lm/m^2 and 149.7lm/m^2 respectively. The Low TC (0.5mg/g) has been found in the month of November at temperature 18.7^{0}C and 17.6^{0}C;

humidity 91.4 and 91.6; light intensity 130lm/m^2 and 127.32lm/m^2. Lowest TC (0.34mg/g) was observed in the month of December at temperature 14.8^{0}C and 16.4^{0}C; humidity 67 and 77.4; light intensity 102.9lm/m^2 and 63.3lm/m^2. Low AA(1.2mg/g and 1.5mg/g) was observed in the month of January at temperature 15.7^{0}C and 18.2^{0}C; humidity 95.2 and 81; light intensity 116.3lm/m^2 and 137.3lm/m^2. The decreasing orders of RWC (seasonally): summer < winter = monsoon. For pH, the order is monsoon: < summer < winter. For AA, the order is summer < monsoon < winter. For TC, the order is, winter < summer < monsoon

5.3.3 *Mentha piperita* exposed to 50% sunlight

As shown, in the Table 5.3 considerable variations was observed in the biochemical parameters of *Mentha piperita* exposed to 50% exposure. Relative water content in leaves was increasing during early months and then decreases and then increases. It is not showing any particular trend. Also, the temperature was first increasing initially then decreasing. Humidity and light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 68% to 94%;96lm/m^2 to 153.9lm/m^2;15^{0}C to 42.5^{o}C; 52.8 to 91.4. High RWC (94% and 93.4%) was found in the month of October at temperature 19.4^{0}C and 20.8^{0}C; humidity74.4 and 85.7; light intensity 96lm/m^2 and 112.9lm/m^2. Highest RWC (94%) was found in the month of September at temperature 25.8^{0}C; humidity 87; light intensity108.7lm/m^2. pH of the *Mentha piperita* throughout the year has been observed in the range of 6.9-8. High pH (7.7) was found in the month of April at temperature 26.3^{0}C; humidity 72.6; light intensity (120.9lm/m^2). Highest pH (7.8) was found in the month of February at temperature 18.2^{0}C, humidity 67.8; light intensity 139.9lm/m^2 respectively.

The chlorophyll content in leaves varied ranged from 0.3-1.6. High chlorophyll (1.5 mg/g and 1.4 mg/g) was found in September at temperature 25.8 and 26.5^{0}C; humidity 87 and 84.2; light intensity (108.7 and 128.8lm/m^2). Furthermore, highest chlorophyll (1.6mg/g and 1.5mg/g) was found in the month of October at temperature 20.8^{0}C and 19.4^{0}C, humidity 85.7 and 74.4, light intensity 112.9lm/$m^2$and 96lm/m^2.The AA content in leaves of *Mentha piperita* ranged from 0.7mg/g to 3.2mg/g. High AA (3.1 mg/g) was found in August at temperature 42.5^{0}C; humidity 62.1; light intensity 118.8lm/m^2.

Table 5.3 *Mentha piperita* exposed to 50% exposure

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
1/01/22 - 15/01/22	88.7	7.1	0.7	0.47	15.4	91.4	96.9
16/01/22- 31/01/22	88.7	7	1	0.47	16.9	78.1	114.4
1/02/22 - 15/02/22	86.5	7.8	3	0.45	18.2	67.8	139.9
16/02/22 - 28/02/22	85.5	7.5	3.2	0.45	18.3	63.9	133.5
1/03/22 -15/03/22	68.9	6.8	2.1	0.89	16.2	85.2	153.9
16/03/22 - 31/03/22	78.6	6.8	2.1	0.89	17.9	79.8	121.2
1/04/22 - 15/04/22	69.5	7.7	1.8	0.67	22.3	70.6	120.9
16/04/22 - 31/04/22	69.5	7.7	1.8	0.67	26.3	72.6	120.9
1/04/22 - 15/04/22	69.5	7	2.8	1.2	30.2	72	126.2
16/04/22 - 31/04/22	67.8	7.1	2.3	1.2	32.6	73.4	134.6
1/06/22 - 15/06/22	70.6	6.9	2	1.2	37.9	62	132.8
16/06/22 - 30/06/22	70.6	7.5	2	1.2	40.6	63.2	124.8
1/07/22 - 15/07/22	87.7	7.5	3.1	1.2	42.5	62.1	118.8
16/07/22 - 31/07/22	87.5	7.5	2.5	1.2	42.4	52.8	122.8
1/08/22 - 15/08/22	87.5	7.5	2.6	0.98	35.5	71.9	120.9
16/08/22 - 31/08/22	70.6	7.6	2.1	0.98	32.2	77.6	139.6
1/09/22 - 15/09/22	92	6.9	2.3	1.4	26.5	84.2	128.8
16/09/22 - 30/09/22	94	7.5	1.8	1.5	25.8	87	108.7
1/10/22 - 15/10/22	92.4	7.5	2.2	1.6	20.8	85.7	112.9
16/10/22 - 31/10/22	93.4	7.5	2.5	1.5	19.4	74.4	96
1/11/22 - 15/11/22	76.3	7.6	1.8	0.50	18.6	91	108.4
16/11/22 - 30/11/22	81.7	7.6	1.8	0.51	17.2	91.1	106
1/12/22 - 15/12/22	83.7	7.3	1.3	0.34	15.72	90.6	102.9
16/12/22 - 31/12/22	83.7	7.4	1.0	0.34	16.9	78.8	105.6

Highest AA (3mg/g and 3.2mg/g) content in leaves were found in the month of February at temperature 18.2^{0}C and 18.3^{0}C; humidity 67.8 and 63.9; light intensity 139.9lm/m^2 and 133.5lm/m^2. The increasing orders for RWC (seasonally): winter > monsoon > summer. For pH, the order is winter > monsoon > summer. For AA, the order is summer > monsoon > winter. For TC, the order is summer > monsoon > winter.

The Low RWC (68.9%) was found in the month of March at temperature 16.2^{0}C; humidity 85.2; light intensity 153.9lm/m^2. Lowest RWC (67.8%) was observed in the month of May at temperature 32.6^{0}C; humidity 73.4; light intensity 134.6lm/m^2. Lowest pH (6.8) has been observed in the month of March at temperature 16.2^{0}C and 17.9^{0}C; humidity 79.8and 85.2; light intensity 121.2lm/m^2 and 153.9lm/m^2 respectively. The Low TC (0.4mg/g) has been found in the month of January, followed by February at temperature 15.4^{0}C to 18.3^{0}C; humidity 63.9 to 91.4; light intensity 96.9lm/m^2 to 139.9lm/m^2 respectively. Lowest TC (0.34mg/g) was observed in the month of December at temperature 15.7^{0}C and 16.9^{0}C; humidity 90.6 and 78.8; light intensity 102.9lm/m^2 and 105.6lm/m^2. Low AA (0.73mg/g and 1mg/g) was observed in the month of January at temperature 15.4^{0}C and 16.9^{0}C; humidity 91.4 and 78.1; light intensity 96.9lm/m^2 and 114.4lm/m^2. The decreasing orders for RWC (seasonally): summer < winter < monsoon. For pH, the order is monsoon = summer < winter. For AA, the order is winter < monsoon < summer. For TC, the order is winter < monsoon < summer.

5.3.4 *Mentha piperita* exposed to 30% sunlight

As shown in the Table 5.4, considerable variations were observed in the biochemical parameters of *Mentha piperita* exposed to 30% exposure. The temperature was initially increasing from March to August and then decreased from September to January. Humidity and light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 51% to 99.6%;57.6lm/m^2 to 102.9lm/m^2;14 ^{0}C to 42.4^{o}C; 62 to 93.

High RWC was observed in the month of September (97.9% and 97.8%) at temperature 19.3^{0}C and 20.6^{0}C; humidity 74 and 83.1; light intensity 57.6lm/m^2 and 67.7lm/m^2. Highest RWC (99.3 % and 99.6%) was found in the month of October at temperature 40.1^{0}C and 37.9^{0}C; Humidity 67.2 and 62.4; light intensity 74.8lm/m^2 and 79.6lm/m^2.pH of the *Mentha piperita* throughout the year has been observed in the range of 6-8. High pH (7.9) was found in the month of September at temperature 32^{0}C; humidity 80.2; light intensity 83.7lm/m^2. The highest pH (8) has been found in the month of November at temperature 18.3^{0}C; humidity 91.2; light intensity 65lm/m^2. Also, it has been observed highest pH has been found at comparatively low temperatures and high humidity. The chlorophyll content in leaves varied ranged from 0.3-1.6.

Table 5.4 *Mentha piperita* exposed to 30% exposure

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
1/01/22 - 15/01/22	51.2	7.1	2.4	0.42	15.1	93	58.1
16/01/22- 31/01/22	54.6	7.4	2.1	0.41	17.9	74.2	68.6
1/02/22 - 15/02/22	61.5	7.1	3.1	0.42	17.9	68.8	83.7
16/02/22 - 28/02/22	63.1	7	3.1	0.42	17.6	71.9	80.1
1/03/22 -15/03/22	64.4	7.8	2.1	0.87	16.4	84.2	92.3
16/03/22 - 31/03/22	63.5	7.3	2.1	0.87	17.6	79	72.7
1/04/22 - 15/04/22	62.5	7.5	1.5	0.67	22.2	69	72.5
16/04/22 - 31/04/22	61.2	7.5	1.5	0.67	26.2	73.2	72.5
1/04/22 - 15/04/22	65.2	7	2.1	1.2	30.8	82.8	75.7
16/04/22 - 31/04/22	65.8	7.1	2.1	1.2	32.6	72.8	80.7
1/06/22 - 15/06/22	85.8	7.1	2	0.98	37.9	62.4	79.6
16/06/22 - 30/06/22	88.5	7.1	2.5	1	40.1	67.2	74.8
1/07/22 - 15/07/22	92.7	7	3.1	1.2	42.1	58.5	71.3
16/07/22 - 31/07/22	92.6	7.1	2.9	1	42.4	53.2	73.7
1/08/22 - 15/08/22	93.1	7.5	2.7	0.95	35.2	73.2	72.5
16/08/22 - 31/08/22	94.3	7.9	2.1	0.97	32	80.2	83.7
1/09/22 - 15/09/22	97.8	6.9	2.4	1.4	26.3	83.6	77.2
16/09/22 - 30/09/22	97.9	7.5	2.4	1.2	24.6	85.6	65.2
1/10/22 - 15/10/22	99.3	7.5	2	1.6	20.6	83.1	67.7
16/10/22 - 31/10/22	99.6	7.5	2.1	1.2	19.3	74	57.6
1/11/22 - 15/11/22	55.7	8	1.7	0.42	18.3	91.2	65
16/11/22 - 30/11/22	64	7.8	1.7	0.45	17.1	89.6	63.6
1/12/22 - 15/12/22	66.1	7.5	1.1	0.34	14.8	67	102.9
16/12/22 - 31/12/22	63	6.9	1.2	0.34	16.4	77.4	63.3

Higher chlorophyll (1.4mg/g) was observed in the month of September, at temperature 26.3^{0}C; humidity 83.6; light intensity 77.2lm/m^2. The highest chlorophyll (2.1mg/g and 2mg/g) was found in the month of October at temperature 20.6^{0}C; humidity 83.1; light intensity 67.7lm/m^2. The AA content in leaves of *Mentha piperita* ranged from 1mg/g to 3.2mg/g. Highest AA (3.1mg/g) content in leaves were found in the month of February and July at temperature 17.9^{0}C and 42.1^{0}C; humidity 68.8 and 58.5; light intensity 83.7lm/m^2 and

71.3lm/m^2. The increasing orders for RWC (seasonally): monsoon > summer > winter. For pH, the order is: summer > monsoon > winter. For AA, the order is: summer > monsoon > winter. For TC, the order is summer > monsoon > winter. The Lowest RWC (51.2%) was found in the month of January at temperature 15.1^{0}C; humidity 93; light intensity 58.1lm/m^2; humidity 91.2 and 89.6; light intensity 65lm/m^2 and 63.6lm/m^2. Lowest pH (6.9) has been observed in the month of December and September at temperature 16.4^{0}C and 26.3^{0}C; humidity 77.4and 83.6; light intensity 63.3lm/m^2 and 77.2lm/m^2 respectively. The Low TC (0.4mg/g) has been found in the month of January, followed by February and November at temperature 15.1^{0}C to 18.3^{0}C; humidity 68.8 to 93; light intensity 58lm/m^2 to 83.7lm/m^2 respectively. Lowest TC (0.34mg/g) was observed in the month of December at temperature 14.8^{0}C and 16.4^{0}C; humidity 67 and 77.4; light intensity 102.9lm/m^2 and 63.3lm/m^2. Low AA (1.7mg/g) was observed in the month of November at temperature 17.1^{0}C and 18.3^{0}C; humidity 91.2 and 89.6; light intensity 63.6lm/m^2 and 65lm/m^2. Lowest AA (1.1mg/g and 1.2 mg/g) was observed in the month of December at temperature 14.8^{0}C and 16.4^{0}C; humidity 67 and 77.4; light intensity 102.9lm/m^2 and 63.3lm/m^2.The decreasing orders for RWC seasonally: winter < summer < monsoon. For pH, the order is monsoon < summer < winter. For AA, the order is winter < summer < monsoon. For TC, the order is monsoon < summer = winter.

5.3.5 *Mentha piperita* exposed to 10% sunlight

As shown in the Table 5.5, considerable variations were observed in the biochemical parameters of *Mentha piperita* exposed to 10% exposure. Also, the temperature was initially increasing to July and then decreased from August to December. Humidity and light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 50% to 87.2%; 90lm/m^2 to 30.7lm/m^2;14 ^{0}C to 42.9^{o}C; 60 to 94.4 respectively. High RWC (86.8 and 86.2%) was found in the month of September at temperature 24^{0}C and 26^{0}C; humidity 87.2 and 85.2; light intensity21.7lm/m^2 and 25.7lm/m2. Highest RWC (90.1% and 92.3%) was found in the month of October at temperature 18.8^{0}C and 20.5^{0}C; humidity77.6 and 77.2; light intensity 19.2lm/m^2 and 22.5lm/m^2. pH of the *Mentha piperita* throughout the year has been observed in the range of 6

Table 5.5 *Mentha piperita* exposed to 10% exposure

Date	RWC	pH	AA	TC	Temperature (ºC)	Humidity	Light Intensity ($lm/m^{2)}$
1/01/22 - 15/01/22	NA	NA	NA	NA	15	94.4	19.3
16/01/22- 31/01/22	NA	NA	NA	NA	17.7	76.8	22.8
1/02/22 - 15/02/22	NA	NA	NA	NA	18	65.8	27.9
16/02/22 - 28/02/22	NA	NA	NA	NA	17.9	71.2	26.7
1/03/22 -15/03/22	66.73	7	3.1	0.55	16.2	84.5	30.7
16/03/22 - 31/03/22	66.3	7	3.5	0.55	17.2	81.2	24.2
1/04/22 - 15/04/22	70.5	8	1.5	0.56	21.3	68.7	24.1
16/04/22 - 31/04/22	70.5	8	1.5	0.56	26.2	71.5	24.1
1/04/22 - 15/04/22	67.8	6.8	3.2	0.56	29.6	89.8	25.2
16/04/22 - 31/04/22	67.5	6.8	3.2	0.89	32.2	75.2	26.9
1/06/22 - 15/06/22	70.2	7	3.2	0.56	39.1	60.6	26.5
16/06/22 - 30/06/22	71.2	7	2.1	1.2	42.4	68	24.9
1/07/22 - 15/07/22	71.9	8.1	2.5	0.78	42.9	60.8	23.7
16/07/22 - 31/07/22	72.9	7.8	2.5	0.78	34.3	55.4	24.5
1/08/22 - 15/08/22	75.6	7.5	1.8	0.78	20.6	75.6	24.1
16/08/22 - 31/08/22	76.1	7.9	1.7	0.89	31	77.8	27.9
1/09/22 - 15/09/22	86.2	7	2.4	0.79	26	85.2	25.7
16/09/22 - 30/09/22	86.8	7.6	2.1	0.79	24	87.2	21.7
1/10/22 - 15/10/22	90.1	7.2	2.4	1	20.5	77.2	22.5
16/10/22 - 31/10/22	92.3	7.2	2.9	1	18.8	77.6	19.2
1/11/22 - 15/11/22	50.3	7.6	1.5	0.55	17.9	92.1	21.6
16/11/22 - 30/11/22	50.5	7.5	1.5	0.56	17.1	87.2	21.2
1/12/22 - 15/12/22	NA	NA	NA	NA	14.7	67.2	20.5
16/12/22 - 31/12/22	NA	NA	NA	NA	16.1	78	21.1

High pH (8) was found in the month of April at temperature 21.3^0C and 26.2^0C, humidity 68.7 and 71.5; light intensity 24.1lm/m^2.Highest pH (8.1) was found in the month of August at temperature 42.9^0C; humidity 60.8; light intensity (23.7lm/m^2). The chlorophyll content in leaves varied ranged from 0.5-1.2mg/g. High chlorophyll (1mg/g) was found in September at temperature 20.5^0C and 18.8^0C; humidity 77.6 and 77.2; light intensity22.5lm/m^2 and

19.2lm/m^2. Furthermore, the highest chlorophyll (1.2mg/g) was found in the month of June at temperature 42.4^{0}C, humidity 68; light intensity 24.9lm/m^2.The AA content in leaves of *Mentha piperita* ranged from 1.5mg/g to 3.5mg/g. High AA (3.2mg/g) was found in May and June at temperature 29.6^{0}C and 39.1^{0}C; humidity 89.8 and 60.6; light intensity 25.2lm/m^2 and 26.5lm/m^2. Highest AA (3.5mg/g) content in leaves was found in the month of March at temperature 17.2^{0}C; humidity81.2; light intensity 24.2lm/m^2. The increasing orders for RWC (seasonally): monsoon > summer > winter. For pH, the order is: monsoon > summer > winter. For AA, the order is: summer > monsoon > winter. For TC, the order is monsoon > summer > winter.

Lowest RWC (50.3% and 50.5%) was observed in the month of November at temperature 17.9^{0}C and 17.1^{0}C; humidity 92.1 and 87.2; light intensity 21.6lm/m^2 and 21.2lm/m^2. Lowest pH (6.8) has been observed in the month of May at temperature 29.6^{0}C and 32.2^{0}C; humidity 89.8 and 75.2; light intensity 25.2lm/m^2 and 26.9lm/m^2respectively. The Low TC (0.5mg/g) has been found in the month of March, April, May and November at temperature 15.2^{0}C to 29.6^{0}C; humidity 68.7 to 92.1; light intensity 21.2lm/m^2 to 30.7lm/m^2 respectively. Low AA (1.5mg/g) was observed in the month of January at temperature 17.1^{0}C and 17.9^{0}C; humidity 87.2 and 92.1; light intensity 21.2lm/m^2 and 21.6lm/m^2.The decreasing order for RWC (seasonally): winter < summer < monsoon. For pH, the order is winter < summer < monsoon. For AA, the order is winter < monsoon < summer. For TC, the order is winter < summer < monsoon.

5.3.6 *Ocimum sanctum* exposed to 100% sunlight

Significant variation has been shown in the biochemical parameters *Ocimum sanctum* growing under different environmental parameters (temperature, light and humidity) exposure. The results are presented in Tables 5.6-5.10. As shown, in the Table 5.6 considerable variations was observed in the biochemical parameters of *Ocimum sanctum* exposed to 100% exposure. The temperature was initially increasing January to July and then decreased from August to December. Humidity and light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 57% to 98.9%;192lm/m^2 to 307.8lm/m^2;15^{0}C to 46^{o}C;55 to 95.3 respectively. High RWC (86.9% and 86%) was found in the month of September at temperature 22.8^{0}C and 26.7^{0}C; humidity85.9 and 82.9; light intensity 217.5lm/m^2 and 257.6lm/m^2. Highest RWC (98.9% and 97.5%) was found in the month of October at temperature 19.9^{0}C and 21^{0}C; humidity 91.1 and 86.9; light intensity192lm/m^2 and 225.8lm/m^2.pH of the *Ocimum sanctum* throughout the year has been

observed in the range of 6-7.6. Highest pH (7.6) was found in the month of December at temperature 17.6^{0}C, humidity 85.9; light intensity 211.2lm/m^2. The chlorophyll content in leaves varied ranged from 0.6-2.8mg/g. Highest chlorophyll (2.8mg/g) was found in the month of August at temperature 32.7^{0}C, humidity 76.6; light intensity 279.2lm/m^2.The AA content in leaves of *Ocimum sanctum* ranged from 1mg/g to 3.5mg/g. High AA (3.4mg/g) was found in May at temperature 30.7^{0}C; humidity 77.9; light intensity 252.4lm/m^2. Highest AA (3.5mg/g) content in leaves was found in the month of June, August and September at temperature 39.2^{0}C , 32.7^{0}C and 22.8^{0}C; humidity 59.9, 76.6 and 85.9; light intensity 265.6lm/m^2, 279.2lm/m^2 and 217.5lm/m^2. The increasing orders for RWC (seasonally): monsoon = summer > winter. For pH, the order is summer > winter > monsoon. For AA, the order is monsoon > summer > winter. For TC, the order is monsoon > summer > winter.

Table 5.6 *Ocimum sanctum* exposed to 100%

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
1/01/22 - 15/01/22	NA	NA	NA	NA	15.7	95.3	193.9
16/01/22- 31/01/22	NA	NA	NA	NA	17.1	87.2	228.9
1/02/22 - 15/02/22	NA	NA	NA	NA	18.1	79.6	279.2
16/02/22 - 28/02/22	NA	NA	NA	NA	19.1	72.4	267.1
1/03/22 -15/03/22	NA	NA	NA	NA	17	90.3	307.8
16/03/22 - 31/03/22	NA	NA	NA	NA	18.1	90.8	242.5
1/04/22 - 15/04/22	77.9	7	2.6	0.8	22.1	74	241.8
16/04/22 - 31/04/22	77.1	7.3	2.1	0.8	27.5	72.4	241.8
1/04/22 - 15/04/22	68.7	7.5	3.4	1.8	30.7	77.9	252.4
16/04/22 - 31/04/22	69.2	7	3.1	1.8	32.9	73.6	269.2
1/06/22 - 15/06/22	68.9	7.1	3.5	1.8	39.2	59.9	265.6
16/06/22 - 30/06/22	68.1	7.1	3.1	1.8	41.02	55.6	249.6
1/07/22 - 15/07/22	69.1	7	3.1	1.8	43.6	62.6	237.7
16/07/22 - 31/07/22	55	7.2	2.9	1.9	46.02	54.3	245.7
1/08/22 - 15/08/22	73.6	6.1	3.1	2.5	35.7	69.2	241.8
16/08/22 - 31/08/22	74.5	6.9	3.5	2.8	32.7	76.6	279.2
1/09/22 - 15/09/22	86	6.9	3	2.1	26.7	82.9	257.6
16/09/22 - 30/09/22	86.9	6.9	3.5	2.5	22.8	85.9	217.5
1/10/22 - 15/10/22	97.5	7.5	2.8	2.5	21	86.9	225.8
16/10/22 - 31/10/22	98.9	6.8	2.8	2	19.9	91.1	192.0

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
1/11/22 - 15/11/22	57.2	7.5	1.9	0.6	19.3	91.6	216.8
16/11/22 - 30/11/22	58.3	7.5	1.9	0.6	18	87	212.1
1/12/22 - 15/12/22	59.1	7.1	1.9	0.6	15	70	205.8
16/12/22 - 31/12/22	62.5	7.6	1.3	0.6	17.6	85.8	211.2

The Lowest RWC (57.2% and 58.3%) was found in the month of November at temperature 19.3^{0}C and 18^{0}C; humidity 91.6 and 87; light intensity 216.8lm/m^2 and 212.1lm/m^2. Lowest pH (6.8) has been observed in the month of October at temperature 19.9^{0}C; humidity 91.1; light intensity 192lm/m^2. Low TC (0.64mg/g) has been found in the month of December at temperature 15^{0}C and 17.6^{0}C; humidity 70 and 85.8; light intensity 205.8lm/m^2 and 211.2lm/m^2. Lowest TC (0.63mg/g) has been found in the month of November at temperature 19.3^{0}C; humidity 91.6; light intensity 216.8lm/m^2. Lowest AA (1.3mg/g) was observed in the month of December at temperature 17.6^{0}C; humidity 85.8; light intensity 211.2lm/m^2. The decreasing orders for RWC (seasonally): winter < summer < monsoon. For pH, the order is monsoon < summer < winter. For AA, the order is winter < summer < monsoon. For TC, the order is winter < summer < monsoon.

5.3.7 *Ocimum sanctum* exposed to 60% sunlight

As shown in the Table 5.7, a considerable variation was observed in the biochemical parameters of *Ocimum sanctum* exposed to 60% exposure. Humidity and light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 51% to 98.1%;123lm/m^2 to 184.2lm/m^2;15 ^{0}C to 42.8^{o}C; 53.9 to 95.2. High RWC (96.9%) was found in the month of September at temperature 26.4^{0}C and 25.4^{0}C; humidity 86.7 and 89.9; light intensity 154.5lm/m^2 and 130.5lm/m^2. Highest RWC (98.1% and 97.4%) was observed in the month of October at temperature 19.5^{0}C and 21.3^{0}C; humidity 87.2 and 85.7; light intensity 115.5lm/m^2 and 135.4lm/m^2. pH of the *Ocimum sanctum* throughout the year has been observed in the range of 6.5-8.High leaf extract pH (7.8) was found in the month of December at temperature 17.5^{0}C and 16.3^{0}C; humidity 81.4 and 82.6; light intensity 123.4lm/m^{2}and 126.7lm/m^2. Highest pH (8) was found in the month of November at temperature 18.7^{0}C; humidity 91.4; light intensity130lm/m^2 respectively. Also, it has been observed highest pH has been found at comparatively low temperatures and high humidity. The chlorophyll content in leaves varied ranged from 0.6-2.5. The highest

Chlorophyll (2.5mg/g and 2.3mg/g) was found in the month of September at temperature 25.4^{0}C and 26.4^{0}C; humidity 89.9 and 86.7; light intensity 130.5lm/m^2 and 154.5lm/m^2. The AA content in leaves of *Ocimum sanctum* ranged from 1.1mg/g to 3.6mg/g. Highest AA (3.6mg/g and 3.4mg/g) content in leaves were found in the month of September and August at temperature 26.4^{0}C and 35.3^{0}C; humidity 86.7 and 70.8; light intensity 154.5lm/m^2 and 145lm/m^2. The increasing order for RWC (seasonally): summer = monsoon > winter. For pH, the order is winter > summer > monsoon. For AA, the order is monsoon > summer > winter. For TC, the order is monsoon > summer > winter.

Lowest RWC (57.2% and 61%) was observed in the month of November at temperature 18.7^{0}C; humidity 91.4; light intensity 130lm/m^2. Lowest pH (6.5) has been observed in the month of September and August at temperature 25.4 ^{0}C and 32.6^{0}C; humidity 89.9 and 80.2; light intensity 130.5lm/m^2 and 167.5lm/m^2 respectively. The Low TC (0.63mg/g and 0.64mg/g) has been found in the month of December at temperature 17.5^{0}C and 16.3^{0}C; humidity 81.4 and 82.6; light intensity 123.4lm/m^2 and 126.7lm/m^2. Lowest TC (0.61mg/g and 0.62mg/g) was observed in the month of November at temperature 18.7^{0}C and 17.6^{0}C; humidity 91.4 and 91.6; light intensity 130lm/m^2 and 127.3lm/m^2. Low AA(1.3mg/g) was observed in the month of November at temperature 17.6^{0}C; humidity 91.6; light intensity 127.3lm/m^2.

Table 5.7 *Ocimum sanctum* exposed to 60% exposure

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
1/01/22 - 15/01/22	NA	NA	NA	NA	15.7	95.2	116.3
16/01/22- 31/01/22	NA	NA	NA	NA	18.2	81	137.3
1/02/22 - 15/02/22	NA	NA	NA	NA	19	68.1	167.5
16/02/22 - 28/02/22	NA	NA	NA	NA	18.5	72.4	160.2
1/03/22 -15/03/22	NA	NA	NA	NA	16.9	84	184.6
16/03/22 - 31/03/22	NA	NA	NA	NA	17.9	86.7	145.5
1/04/22 - 15/04/22	93.8	7.3	2.1	0.83	22.4	70	145
16/04/22 - 31/04/22	94.3	7.1	2.5	0.83	26.9	70.2	145
1/04/22 - 15/04/22	95.1	7.3	2.1	0.98	30.7	64.6	151.2
16/04/22 - 31/04/22	93.8	7.3	2.2	0.96	32.5	64.3	161.5
1/06/22 - 15/06/22	94.9	7.5	3.1	1.2	37.9	64	159.3
16/06/22 - 30/06/22	78	7.5	2.9	1.1	40.6	59.5	149.7
1/07/22 - 15/07/22	78.1	6.9	3.1	1.8	42.8	53.9	142.6

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
16/07/22 - 31/07/22	78.5	6.8	3	1.4	40.1	62.4	147.4
1/08/22 - 15/08/22	68.7	6.8	3.4	2.1	35.3	70.8	145
16/08/22 - 31/08/22	68.8	6.5	3.1	2.1	32.6	80.2	167.5
1/09/22 - 15/09/22	96.9	6.9	3.6	2.3	26.4	86.7	154.5
16/09/22 - 30/09/22	93.1	6.5	3.2	2.5	25.4	89.9	130.5
1/10/22 - 15/10/22	97.4	7.1	2.9	2.1	21.3	85.7	135.4
16/10/22 - 31/10/22	98.1	7.5	2.8	2	19.5	87.2	115.2
1/11/22 - 15/11/22	57.2	8	1.4	0.61	18.7	91.4	130
16/11/22 - 30/11/22	61	7.5	1.3	0.62	17.6	91.6	127.32
1/12/22 - 15/12/22	68.3	7.8	1.1	0.63	17.5	81.4	123.4
16/12/22 - 31/12/22	64.5	7.8	1.3	0.64	16.3	82.6	126.7

Lowest AA (1.1mg/g) was observed in the month of December at temperature 17.5^{0}C; humidity 81.4; light intensity 123.4lm/m^2. The decreasing orders for RWC (seasonally): winter < summer = monsoon. For pH, the order is monsoon < summer < winter. For AA, the order is: winter < summer < monsoon. For TC, the order is winter < summer < monsoon.

5.3.8 *Ocimum sanctum* exposed to 50% sunlight

As shown in the Table 5.8, considerable variations were observed in the biochemical parameters of *Ocimum sanctum* exposed to 50% exposure. The temperature was first increasing initially then decreasing. Humidity and light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 60% to 78.7%;96lm/m^2 to 153.9lm/m^2;15^{0}C to 42.5^{o}C; 52.8 to 91.4. High RWC (76.9%) was found in the month of September at temperature 26.5^{0}C and 25.8^{0}C; humidity87 and 84.2; light intensity 108.7lm/m^2 and 128.8lm/m^2. Highest RWC (78.7% and 77.6%) was found in the month of October at temperature 20.8^{0}C and 19.4^{0}C; humidity 85.7 and 74.4; light intensity 112.9lm/m^2 and 96lm/m^2. pH of the *Ocimum sanctum* throughout the year has been observed in the range of 6-7.7. Highest pH (7.7) was found in the month of June and November at temperature 18.6^{0}C and 37.9^{0}C; humidity 62 and 91; light intensity 108.4lm/m^2 and 132.8lm/m^2.The chlorophyll content in leaves varied ranged from 0.5-1.8.

The highest chlorophyll (1.7mg/g and 1.8mg/g) was found in the month of October at temperature 20.8^{0}C and 19.4^{0}C, humidity 85.7 and 74.4, light intensity 112.9lm/m^{2}and

96lm/m^2.The AA content in leaves of *Ocimum sanctum* ranged from 1mg/g to 4.2mg/g. High AA (3.9mg/g and 3.5mg/g) was found in September at temperature 25.8^{0}C and 26.5^{0}C; humidity 87 and 84.2; light intensity 108.7lm/m^2 and 128.8lm/m^2. Highest AA (4.2mg/g and 4.1mg/g) content in leaves were found in the month of August at temperature 35.5^{0}C and 32.2^{0}C; humidity 77.6 and 71.9; Light intensity 120.9lm/m^2 and 139.6lm/m^2. The increasing orders for RWC seasonally: summer > winter > monsoon. For pH, the order is winter > summer > monsoon. For AA, the order is monsoon> summer > winter. For TC, the order is monsoon > summer > winter.

Table 5.8 *Ocimum sanctum* exposed to 50% exposure

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
1/01/22 - 15/01/22	NA	NA	NA	NA	15.4	91.4	96.9
16/01/22- 31/01/22	NA	NA	NA	NA	16.9	78.1	114.4
1/02/22 - 15/02/22	NA	NA	NA	NA	18.2	67.8	139.9
16/02/22 - 28/02/22	NA	NA	NA	NA	18.3	63.9	133.5
1/03/22 -15/03/22	NA	NA	NA	NA	16.2	85.2	153.9
16/03/22 - 31/03/22	NA	NA	NA	NA	17.9	79.8	121.2
1/04/22 - 15/04/22	70.8	7.4	1.8	0.7	22.3	70.6	120.9
16/04/22 - 31/04/22	70.1	7.2	1.8	0.7	26.3	72.6	120.9
1/04/22 - 15/04/22	71.5	7.2	1.5	0.89	30.2	72	126.2
16/04/22 - 31/04/22	71.2	7.5	1.4	0.92	32.6	73.4	134.6
1/06/22 - 15/06/22	75.6	7.7	2.8	1.1	37.9	62	132.8
16/06/22 - 30/06/22	76.5	7.6	2.6	1.1	40.6	63.2	124.8
1/07/22 - 15/07/22	60.1	7.4	2.5	1.5	42.5	62.1	118.8
16/07/22 - 31/07/22	62.3	7.4	2.1	1.3	42.4	52.8	122.8
1/08/22 - 15/08/22	68.7	6.9	4.2	1.9	35.5	71.9	120.9
16/08/22 - 31/08/22	72.3	6.9	4.1	1.6	32.2	77.6	139.6
1/09/22 - 15/09/22	76.9	6.7	3.9	1.5	26.5	84.2	128.8
16/09/22 - 30/09/22	76.9	6.7	3.5	2	25.8	87	108.7
1/10/22 - 15/10/22	78.7	7	2.5	1.8	20.8	85.7	112.9
16/10/22 - 31/10/22	77.6	7.6	2.5	1.7	19.4	74.4	96
1/11/22 - 15/11/22	70.5	7.7	1.7	0.5	18.6	91	108.4
16/11/22 - 30/11/22	70.5	7.1	1.7	0.5	17.2	91.1	106
1/12/22 - 15/12/22	71.6	7.1	1.5	0.6	15.7	90.6	102.9

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
16/12/22 - 31/12/22	71.6	6.9	1.9	0.6	16.9	78.8	105.6

The Low RWC (109.9%) was found in the month of November at temperature 17.2^{0}C and 18.6^{0}C; humidity 91.1 and 91; light intensity 106lm/m^2 and 108.4lm/m^2. Lowest RWC (110.1% and 110.4%) was observed in the month of December at temperature 16.9^{0}C and 15.7^{0}C; humidity 78.8 and 90.6; light intensity 105.6lm/m^2 and 102.9lm/m^2. Lowest pH (6.7) has been observed in the month of September at temperature 25.8^{0}C and 26.5^{0}C; humidity 87 and 84.2; light intensity 108.7lm/m^2 and 128.8lm/m^2 respectively. The Low TC (0.6mg/g) has been found in the month of December at temperature 15.7^{0}C and 16.9^{0}C; humidity 90.6 to 78.8; light intensity 102.9lm/m^2 to 105.6lm/m^2 respectively. Lowest TC (0.5mg/g) was observed in the month of November at temperature 17.2^{0}C and 18.6^{0}C; humidity 91.1 and 91; light intensity 106lm/m^2 and 108.4lm/m^2. Lowest AA(1.4mg/g and .5mg/g) was observed in the month of April and December at temperature 32.6^{0}C and 15.7^{0}C; humidity 73.4 and 90.6; light intensity 134.6lm/m^2 and 102.9lm/m^2 respectively.

The decreasing orders for RWC (seasonally): monsoon < winter = summer. For pH the order is monsoon < winter < summer. For AA, the order is winter < summer < monsoon. For TC, the order is winter < summer < monsoon.

5.3.9 *Ocimum sanctum* exposed to 30% sunlight

As shown, in the Table 5.9 a considerable variation was observed in the biochemical parameters of *Ocimum sanctum* exposed to 30% exposure. The temperature was initially increasing from March to August and then decreased from September to January. Humidity and light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 56% to 88.5%; 57.6 lm/m^2 to 102.9lm/m^2; 14^{0}C to 42.4^{o}C; 62 to 93. High RWC was observed in the month of September (83.4% and 79.1%) at temperature 19.3^{0}C and 20.6^{0}C; humidity 74 and 83.1; light intensity 57.6lm/m^2 and 67.7lm/m^2. Highest RWC (88.5% and 87.6%) was found in the month of October at temperature 24.6^{0}C and 26.3^{0}C; humidity 85.6 and 83.6; light intensity 65.2lm/m^2 and 77.2lm/m^2. pH of the *Ocimum sanctum* throughout the year has been observed in the range of 7-7.9. Highest pH (7.9 and 7.8) was found in the month of June at temperature 40.1^{0}C and 37.9^{0}C; humidity 67.2 and 62.4; light intensity 74.8lm/m^2 and 79.6lm/m^2.

Table 5.9 *Ocimum sanctum* exposed to 30% exposure

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
1/01/22 - 15/01/22	NA	NA	NA	NA	15.1	93	58.1
16/01/22- 31/01/22	NA	NA	NA	NA	17.9	74.2	68.6
1/02/22 - 15/02/22	NA	NA	NA	NA	17.9	68.8	83.7
16/02/22 - 28/02/22	NA	NA	NA	NA	17.6	71.9	80.1
1/03/22 -15/03/22	NA	NA	NA	NA	16.4	84.2	92.3
16/03/22 - 31/03/22	NA	NA	NA	NA	17.6	79	72.7
1/04/22 - 15/04/22	68.5	7.2	1.8	0.7	22.2	69	72.5
16/04/22 - 31/04/22	64.1	7.1	1.8	0.7	26.2	73.2	72.5
1/04/22 - 15/04/22	74.5	7.3	2.4	0.7	30.8	82.8	75.7
16/04/22 - 31/04/22	74.5	7.2	2.3	0.7	32.6	72.8	80.7
1/06/22 - 15/06/22	75.8	7.8	2.6	0.9	37.9	62.4	79.6
16/06/22 - 30/06/22	78.1	7.9	2.6	1	40.1	67.2	74.8
1/07/22 - 15/07/22	63.1	7.6	2.1	0.9	42.1	58.5	71.3
16/07/22 - 31/07/22	64.6	7.1	2.1	0.9	42.4	53.2	73.7
1/08/22 - 15/08/22	75.4	7.5	3.5	1.7	35.2	73.2	72.5
16/08/22 - 31/08/22	64.6	7.1	3.2	1.5	32	80.2	83.7
1/09/22 - 15/09/22	79.1	7.4	3.2	1.5	26.3	83.6	77.2
16/09/22 - 30/09/22	83.4	7.1	3.5	1.8	24.6	85.6	65.2
1/10/22 - 15/10/22	87.6	7.5	2.4	1.2	20.6	83.1	67.7
16/10/22 - 31/10/22	88.5	7.6	2.6	1.5	19.3	74	57.6
1/11/22 - 15/11/22	56.7	7.4	2	0.5	18.3	91.2	65
16/11/22 - 30/11/22	56.9	7.5	2	0.5	17.1	89.6	63.6
1/12/22 - 15/12/22	64.5	7.5	0.9	0.3	14.8	67	102.9
16/12/22 - 31/12/22	63.4	7.5	0.9	0.3	16.4	77.4	63.3

Also, it has been observed highest pH has been found at comparatively high temperatures and low humidity. The chlorophyll content in leaves varied ranged from 0.3-1.8. The highest chlorophyll (1.8mg/g and 1.7mg/g) was found in the month of September and August at temperature 24.6^{0}C and 35.2^{0}C; humidity 85.6 and 73.2; light intensity 65.2lm/m^2 and 72.5lm/m^2.The AA content in leaves of *Ocimum sanctum* ranged from 1mg/g to 3.5mg/g. Highest AA (3.5mg/g) content in leaves was found in the month of August and September at temperature 35.2^{0}C and 24.6^{0}C; humidity 73.2 and 85.6; light intensity 72.5lm/m^2 and

65.2lm/m^2. The increasing orders for RWC (seasonally): monsoon > summer > winter. For pH the order is summer > monsoon > winter. For AA the order is monsoon > summer > winter. For TC the order is monsoon> summer > winter. The Lowest RWC (56.7% and 56.9%) was found in the month of November at temperature 18.3^{0}C and 17.1^{0}C; humidity 91.2 and 89.6; light intensity 65lm/m^2 and 63.6lm/m^2. The Low TC (0.5mg/g) has been found in the month of November at temperature 17.1^{0}C and 18.3^{0}C; humidity 89.6 and 91.2; light intensity 63.6lm/m^2 and 65lm/m^2 respectively. Lowest TC (0.3mg/g) was observed in the month of December at temperature 14.8^{0}C and 16.4^{0}C; humidity 67 and 77.4; light intensity 102.9lm/m^2 and 63.3lm/m^2. Lowest AA (0.9mg/g) was observed in the month of December at temperature 14.8^{0}C and 16.4^{0}C; humidity 67 and 77.4; light intensity 102.9lm/m^2 and 63.3lm/m^2. The decreasing orders for RWC (seasonally): winter < summer < monsoon. For pH, the order is monsoon < winter < summer. For AA, the order is winter < summer < monsoon. For TC, the order is summer < winter < monsoon.

5.3.10 *Ocimum sanctum* exposed to 10% sunlight

As shown in the Table 5.10, a considerable variation was observed in the biochemical parameters of *Ocimum sanctum* exposed to 10% exposure. The temperature was initially increasing to July and then decreased from August to December. Humidity and Light intensity show no particular trend, varying throughout the year. The RWC, light intensity, temperature and humidity ranged from 63% to 88.5%; 90lm/m^2 to 30.7lm/m^2;14 ^{0}C to 42.9^{o}C; 60 to 94.4 respectively. Highest RWC (88.5% and 87.6%) was found in the month of October at temperature 18.8^{0}C and 20.5^{0}C; humidity77.6 and 77.2; high lightt intensity 19.2lm/m^2 and 22.5lm/m^2.pH of the *Ocimum sanctum* throughout the year has been observed in the range of 6-8.1. High pH (7.8) was found in the month of April at temperature 17.9^{0}C; humidity 92.1; light intensity 21.6lm/m^2. Highest pH (8) was found in the month of June at temperature 39.1^{0}C; humidity 60.6; light intensity 26.5lm/m^2.The chlorophyll content in leaves varied ranged from 0.2-1mg/g. The highest chlorophyll (1mg/g) was found in the month of September and November at temperature 26^{0}C and 17.9^{0}C, humidity 85.2 and 92.1; light intensity 25.7lm/m^2 and 21.6lm/m^2.The AA content in leaves of *Ocimum sanctum* ranged from 1.5mg/g to 3.4mg/g.

Table 5.10 *Ocimum sanctum* exposed to 10% exposure

Date	RWC	pH	AA	TC	Temperature (^{0}C)	Humidity	Light Intensity (lm/m^2)
1/01/22 - 15/01/22	NA	NA	NA	NA	15	94.4	19.3
16/01/22- 31/01/22	NA	NA	NA	NA	17.7	76.8	22.8
1/02/22 - 15/02/22	NA	NA	NA	NA	18	65.8	27.9
16/02/22 - 28/02/22	NA	NA	NA	NA	17.9	71.2	26.7
1/03/22 -15/03/22	NA	NA	NA	NA	16.2	84.5	30.7
16/03/22 - 31/03/22	NA	NA	NA	NA	17.2	81.2	24.2
1/04/22 - 15/04/22	NA	NA	NA	NA	21.3	68.7	24.1
16/04/22 - 31/04/22	NA	NA	NA	NA	26.2	71.5	24.1
1/04/22 - 15/04/22	71.2	7.2	2	0.4	29.6	89.8	25.2
16/04/22 - 31/04/22	64.8	7	2.1	0.4	32.2	75.2	26.9
1/06/22 - 15/06/22	78.8	8	3.1	0.6	39.1	60.6	26.5
16/06/22 - 30/06/22	81.2	7	2.8	0.7	42.4	68	24.9
1/07/22 - 15/07/22	83.4	7	2.9	0.7	42.9	60.8	23.7
16/07/22 - 31/07/22	82.9	7	3	0.7	34.3	55.4	24.5
1/08/22 - 15/08/22	64.7	7.1	2.5	0.7	20.6	75.6	24.1
16/08/22 - 31/08/22	64.6	7.1	2.8	0.7	31	77.8	27.9
1/09/22 - 15/09/22	74.1	7.3	3.4	1	26.3	85.2	25.7
16/09/22 - 30/09/22	72.3	7	3.4	1	24.6	87.2	21.7
1/10/22 - 15/10/22	87.6	7.5	2.4	0.9	20.5	77.2	22.5
16/10/22 - 31/10/22	88.5	7.1	2.1	0.9	18.8	77.6	19.2
1/11/22 - 15/11/22	87.5	7.8	2.5	1	17.9	92.1	21.6
16/11/22 - 30/11/22	80.9	6.8	2.5	0.9	17.1	87.2	21.2
1/12/22 - 15/12/22	64.5	7.6	1.5	0.9	14.7	67.2	20.5
16/12/22 - 31/12/22	63.4	7.6	1.5	0.2	16.1	78	21.1

High AA (3.1mg/g) was found in June at temperature 39.1^{0}C; humidity 60.6; light intensity 26.5lm/m^2. Highest AA (3.4mg/g) content in leaves was found in the month of September at temperature 26^{0}C and 24^{0}C; humidity 85.2 and 87.2; light intensity 25.7lm/m^2 and 21.7lm/m^2.The increasing order for RWC (seasonally): monsoon < summer < winter. For pH, the order is winter > monsoon > summer. For AA, the order is monsoon > summer > winter.

For TC, the order is monsoon > winter > summer.

Lowest RWC (63.4% and 64.5%) was observed in the month of December at temperature 14.7^0C and 16.1^0C; humidity 67.2 and 78; light intensity 20.5lm/m^2 and 21.1lm/m^2. Lowest pH (6.8) has been observed in the month of November at temperature 17.1^0C; humidity 87.2; light intensity 21.2lm/m^2 respectively. Low TC (0.4mg/g) has been found in the month of May at temperature 29.6^0C and 32.2^0C; humidity 89.8 to 75.2; light intensity 25.2lm/m^2 to 26.9lm/m^2 respectively. The Lowest TC (0.25mg/g and 0.39mg/g) has been found in the month of December at temperature 16.1^0C and 14.7^0C; humidity78 to 67.2; light intensity 21.1lm/m^2 to 20.5lm/m^2 respectively. Lowest AA (1.5mg/g) was observed in the month of December at temperature 14.7^0C and 16.1^0C; humidity 67.2 and 78; light intensity 20.5lm/m^2 and 21.1lm/m^2.The decreasing order for RWC (seasonally): winter < summer < monsoon. For pH, the order is summer < monsoon < winter. For AA, the order is winter < summer < monsoon. For TC, the order is summer < monsoon= winter.

5.4 Discussion

The current study investigated the impact of three environmental factors namely temperature, light intensity and humidity on biochemical parameters in two different plant species (*Ocimum sanctum* and *Mentha piperita)*. Significant variation in biochemical parameters of *Ocimum sanctum* and *Mentha piperita* has been observed when exposed to different environmental factors under controlled conditions. The variation in each biochemical parameters of both plants with each environmental factor are discussed below. Additionally, statistically analyses used to elucidate relationship between biochemical parameters and environmental factors.

Pearson's correlation, multiple linear regressions and non linear regression methods were used. Pearson's correlation measures the strength of the linear relation between two variables. Multiple linear and non linear regressions are a prediction method for statistical analysis for defining the quantitative relationships between multiple independent variables. Pearson correlation coefficient (R_p) values for the data have been obtained to examine the variability level of the data under investigation. Multiple linear regression coefficients have been representing by R_L. A best fitted non linear regression line has been used to predict the estimates of dependent variables from independent variables. Coefficients of non linear multiple determination (R_{nl}) are the validation estimates provided by the regression coefficient.

5.4.1 Effect of Environmental factors (temperature, humidity and light intensity) on total chlorophyll (mg/g)

In natural environments, temperature usually has damaging impacts on plant photosynthesis (Hou et al., 2015). As shown in Figure 5.1 higher chlorophyll found generally in the month of September and October for *Mentha piperita*. Similar trend has been observed for *Ocimum sanctum* (Figure 5.2) including August. The temperature, humidity and light intensity recorded during these months ranged between 19^0C to 26^0C (which is comparatively moderate temperature); 74 to 91 (which is comparatively higher) and 112 lm/m^2 to 241 lm/m^2 (which is comparatively moderate according to selected different light intensities shade nets) respectively. Thus, it has been observed higher chlorophyll has been found at lower temperatures and high humidity and moderate light intensities. This is may be due to photochemical reactions in thylakoid membranes and carbon metabolism in stroma of chloroplasts has been reported primary sites of an injury due to high temperature (Yamori et al., 2008; Hou et al., 2015). However, low temperature also disrupts essentially all major components of photosynthesis including thylakoid electron transport, carbon reduction cycle and control of stomatal conductance (Hou et al., 2015). Djanaguiraman et al., 2010 found in their study that chlorophyll decreases when exposed to low and high temperature (Djanaguiraman et al., 2010). The aforementioned findings are consistent to our current findings that high temperature cause slight decrease in chlorophyll. Moreover, similar findings have been reported by Hou et al., (2015) and Djanaguiraman et al., (2010). Increase in humidity increases photosynthesis as studied by Forde and Thorne, 1973; Rawson and Begg 1977 and interestingly same findings have been drawn in current study. In the previous literature it was well known that stomatal changes directly associated to humidity. So this may be because stomatal conductance increases at higher humidity resulting in greater carbon dioxide fixation which may result in increased photosynthesis. Bunce, (1982) also made similar findings even in C4 plants.

Light intensity is considered as a significant factor for determining the rate of photosynthesis (Chapman et al., 1976; Taiz and Zeiger, 2002). Generally, at low light intensities chlorophyll gets larger as a results photosynthetic rate increases. On other side, at high light intensity chlorophyll get damaged which result in decrease in photosynthetic rate. Excessive light intensity would inactivate the reaction center of photo systems, damage photosynthetic organs and inhibit photosynthesis (Wimalasekera, 2019; Zhang et al., 2019; Lovell et al., 1972' Hazrati et al., 2016;Fu et al., 2012). The aforementioned findings are consistent with the current study, as high chlorophyll was observed at moderate and low light intensities. Jeon et

al (2016) had also observed increased in chlorophyll content in *Doritaenopsis* at low light intensities. In the current study high chlorophyll was observed at moderate and low light intensities. Pearson correlation analysis (as shown in Table 5.11and 5.12) was performed to assess the correlation between the biochemical parameters TC and environmental factors (temperature, humidity and light intensity). A weak positive correlation at $p < 0.005$ level was observed between TC (mg/g) of *Mentha piperita* and temperature ($R_p = 0.45$) and weak negative correlation with humidity ($R_p = -0.15$) but significant positive correlation was found with light intensity ($R_p = 0.52$).

Similarly, *Ocimum sanctum* has also exhibited a weak positive correlation of TC (mg/g) with temperature ($R_p = 0.30$) and humidity ($R_p = 0.01$) but significant positive correlation was observed with light intensity ($R_p = 0.51$). A significant and higher R_p value highlights the major role of temperature and light intensity in influencing the total chlorophyll content in plants. This is consistent with previous literature. However, the results obtained from multiple linear regressions also revealed the relationship between the TC (mg/g) (dependent variable) with temperature, humidity and light intensity (independent variables). The influence of temperature, light intensity and humidity on TC (mg/g) was predicted with great significant p values (< 0.05). The Regression coefficients were examined for both plants to evaluate the impact of individual environmental factors on TC (mg/g) has been observed <0.01. These findings suggest that individual environmental factors have minimal influence on TC (mg/g) variation. However, when considering the combined effect of environmental factors namely temperature, humidity and light intensity on TC (mg/g) of *Mentha piperita* and *Ocimum sanctum*, a notable and statistically significant impact was observed ($R_L = 0.52$ and $R_L = 0.41$respectively).Similar results were obtained from non linear multiple regression analysis. Individual environmental factors did not exhibit a strong relationship with TC (mg/g) for both plant *Mentha piperita* and *Ocimum sanctum* ($R^2_{nL}= 0.33$, $R^2_{nL}= 0.3$, $R^2_{nL}= 0.13$ respectively).

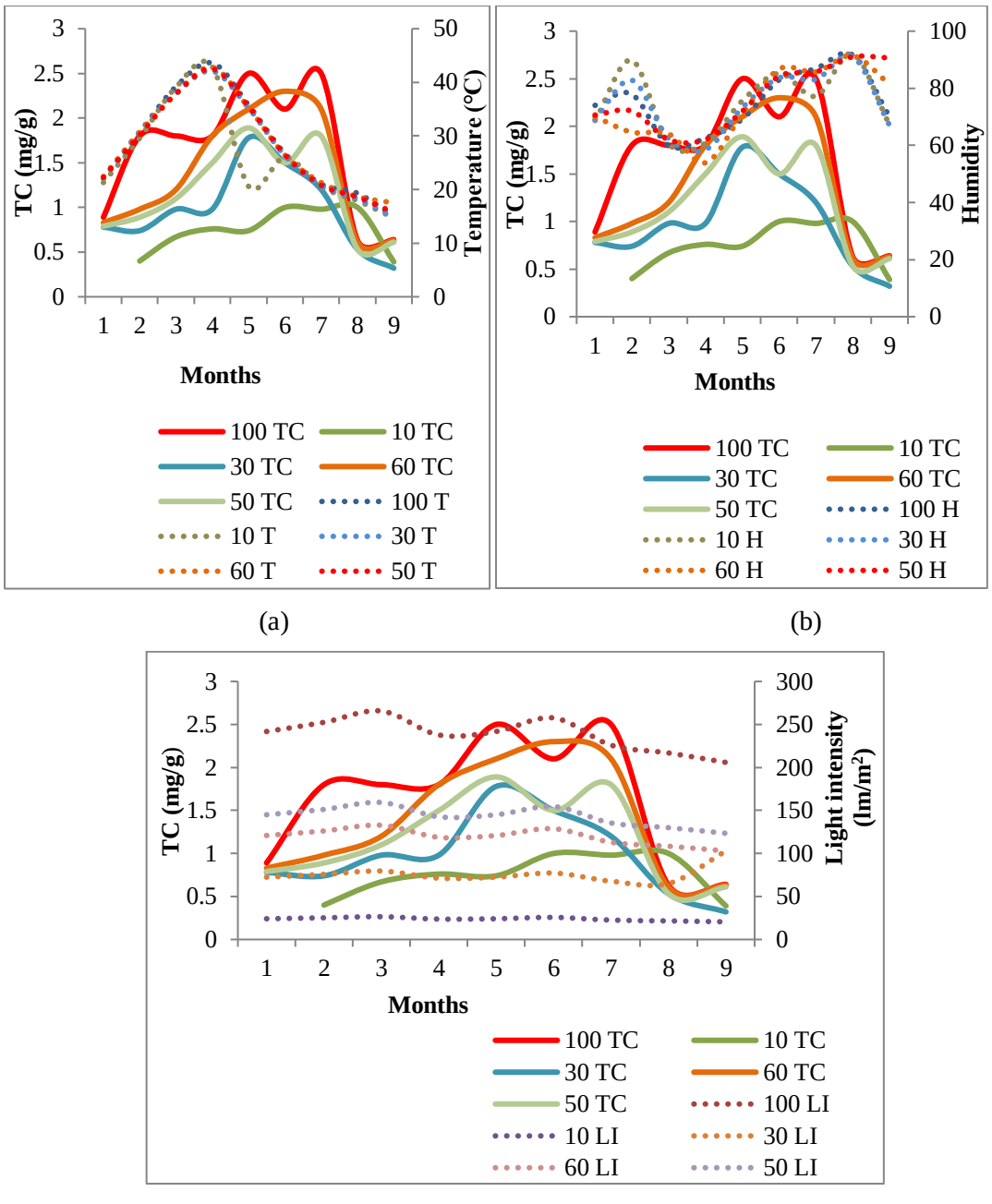


Figure 5.1 Variation in the TC (mg/g) of grown *Ocimum sanctum* under different environmental factors (temperature, humidity and light intensity respectively)

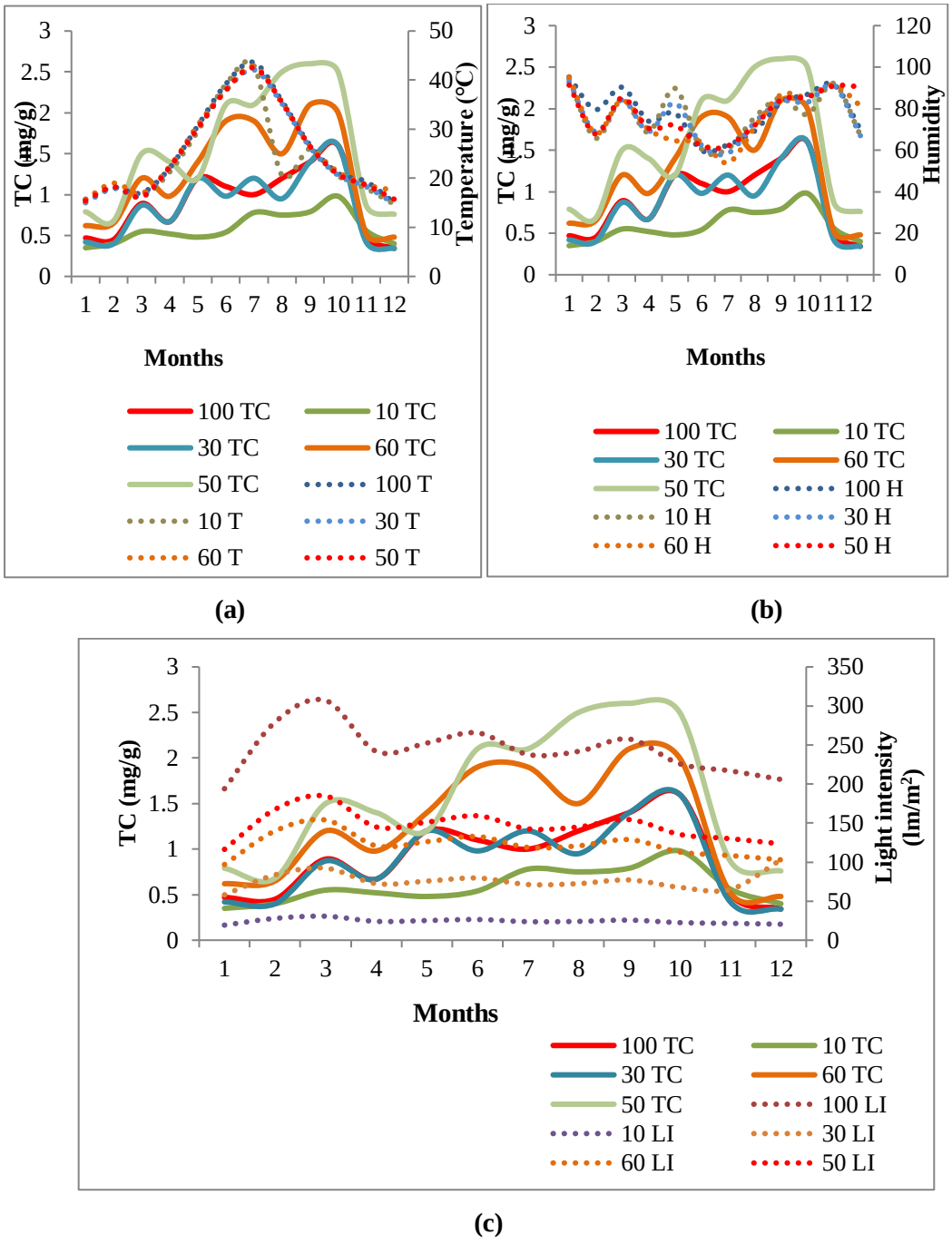


Figure 5.2 Variation in the TC (mg/g) of grown *Mentha piperita* under different environmental factors (temperature, humidity and light intensity respectively)

However, when considering all three environmental factors together, a markedly significant effect on TC (mg/g) was observed for both *Mentha piperita* and *Ocimum sanctum* (R^2_{nL}= 0.76 and R^2_{nL}= 0.7 respectively) at $p < 0.05$. The current study emphasize the significance of considering multiple environmental factors collectively rather than a focusing solely on individual parameters when assessing their impact on TC (mg/g) in plants. These findings

deepen understanding the complex relationship between environmental factors and biochemical parameters in plant.

5.4.2 Effect of Environmental factors (temperature, humidity and light intensity) on relative water content (%)

Various factors affect the water status of the plants including temperature difference, sunlight affecting the temperature differential between plants and the surrounding air, wind speed etc. Humidity is also considered a determinant factor for water loss in plants (Thut, 1938). As shown in the Figure (5.3a), RWC increases significantly with increase in temperature throughout the year. It has also been observed that RWC in *Mentha piperita* was found higher in the month of September and October under different exposure (90%, 70%, 50%, 40% and 100%) shade nets. The same pattern was found in *Ocimum sanctum* (Figure 5.4a). This may possibly be due to lower transpiration and evaporation rates at lower temperature allowing leaves to hold more water. The temperature during this period is considered comparatively moderate. Ghafari et al., (2021), Eslamdoust et al. (2023), and Kaur and Nagpal (2017) were also found higher RWC at lower temperature. Several researchers highlight the significance of higher relative Water Content (RWC) in maintaining physiological balance and enhancing stress tolerance in plants. However, current study highlighted the role of temperature and humidity on RWC of selected plants. Similarly, Zhang et al. (2016) emphasized the role of temperature and humidity as major factors influencing RWC preservation in plant leaves.

They studied that lower temperatures and higher humidity levels contribute to higher RWC in common plants, which is also consistent with our current findings for *Mentha piperita* and *Ocimum sanctum*. The resistance processes observed in plants are predominantly attributed to physiological and biochemical mechanisms, enabling them to withstand the impacts of temperature variations (Nievola et al., 2017). Higher temperature increases the metabolic activity that may lead to increased water uptake by the roots and subsequent transportation to the leaves. It is certainly reported that leaf water status interacts with stomatal conductance and Transpiration (Jackson, 2000; Larkindale et al., 2005; Damour et al., 2010; Rawson and Begg 1977). As temperature increases, the rate of transpiration typically increases due to increased evaporation from the leaf surface. It causes stomata to open wider, facilitating more water loss from the leaves, potentially cause low water content in leaves (Damour et al., 2010; Medrano et al., 2002; Moore et al., 2021). The rate of evaporation at higher temperature is greater not only from the leaf surface but also from the surrounding soil which creates a larger

gradient for the movement of water from the roots to the leaves, allowing the plant to take up more water (Pallas et al., 1967).

Thus, in the current study transpiration and evaporation can be considered at occur moderate rates at moderate temperature due to which leaves of both the species had hold higher water content (Dastur et al., 1933). Generally, under temperature stress plants usually show reduction in photosynthesis due to which stomata get closed and CO_2 assimilation is also limited (Hou et al., 2016; Donald and Paulsen, 1997; Roden and Ball, 1996). Since photosynthesis indirectly affects the water content of the leaf (Hilosaka et al., 2006). This could be another reason that the rate of photosynthesis is optimum at optimum temperature thus reducing water loss. Additionally, the leaves have a high water content to prevent additional loss. It is well explained in the previous literature that high temperature reduces the stomatal conductance and affects the plant's water usage efficiency and potentially its overall water content (Grantz, 1990). Besides temperature high humidity also reduce stomatal conductance that may limit the availability of carbon dioxide (CO_2) for photosynthesis. Thus, it affects the plant's water usage efficiency and potentially its overall water content (Yarwood and Hazen, 1944; Grantz, 1990; Pallas et al., 1966). In the present study, RWC has been found higher in both (*Mentha piperita* and *Ocimum sanctum*) the plant species at higher humidity.

It may be because high humidity reduced the gradient for water vapor and slowing down the transpiration and potentially preserves the water content in the leaves. Another reason could be high humidity increases the transpiration rate which signals the plant and cause stomatal closure. As a response, plant reduces the water loss from the leaves and maintains high water content (Thut, 1938; Kaiser 1987). However, the Present findings are inconsistent with Arve et al., (2013) in which they reported that higher humidity results in greater water loss. Besides temperature, humidity, light intensity also influences the water content in leaves, primarily through its effects on photosynthesis and transpiration. Since, higher light intensity leads to higher rates of photosynthesis, as more energy is available for the plant to drive this process. During photosynthesis water is drawn up from the roots to the leaves. Therefore, higher light intensity can increase the demand for water uptake by the plant, potentially leading to higher water content in the leaves. Light intensity is often positively correlated with temperature as both tend to increase with greater solar radiation. Higher light intensity generally leads to higher rates of transpiration because it increases the temperature of the leaf surface and stimulates stomatal opening (Pallas et al., 1967).Higher temperature can increase the rate of transpiration and metabolic activity within plant cells potentially influencing water content in leaves. This increased transpiration can result in greater water loss from the leaves and

potentially lower water content (Jackson, 2000; Damour et al., 2010). These observations were also in line with the results of present study that at low light intensities higher water content has been observed in the leaves of *Mentha piperita* and *Ocimum sanctum.* However, plants can regulate stomatal aperture in response to light intensity to optimize water use efficiency. For example, some plants may partially close their stomata under high light intensity to reduce water loss while still allowing for sufficient CO_2. Sahu et al., 2020 reported that fluctuations in environmental conditions like temperature, water content, humidity, and soil acidification may alter the plants tolerance (Sahu et al., 2020). Pearson correlation analysis (as shown in Table 5.11 and 5.12) was performed to assess the correlation between the biochemical parameter (RWC) with environmental factors (temperature, humidity and light intensity). A weak correlation at $p < 0.005$ level was observed between RWC (%) of *Mentha piperita* with temperature ($R_p = 0.19$) and other side weakest correlation was found between humidity and light intensity ($R_p = -0.06$ and $R_p = 0.07$ respectively). Similarly, RWC (%) of *Ocimum sanctum* has also exhibited insignificant correlation with temperature, light intensity and humidity ($R_p = -0.01$, $R_p = 0.002$ and $R_p = 0.07$ respectively). A low R_p values indicates the weak and insignificant relationship between the environmental factors and biochemical parameter (RWC).

However, multiple linear analyses was used and the results obtained from multiple linear regressions also revealed the insignificant relationship between RWC (%) (Dependent variable) with temperature, humidity and light intensity (independent variables) for both the plant species. The Regression coefficients were examined for both plants to evaluate the impact of individual environmental factors on RWC (%) has been observed <0.01. However, when considering the combined effect of environmental factors namely temperature, humidity and light intensity on RWC (%)*Mentha piperita* and *Ocimum sanctum*, an minimal impact was observed ($R_L = 0.52$ and $R_L = 0.41$respectively). Non linear regression method was employed to further explicate the effects of temperature, humidity and light intensity on RWC (%). Interestingly, the individual effects of each environmental factor (humidity, light intensity and temperature) on RWC (%) was also found minimal <0.01. However, when considering all three environmental factors together, a markedly significant effect on RWC (%) was observed for both *Mentha piperita* and *Ocimum sanctum* ($R^2_{nL}= 0.42$ and $R^2_{nL}= 0.56$ respectively) at $p < 0.05$.

The study underscores the significance of considering multiple environmental factors collectively rather than a individual parameter when assessing their impact on RWC (%) in plants.

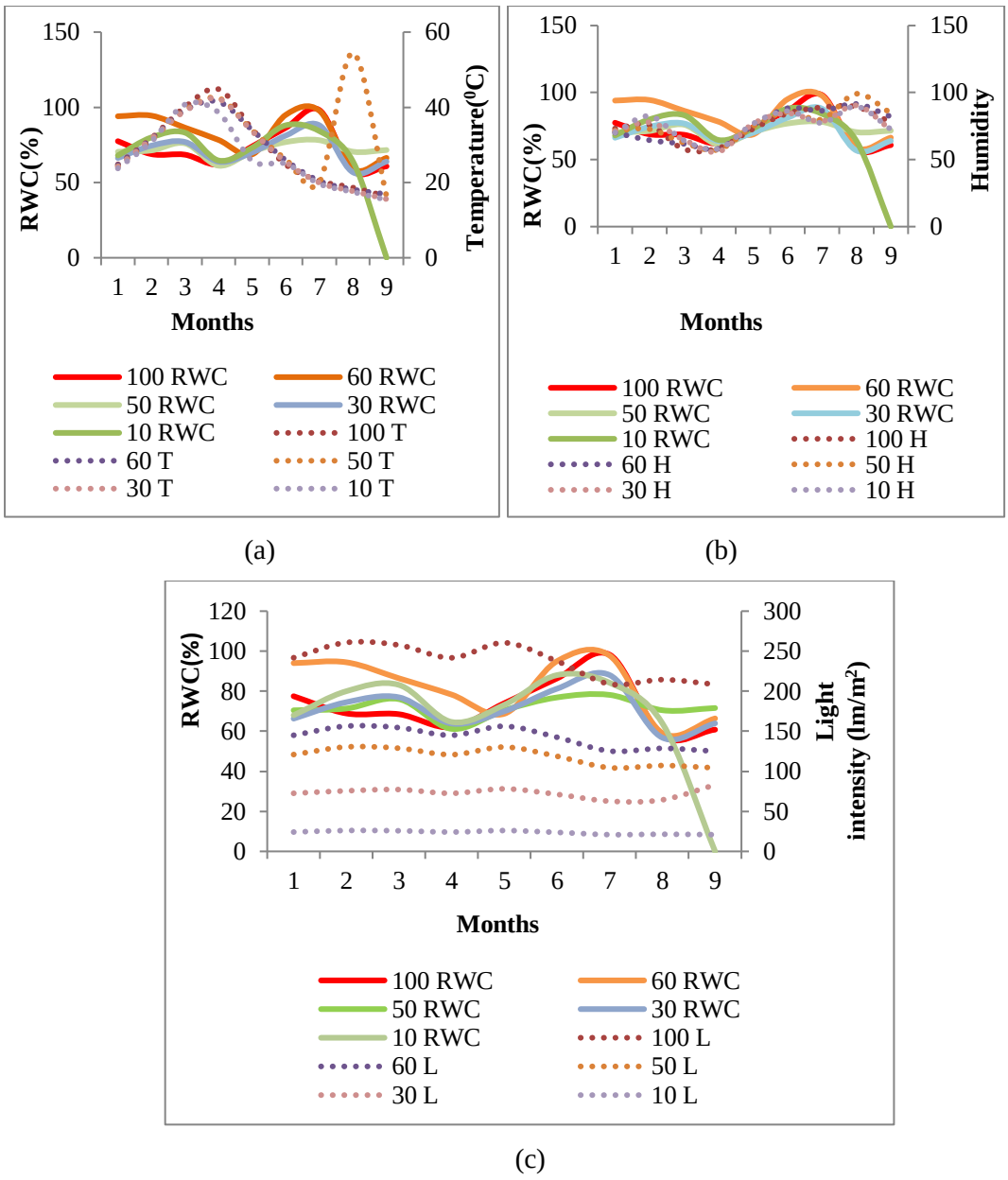


Figure 5.3 Variation in the RWC of grown *Ocimum sanctum* under different environmental factors (temperature, humidity and light intensity respectively).

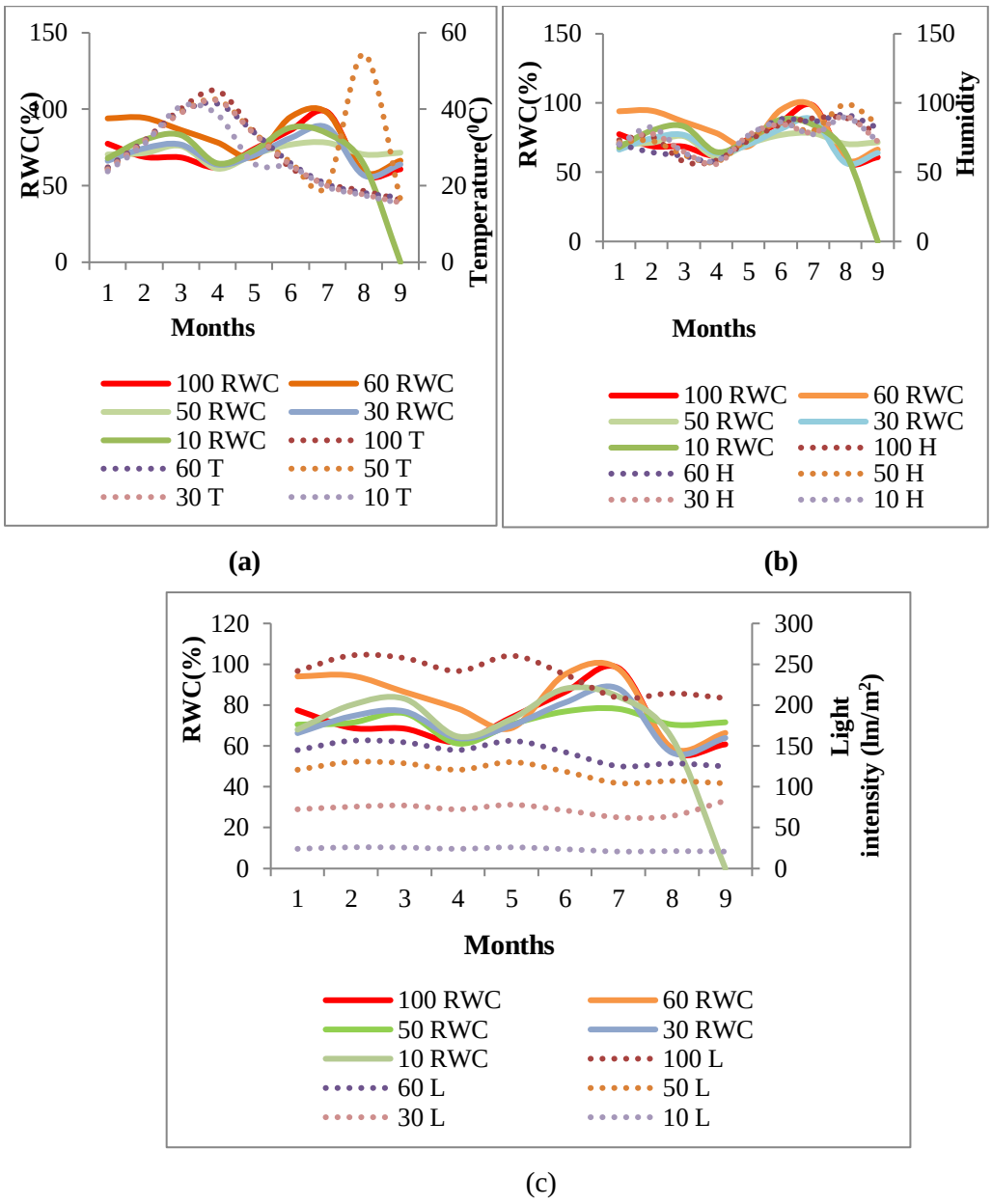


Figure 5.4 Variation in the RWC of grown *Mentha piperita* under different environmental factors (temperature, humidity and light intensity respectively)

The current study emphasize the significance of considering multiple environmental factors collectively rather than a focusing solely on individual parameters when assessing their impact on RWC (%) in plants.

5.4.3 Effect of Environmental factors (temperature, humidity and light intensity) on pH

As shown in the Figure 5.5 and 5.6, pH of the both the plant species (*Mentha piperita* and *Ocimum sanctum*) is not showing any specific trend. However it was observed that maximum pH has been showing at lower temperature and higher humidity and light intensity. Since temperature varies across the different shade nets (90%, 70%, 50%, 40%, and 100%). Generally, high temperature was recorded in the range of 32^0C to 42.9^0C. Lower pH was recorded at higher temperature and lower humidity and light intensity. Low temperature was recorded 15^0C to 20^0C. Both the species generally, exhibiting pH 7 and above 7 throughout the year. Temperature and pH are physicochemical parameters and relationship between them may be limited. Studies provide deep insights into temperature affects pH may be limited. However, temperature affects pH indirectly. High temperature affects pH indirectly by inducing oxidative stress (Djanaguiraman et al., 2010). The temperature affects the anti oxidative activity and polyphenols in plants (Akowuah et al., 2010; Larrauri et al., 1997). However, the anti oxidative property evidently associated with phenol levels which significantly higher in acidic pH and lower in alkaline pH. The pH value significantly affected accumulation of total Phenolics (Radic et al., 2016; Bayliak et al., 2016). This is not consistent with the current findings. In the current study, pH does not show any direct or indirect relationship with environmental factors. It was observed leaf extract pH is unaffected at different temperature, humidity and Light intensity. Some other parameters such as soil conditions, leaves morphological parameters may account for the deviation in the present study results.

Pearson correlation analysis (as shown in Table 5.11 and 5.12) was performed to assess the correlation between biochemical parameter (pH) and environmental factors (temperature, humidity and light intensity). A weak positive correlation at $p < 0.005$ level was observed between pH of *Mentha piperita* with temperature ($R_p = 0.02$), light intensity ($R_p = 0.14$) and humidity ($R_p = -0.01$). Similarly, *Ocimum sanctum* has also exhibited a weak negative correlation of pH with temperature ($R_p = -0.16$) humidity ($R_p = -0.02$) and light intensity ($R_p = -0.23$). Low Rp values indicate that environmental factors have weak correlation with pH, which is consistent with previous literature. However, the results obtained from multiple linear regressions also revealed the insignificant relationship between the pH (dependent variable) with temperature, humidity and light intensity (independent variables). The influence of

temperature, light intensity and humidity on pH was predicted with great significant p values (< 0.05). The Regression coefficients were examined for both plants to evaluate the impact of individual environmental factors on pH has been observed <0.01. However, when considering the combined effect of environmental factors on pH of *Mentha piperita* and *Ocimum sanctum*, an insignificant results was observed (R_L = 0.01 and R_L = 0.11).

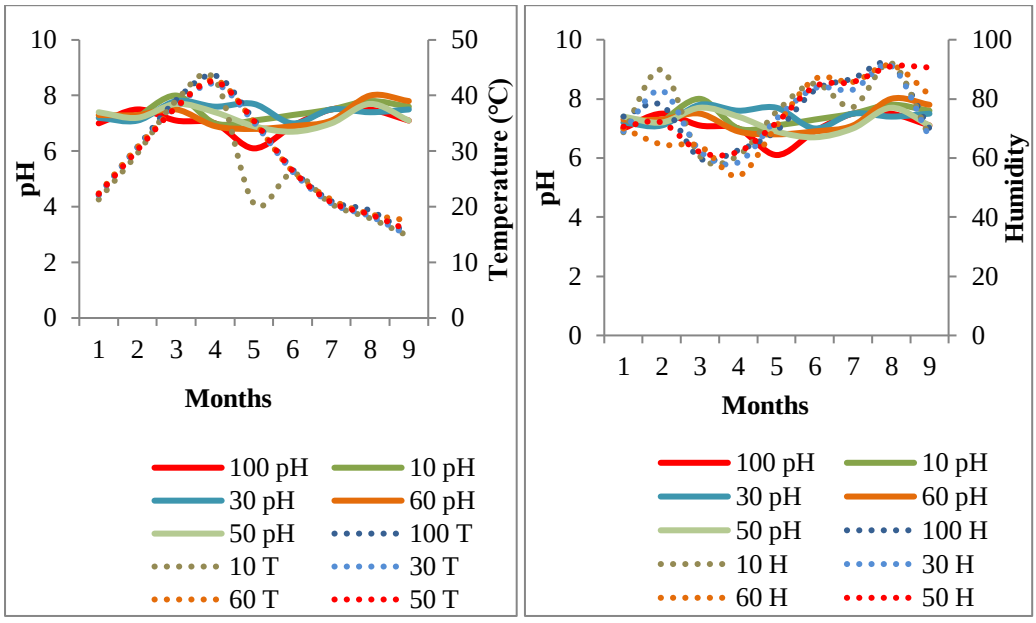
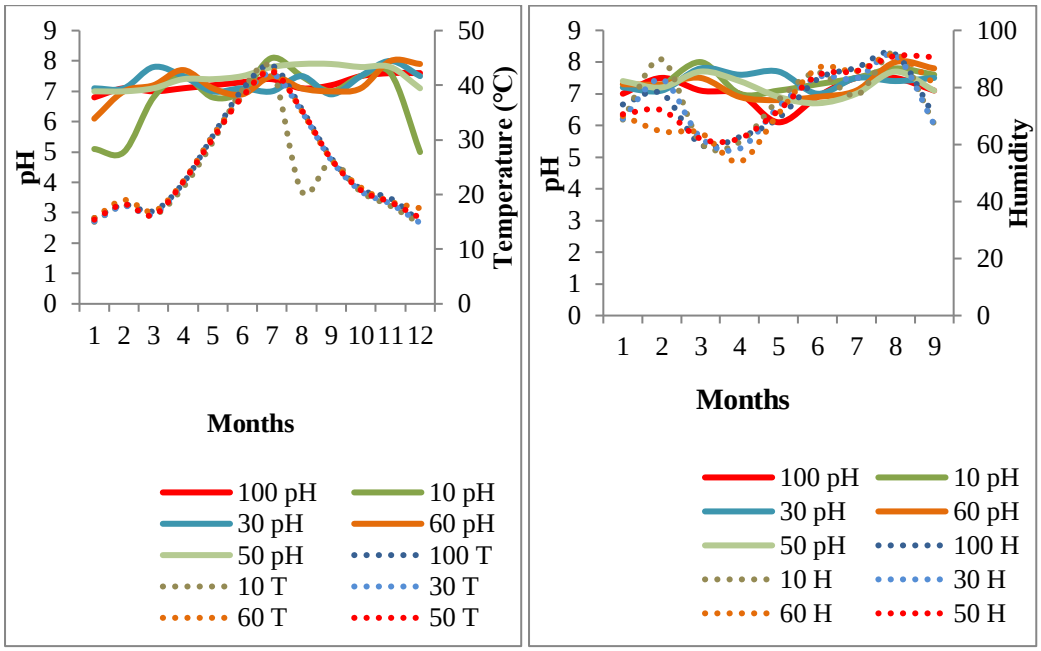


Figure 5.5 Variation in the pH of grown *Ocimum sanctum* under different environmental factors (temperature, humidity and light intensity respectively)

Non linear regression method was employed to further explicate the effects of temperature, humidity and light intensity on pH. Similarly, the individual effects of each environmental

factor (light intensity, temperature and humidity) on pH, was also found less than <0.01. However, when considering all three environmental factors together, a markedly significant effect on pH of *Ocimum sanctum* was observed (R^2 = 0.55) at p <0.05 and slightly weak in *Mentha piperita* (R^2_{nL} = 0.41) was found. The current study suggested that various other factors influence pH more strongly rather than environmental factors.



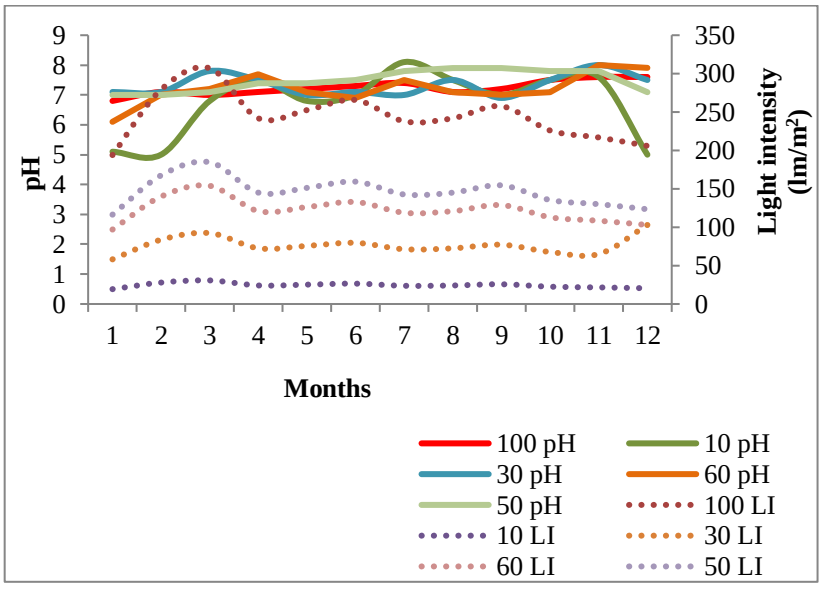


Figure 5.6 Variation in the pH of grown *Mentha piperita* under different environmental factors (temperature, humidity and light intensity respectively)

5.4.4 Effect of Environmental factors (temperature, humidity and light intensity) on ascorbic acid (mg/g)

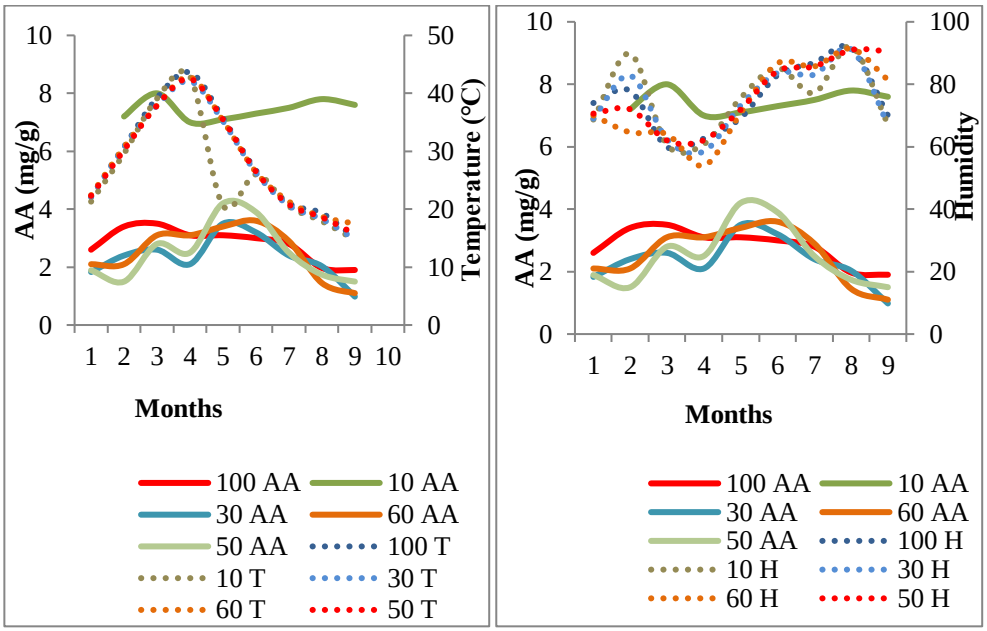
Ascorbic acid is strongly influenced by temperature (Schonhof et al., 2007). As shown in the Figures 5.7 and 5.8, the higher ascorbic acid was found in the February and March month for *Mentha piperita*. The temperatures during these months were recorded low with moderate humidity and light intensity. Since, *Ocimum sanctum* does not grow during December to March. The amount of Ascorbic acid is highest in the month of August and September including May and July. The temperature, humidity and light intensity recorded during these months was moderate and high. Schonhof et al. (2007) discovered in their research that lower temperatures combined with moderate light intensity led to the accumulation of ascorbic acid in broccoli heads. Their findings emphasized the predominant influence of temperature on ascorbic acid levels. This aforementioned finding is inconsistent with the current findings. *Mentha piperita* exhibited higher ascorbic content during lower temperature. The increase in ascorbic acid content at lower temperatures indicated that the plant was experiencing stress conditions. Schonhof et al., 2007 also studied the same. Smirnoff (1995) noted that the impact of low temperatures on ascorbic acid levels seems to involve restricting the utilization of excitation energy in photosynthetic carbon dioxide fixation. Evers (1994) proposed that the rise in ascorbic acid levels at low temperatures is attributed to reduced carbohydrate metabolism. Additionally, some studies have associated the rise in ascorbic acid with radiation-induced stress (Eskling et al., 1998). These results from previous literature can be considered uncertainties for current study results. However, David et al. (2001) reported that ascorbic acid biosynthesis is not strictly dependent on light. According to previous literature, it (ascorbic acid) has relationship with environmental factors which is inconsistent with the current findings. *Ocimum sanctum* plant species has been shown to have higher ascorbic acid content under high temperatures which is dissimilar to *Mentha piperita*. This may be because the most favorable temperature for ascorbic acid synthesis varies in different types of plant. Rield et al., (1941) have also drawn similar conclusions. Similarly effect of humidity on ascorbic acid was not addressed properly in previous study. However, some authors reported and some did not address well. Humidity did not directly affect ascorbic acid levels (Albrecht et al., 1991; Patykowski et al., 2007).

The results of the two different species considered in current study are contradictory with the previous literature. Thus, *Mentha piperita* exhibited higher ascorbic acid content at moderate

humidity in contrast to *Ocimum sanctum* having high ascorbic at high humidity. It may be due to high humidity increased antioxidant enzyme activity which results in high ascorbic acid content in leaves. Similarly, Xu et al., (2020) studied high humidity increased antioxidant enzyme activity reducing oxidative stress in tomato plants. Herrera et al., (2014) observed increased ascorbic acid levels in response to high humidity, indicating stress tolerance.The results of the current study are consistent with the previous literature. Sunmonu et al., (2012) also reported higher humidity correlated with higher ascorbic level. However, specific impact on ascorbic acid levels was not addressed in the study. Also it has been observed that *Mentha piperita* exhibited high ascorbic acid under low and moderate light intensity. However, *Ocimum sanctum* exhibited high ascorbic acid at high light intensity. It may be because light influences antioxidative enzymes in plants such as ascorbate peroxidase (APX). So, higher light intensity increases APX activity in leaves and roots which results in the increase in ascorbic acid content. Similar conclusions have been drawn by Onwona-Agyeman, (2006). According to previous literature light intensity variations did not significantly affect ascorbic acid concentration in plants. Similarly conclusions have drawn by Hikosaka et al., (2013). While, other researchers, Verkerke et al., 2014 observed in their study that light intensity logarithmically enhances Vitamin C (ascorbic acid) concentration in tomato fruits. Akpan and Essien (2005), Bartole et al., 2006; Utasi et al., (2019) and Yabuta et al., (2007) also observed that higher light intensity results in increased ascorbic acid levels.

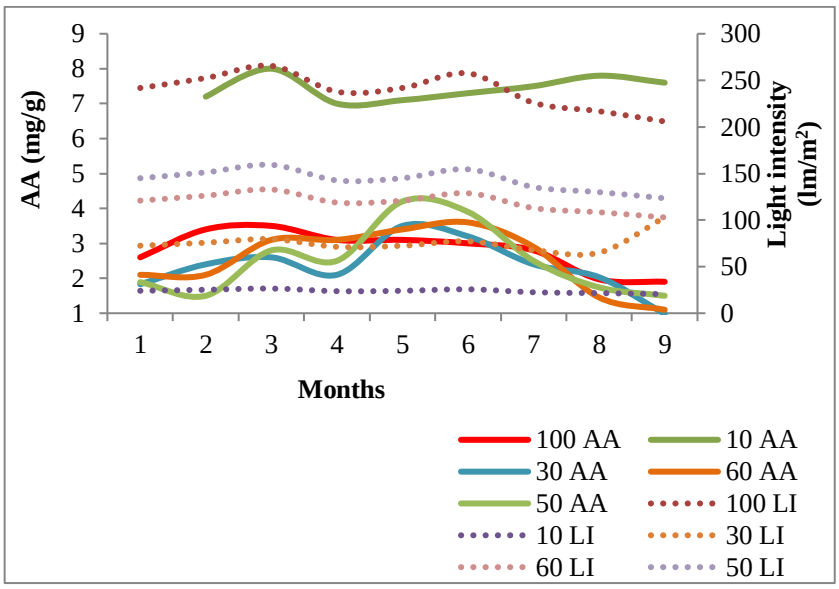
Pearson correlation analysis (as shown in Table 5.11 and 5.12) was performed to assess the correlation between biochemical parameter (AA) with environmental factors (temperature, humidity and light intensity). A weak positive correlation at $p < 0.005$ level was observed between AA (mg/g) of *Mentha piperita* with temperature ($R_p = 0.14$) and light intensity ($R_p = 0.14$) while weak negative correlation was found with humidity ($R_p = -0.22$). Similarly, *Ocimum sanctum* has also exhibited a positive correlation of AA (mg/g) with Temperature ($R_p = 0.48$) and Light intensity ($R_p = 0.11$) while weak negative correlation with humidity ($R_p = -0.01$). A low value of R_p suggests the minimal effect of individual environmental factor on AA (mg/g). The results obtained from multiple linear regressions also revealed the insignificant relationship between the AA (mg/g) (dependent variable) with temperature, humidity and light intensity (independent variables). The influence of temperature, light intensity and humidity on AA (mg/g) was predicted with great significant p values (< 0.05). The Regression coefficients were examined for both *Mentha piperita* and *Ocimum sanctum* to evaluate the impact of individual environmental factors on AA (mg/g) has been observed <0.01. However, when considering the combined effect of environmental factors namely temperature, humidity and light intensity on AA (mg/g), a notable insignificant impact was observed ($R_L = 0.07$ and

R_L = 0.38 respectively).Non linear regression method was employed to further explicate the effects of temperature, humidity and light intensity on AA (mg/g). The individual effects of each environmental factor (temperature, light intensity and humidity) on AA (mg/g), was also found insignificant and < 0.01.



(a)

(b)



(c)

Figure 5.7 Variation in the AA(mg/g) of grown *Ocimum sanctum* under different environmental factors (temperature, humidity and light intensity respectively).

The current study emphasize the significance of considering multiple environmental factors collectively rather than a focusing solely on individual parameters when assessing their impact

on AA (mg/g) in plants. However, when considering all three environmental factors together, a markedly significant effect on AA (mg/g) was observed for Ocimum sanctum (R^2_{nL}= 0.75) and slightly weak coefficients was observed for *Mentha piperita* (R^2_{nL}= 0.44) respectively) at p <0.05.

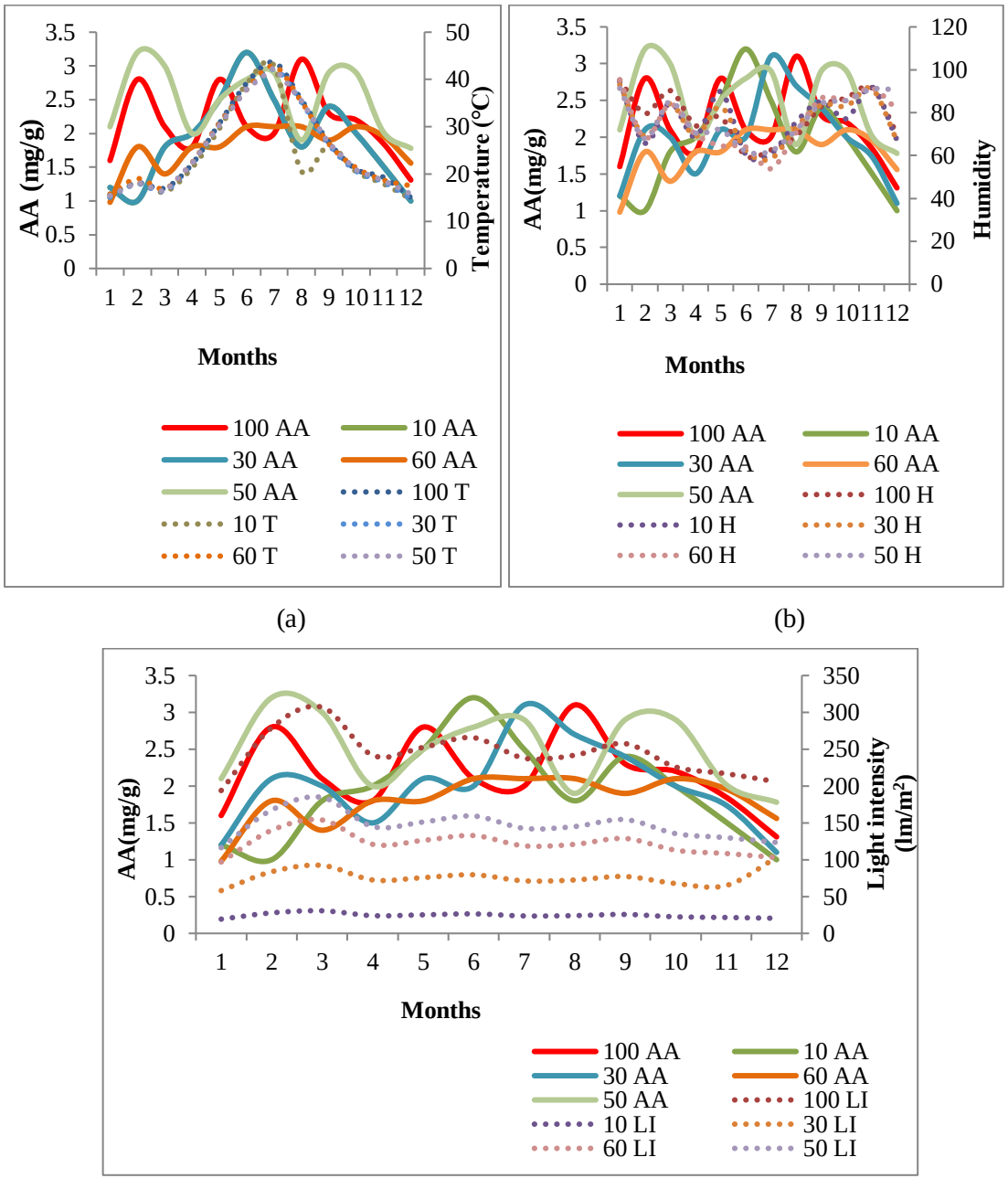


Figure 5.8 Variation in the AA(mg/g) of grown *Mentha piperita* under different environmental factors (temperature, humidity and light intensity respectively)

Table 5.11 Pearson correlation coefficients between biochemical parameters of *Mentha piperita* and environmental parameters.

	RWC	pH	TC	AA	T	H	LI
RWC	1						
pH	0.14	1					
TC	0.77	0.04	1				
AA	0.05	-0.25	0.12	1			
T	0.19	0.02	0.45	0.14	1		
H	-0.06	-0.01	-0.15	-0.22	-0.71	1	
LI	0.07	0.11	0.52	0.144	0.04	-0.01	1

Table 5.12 Pearson correlation coefficients between biochemical parameters of *Ocimum sanctum* leaves and environmental parameters.

	RWC	pH	AA	TC	H	LI	T
RWC	1						
pH	-0.32	1					
AA	0.62	-0.47	1				
TC	0.83	-0.49	0.74	1			
H	0.07	-0.02	-0.10	0.01	1		
LI	0.002	-0.23	0.19	0.51	-0.08	1	
T	-0.01	-0.16	0.48	0.30	-0.74	0.15	1

A general equation for 4th order polynomial with three independent variables

$f(x_1,x_2,x_3)=a_0+a_1x_3+a_2(x_3)^2+a_3(x_3)^3+a_4x_2+a_5x_2x_3+a6x_2(x_3)^2+a_7x_2(x_3)^3+a_8(x_2)^2+a_9(x_2)^2x_3+a_{10}(x_2)^2(x3)^2+a_{11}(x_2)^3+a_{12}(x_2)^3x_3+a_{13}x_1+a_{14}x_1x_3+a_{15}x_1(x_3)^2+a_{16}x_1(x_3)^3+a_{17}x_1x_2+a_{18}x_1x_2x_3+a_{19}x_1x_2(x_3)^2+a_{20}x_1(x_2)^2+a_{21}x_1(x_2)^2x_3+a_{22}x_1(x_2)^3+a_{23}(x_1)^2+a_{24}(x_1)^2x_3+a_{25}(x_1)^2(x_3)^2+a_{26}(x_1)^2x_2+a_{27}(x_1)^2x_2x_3+a_{28}(x_1)^2(x_2)^2+a_{29}(x_1)^3+a_{30}(x_1)^3x_3+a_{31}(x_1)^3x_2+a_{32}(x_1)^4+ a_{33}(x_2)^4+ a_{34}(x_3)^4.$

Where, $f(x_1,x_2,x_3)$ represent the polynomial

x_1 = Light intensity

x_2 = Temperature

x_3 = Humidity

Xabc = coefficients associated with the respective powers of x_1,x_2,x_3

Table 5.13 Multiple non linear regression coefficients associated with the respective powers of independent variables (Temperature, Humidity, Light intensity) for *Mentha piperita.*

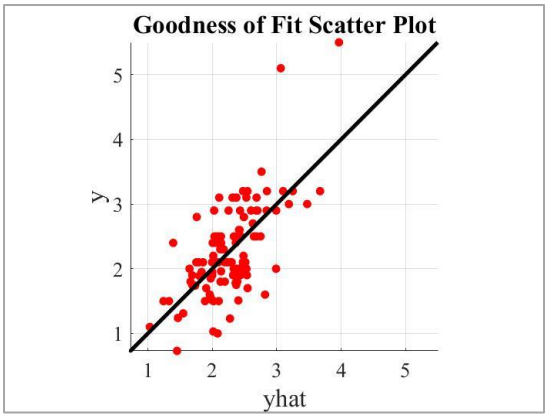
RWC		**pH**		**TC**		**AA**	
Coefficients	Values	Coefficients	Values	Coefficients	Values	Coefficients	Values
a_0	778.86	a_0	-32.29	a_0	-29.02	a_0	89.29
a_1	-10.61	a_1	0.45	a_1	0.38	a_1	-1.19
a_2	0.06	a_2	-0.00	a_2	-0.00	a_2	0.00
a_3	869.47	a_3	-35.65	a_3	-34.01	a_3	129.87
a_4	-24.26	a_4	1.00	a_4	0.98	a_4	-3.35
a_5	0.22	a_5	-0.00	a_5	-0.00	a_5	0.02
a_6	-0.00	a_6	2.45	a_6	2.73	a_6	-8.26
a_7	-12.33	a_7	0.62	a_7	0.54	a_7	-2.72
a_8	0.22	a_8	-0.01	a_8	-0.01	a_8	0.04
a_9	-0.00	a_9	5.49	a_9	4.95	a_9	-0.00
a_{10}	0.79	a_{10}	-0.00	a_{10}	-0.00	a_{10}	0.02
a_{11}	-0.00	a_{11}	5.10	a_{11}	5.26	a_{11}	-0.00
a_{12}	81.1	a_{12}	-1.29	a_{12}	-1.62	a_{12}	3.85
a_{13}	-2.42	a_{13}	0.04	a_{13}	0.05	a_{13}	-0.12
a_{14}	0.02	a_{14}	-0.00	a_{14}	-0.00	a_{14}	0.00
a_{15}	-7.74	a_{15}	1.28	a_{15}	2.29	a_{15}	-3.60
a_{16}	-2.19	a_{16}	0.03	a_{16}	0.01	a_{16}	-0.08
a_{17}	0.043	a_{17}	-0.00	a_{17}	-0.00	a_{17}	0.00
a_{18}	-0.00	a_{18}	4.63	a_{18}	3.59	a_{18}	-1.26
a_{19}	0.16	a_{19}	-0.00	a_{19}	0.00	a_{19}	-3.19
a_{20}	-0.0	a_{20}	5.24	a_{20}	-7.58	a_{20}	-7.86
a_{21}	-212	a_{21}	4.80	a_{21}	-2.33	a_{21}	5.75
a_{22}	-0.00	a_{22}	0.00	a_{22}	0.00	a_{22}	-0.00
a_{23}	0.00	a_{23}	-6.93	a_{23}	-2.56	a_{23}	3.02
a_{24}	-1.26	a_{24}	1.39	a_{24}	8.86	a_{24}	-2.08
a_{25}	0.00	a_{25}	4.96	a_{25}	-2.58	a_{25}	-5.90
a_{26}	-6.59	a_{26}	-3.39	a_{26}	2.33	a_{26}	-2.55
a_{27}	-1.21	a_{27}	-5.77	a_{27}	2.07	a_{27}	1.18
a_{28}	-4.87	a_{28}	-2.84	a_{28}	-5.94	a_{28}	-4.59

RWC		pH		TC		AA	
a_{29}	2.63	a_{29}	3.62	a_{29}	1.67	a_{29}	2.07
a_{30}	6.73	a_{30}	2.01	a_{30}	-5.42	a_{30}	4.55
a_{31}	2.90	a_{31}	-9.07	a_{31}	-1.18	a_{31}	2.82
a_{32}	-0.00	a_{32}	1.56	a_{32}	-8.04	a_{32}	-0.00
a_{33}	-0.00	a_{33}	7.44	a_{33}	3.852	a_{33}	-1.61
a_{34}	-21274.1	a_{34}	850.87	a_{34}	788.40	a_{34}	-2509

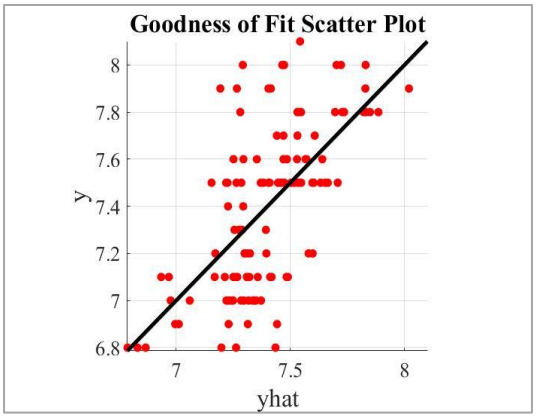
Table 5.14 Multiple non linear regression coefficients associated with the respective powers of independent variables (Temperature, Humidity, Light intensity) for *Ocimum sanctum.*

RWC		pH		TC		AA	
Coefficients	Values	Coefficients	Values	Coefficients	Values	Coefficients	Values
a_0	-1881.84	a_0	79.50	a_0	-32.89	a_0	90
a_1	16.22	a_1	-1.28	a_1	0.55	a_1	-1.47
a_2	-0.09	a_2	0.00	a_2	-0.00	a_2	0.01
a_3	-678.97	a_3	23.92	a_3	14.14	a_3	96.15
a_4	27.29	a_4	-1.17	a_4	0.08	a_4	-2.58
a_5	-0.31	a_5	0.01	a_5	-0.00	a_5	0.02
a_6	0.00	a_6	-5.90	a_6	2.13	a_6	-8.38
a_7	-0.70	a_7	0.45	a_7	-1.11	a_7	-2.03
a_8	-0.13	a_8	-0.00	a_8	0.01	a_8	0.030
a_9	0.00	a_9	-2.01	a_9	-3.57	a_9	-0.00
a_{10}	0.13	a_{10}	-0.00	a_{10}	0.01	a_{10}	0.02
a_{11}	0.00	a_{11}	6.91	a_{11}	-8.39	a_{11}	-0.00
a_{12}	-17.43	a_{12}	1.46	a_{12}	0.00	a_{12}	-0.59
a_{13}	0.60	a_{13}	-0.05	a_{13}	0.00	a_{13}	0.02
a_{14}	-0.00	a_{14}	0.00	a_{14}	-1.39	a_{14}	-0.00
a_{15}	3.39	a_{15}	-2.68	a_{15}	-1.33	a_{15}	1.06
a_{16}	0.17	a_{16}	-0.01	a_{16}	-0.00	a_{16}	-0.01
a_{17}	-0.00	a_{17}	0.00	a_{17}	0.00	a_{17}	0.00
a_{18}	3.91	a_{18}	-3.16	a_{18}	1.69	a_{18}	1.58
a_{19}	-0.00	a_{19}	-0.00	a_{19}	0.00	a_{19}	0.00
a_{20}	-6.56	a_{20}	-3.91	a_{20}	-4.43	a_{20}	-4.99
a_{21}	-1.60	a_{21}	3.17	a_{21}	-2.68	a_{21}	-2.56

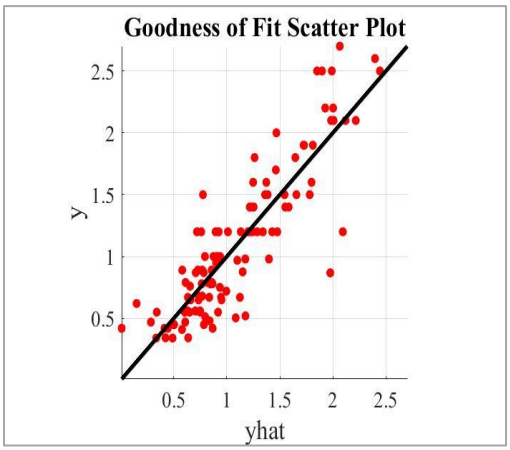
RWC		pH		TC		AA	
a_{22}	0.03	a_{22}	0.00	a_{22}	0.00	a_{22}	0.00
a_{23}	-8.80	a_{23}	1.47	a_{23}	-1.44	a_{23}	-1.95
a_{24}	-1.40	a_{24}	-4.03	a_{24}	6.68	a_{24}	1.01
a_{25}	-0.00	a_{25}	3.05	a_{25}	-5.99	a_{25}	-1.98
a_{26}	2.44	a_{26}	-3.64	a_{26}	4.17	a_{26}	1.49
a_{27}	6.45	a_{27}	-5.10	a_{27}	4.75	a_{27}	3.31
a_{28}	-9.69	a_{28}	-4.19	a_{28}	1.44	a_{28}	-9.26
a_{29}	7.70	a_{29}	3.72	a_{29}	-1.45	a_{29}	-1.42
a_{30}	1.07	a_{30}	6.45	a_{30}	1.27	a_{30}	-2.81
a_{31}	-5.78	a_{31}	-5.08	a_{31}	-1.55	a_{31}	2.90
a_{32}	-0.00	a_{32}	3.85	a_{32}	-0.00	a_{32}	-0.00
a_{33}	0.00	a_{33}	-2.24	a_{33}	9.49	a_{33}	-3.07
a_{34}	26225.47	a_{34}	-1740	a_{34}	629.36	a_{34}	-2169.3



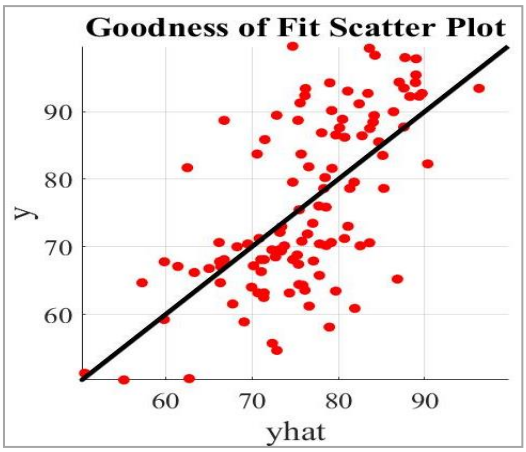
(a) Ascorbic acid (mg/g)



(b) pH

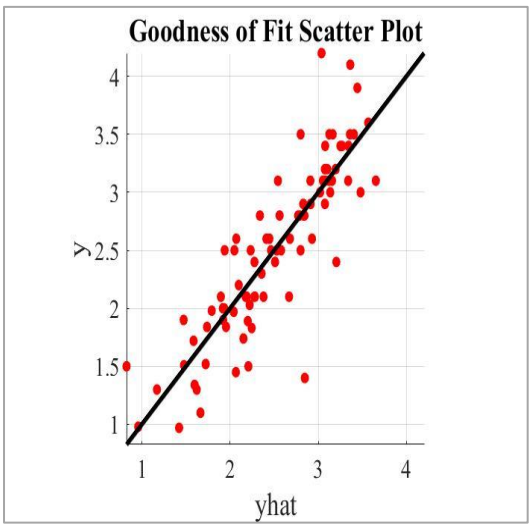


(c) TC (mg/g)

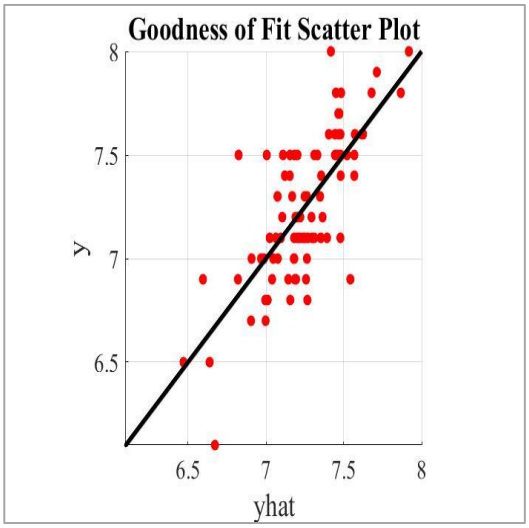


(d) RWC (%)

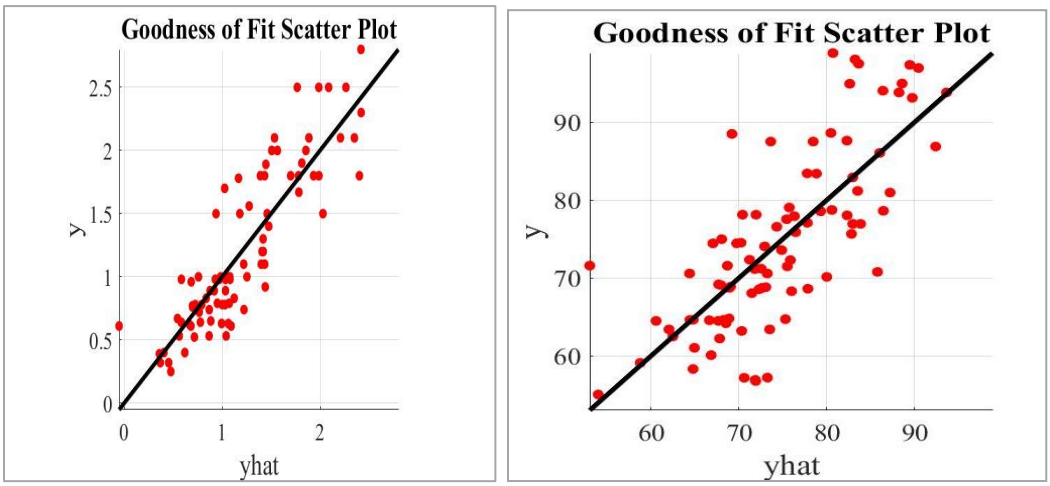
Figure 5.9 Measured (yhat) vs. predicted (y) values for biochemical parameters of *Mentha piperita*.



(a) Ascorbic acid (mg/g)

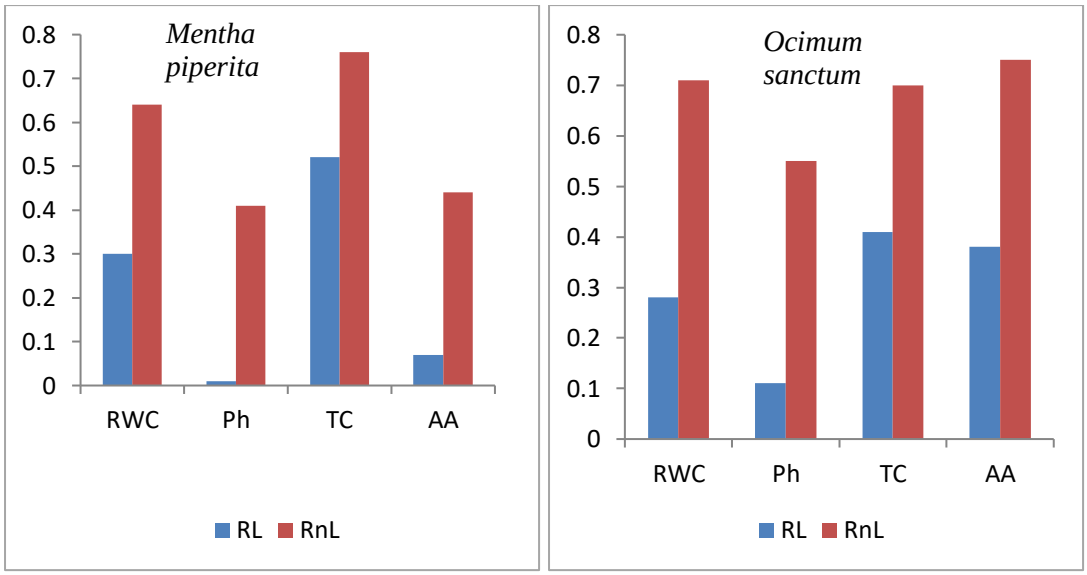


(b) pH



(c)Total chlorophyll (mg/g) (d) RWC(%)

Figure 5.10 Measured (yhat) vs. predicted (y) values for biochemical parameters of *Ocimum sanctum.*



(a) (b)

Figure 5.11 Comparison of Linear (R_L) and non linear regression coefficients (R_{nL})

CHAPTER 6 EFFECT OF SULPHUR DIOXIDE AND NITROGEN DIOXIDE ON BIOCHEMICAL PARAMETERS OF PLANTS

6.1 Background

Due to the rapid urbanization and economic growth in the developing nations, air quality has become a major focus of environmental policies (Khaniabadi et al. 2017). Among various air pollutants, SO_2 and NO_2 are most toxic to plants (Hamid and Jawid, 2009). Over the past few decades, emissions $SO_2$and NO_2 into the atmosphere have increased in many nations, particularly in some Asian nations. The concentrations of sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) emitted from the industries and vehicular emissions will continue to rise and surpass the standard levels of these pollutants (Sheng and Zhu 2019). It has been reported that, SO_2 was one of the earliest air pollutants to harm plants and the environment.

The amount of SO_2 in the air has significantly increased due to the combustion of fossil fuels (Wei et al. 2017). Until the 1970s, SO_2 was widely recognized as a significant cause of forest damage due to acid rain. On the other hand, when the Clean Air Act came into effect in the 1980s, it reduced SO_2 levels in the atmosphere, causing a sulphur (S) deficiency in crop plants (Bloem et al. 2015). High SO_2 concentrations may injure plants, prompting them to rapidly incorporate SO_2 and H_2S into low S pools like cystene and sulfates (Wei et al. 2017). Atmospheric NO_2 and SO_2 enter leaves through stomata and follow the same diffusion pathways as carbon dioxide (CO_2). After entering through the leaf stomata, SO_2 dissolves in the cells and oxidized to bisulphite (HSO^{3-}) and sulphite ions (SO_3^{2-}). SO_3^{2-} is highly toxic; chloroplasts convert small amounts of it into SO_4^{2-} (Hamid and Jawaid 2009; Rahul and Manish 2014).

The accumulation of $SO_3^{2-}$and SO_4^{2-} in high concentration causes SO_2 toxicity by inhibiting photosynthesis and energy metabolism (Bloem et al. 2015; Wei et al. 2017). As a result, plants experience lack of chlorophyll and necrosis on their leaf surfaces and in pine conifers there are chlorotic spots and red to brown tips and margins. Besides, it decreased leaf pH and disturbance of oxidation–reduction balancing in plant tissue that causes disruption of photosynthesis process at the enzymatic level of electron transportation and as a result in the decreased assimilation of CO_2 (Baciak et al. 2015).

Several oxides of nitrogen (NO_X), including NO_2, nitric oxide (NO), nitrous oxide (N_2O) and nitrogen trioxide (N_2O_3) may be present in the atmosphere. Plants absorb gaseous NO_2 more rapidly and NO_2 is known to be more toxic than other NO_X. NO_2 has a serious impact on plants either directly after being deposited on plants, soil or water, or indirectly through chemical reactions in the atmosphere (Haamid and Jawaid 2009). For instance, it dissolves in cells and produces nitrite ions (NO_2, which are toxic at high concentrations), cell acidification (which results in generation of reactive oxygen species) and nitrate ions (NO^{3-}). Large, erratic brown or black spots are the most obvious symptoms of NO_2. Besides, it reduces plant growth in high concentration and also inhibits photosynthesis (Haamid and Jawaid 2009; Sheng and Zhu 2019). However, NO_2 does not always act as pollutant. It also plays an important role in nutrient uptake, leaf area, higher growth, above ground biomass production, flower and fruit production (Oksanen and Soppela 2021).

Despite the fact that numerous policies, laws, and technological advancements have been made to deal with air pollution, the problem still persists (Wei et al. 2017). Due to an increase in sources, sustainable development efforts to mitigate air pollution have turned out to be a long term solution (Kour and Adak 2021). Thus, increasing the sink is the only way out of this situation to mitigate the toxic emission into the air (Ram et al. 2015).The use of ecological methods to reduce the concentration of air pollutants, especially the plant uptake of atmospheric NO_2 and SO_2, is more effective. Plants provide natural ways to reduce atmospheric pollution by absorbing gaseous pollutants (Sahu and Sahu 2015; Bharti et al. 2018).

The smooth/rough surfaces of plants are able to absorb pollutants or biodegrade pollutants into less or nontoxic molecules (Wei et al. 2017). It helps in reducing the air pollution but in the process they are also harmed by the constant exposure to air pollutants. Under polluted conditions, photochemical reactions like oxidation, reduction, reversible bleaching, and generation of reactive oxygen species (ROS) in the chloroplast reduce the chlorophyll content in plants (Karmakar et al. 2020; Eslamdoust et al., 2023). During stress conditions, the relative water content of the plants balances the water uptake (Yadav and Pandey 2020; Mahmood et al. 2023).

The presence of AA in plants prevents oxidative damage to thylakoid membranes and regulates cell division and growth under stressful conditions (Gupta et al. 2020). The measurement of biochemical parameters such as RWC, pH, AA and TC contribute in estimating the tolerance of plants against air pollution. This estimation is important for classifying plants as tolerant, intermediate and sensitive categories. However, tolerant plants can be used as urban planning management programs, as a sustainable method to reduce air pollution while sensitive plants

can be used as bio-indicators for air pollutants (Verma et al. 2022). In the present study, it has been assumed that studying the effect of SO_2 and NO_2 on biochemical parameters of the plants can provide useful information in selecting the right species of plants and developing a sustainable landscape management strategy. Therefore, in the present study, the effect of SO_2 and NO_2 on the biochemistry of plants has been studied.

6.2 Results and Discussion

The plant species under study displayed a significant variation in their biochemical parameters at all six locations (as shown in the Table 6.1 - 6.4 and Figures 6.1-6.6). The current study demonstrated that the differences in tolerance of different plant species to pollutant absorption are accounted for variations in their biochemical parameters. The Pearson correlation coefficient (r) has been calculated between independent variables (atmospheric concentration of SO_2 and NO_2) and dependent variable (RWC, pH, AA, and TC) to determine the degree of correlation between the variables (table 6.5 and table 6.6). Significance was tested at the 5% level of significance (i.e., $p = 0.05$).

6.2.1 Relative water content

The RWC is an important physiological parameter that is directly affected by air pollution (Ghafari et al. 2020). In Jalandhar, RWC was found to be highest in *Ficus religiosa* (96.6 %) followed by *Morus alba* (92.3%) and *Ficus benghalensis* (91.3%) and it was the lowest in *Melia azedarach* (56.6%) *and Psidium guajava* (64.4). In Amritsar, *Ficus religiosa* (91.7%) exhibited higher levels, followed by *Mangifera indica* (88.8%) and *Mentha piperita* (85.6%) and lower levels in *Polyalthia longifolia* (53.7%) and *Syzygium cumini* (68.1). In Ludhiana, *Ficus religiosa* (93.4%) had the highest RWC, followed by *Morus alba* (90.8%) and *Mangifera indica* (88.8%). It was at the lowest level in Z*iziphus mauritiana* (31.8%) and *Moringa oleifera* (31.8%) at the same site. In sector 22, higher RWC was found in *Ficus benghalensis* (98.3%) followed by *Murraya koenigii* (88.8%) and *Ficus religiosa* (85%) and it was lower in *Moringa oleifera* (78%) and *Melia azedarach* (68.4%). In sector 25, the highest RWC was observed in *Ficus benghalensis* (96.6%), followed by *Ficus religiosa* (95.6%) and *Syzygium cumini* (94.6) it was the lowest in *Cascabela thevetia* (70.2%).Similarly, in sector 53, *Syzygium cumini* (93.4%) has the highest RWC followed by *Ficus benghalensis* (92.3%) and *Ziziphus mauritiana* (91.4%.) and it was found to be the lowest in *Moringa oleifera* (72.1%).

Table 6.1 RWC of plants species from different locations of Punjab.

Plants	Jalandhar	Amritsar	Ludhiana	Sec 22	Sec 25	Sec 53
Ficus benghalensis	96.6	82.3	82.5	98.3	96.6	92.3
Ficus religiosa	90.3	91.7	93.4	85.4	95.6	90.3
Polyalthia longifolia	87.7	53.7	75.3	82.1	90.5	90.7
Mangifera indica	82.2	88.8	88.8	82.3	89.7	87.6
Alstonia scholaris	73.3	75.2	63.9	84.3	85.6	87.6
Moringa oleifera	72.2	69.2	31.8	78	90.7	72.1
Cascabela thevetia	63.6	69.1	54.8	82.9	70.2	90.6
Ocimum sanctum	69.2	73.2	59.3	80.5	82.3	85.4
Ziziphus mauritiana	61.4	70	31.8	81.9	82.3	91.4
Mentha piperita	68.3	85.6	50.4	79.6	78.6	89.1
Syzygium cumini	75.3	68.1	52.5	79	94.6	93.4
Murraya koenigii	75.2	70	53.5	88.8	72.5	75.5
Melia azedarach	56.6	69.1	78.5	68.4	93.4	84.9
Psidium guajava	64.4	71	73.4	79.3	78.4	83.4
Morus alba	92.3	70.4	90.8	79.8	91.5	81.5

High level of RWC in plants provides the balance needed for physiological activities under pollution and environmental stress (Karmakar et al. 2021; Eslamdoust et al. 2023). Besides, it makes the plant more tolerant to pollution-induced stress conditions (Karmakar et al. 2021). As shown in Table 6.1, Sector 22, Sector 53 and Sector 25 generally have the highest RWC among all the plants. This may be due to the low concentration of SO_2 at those sampling sites. Besides, it points out that RWC has an indirect relationship with SO_2.

Air pollutants increase cell permeability, dissolved nutrients, increasing the risk of early senescence (Sen et al. 2017). Variation in the leaf water content reflects the impact of air pollution and the sensitivity of the plant. Generally, it was noticed that most of the plant species exhibited maximum relative water content at Chandigarh sectors 22, 25 and 53. It may be because at those sites, SO_2 concentrations were lower. However, RWC (%) of all the studied species had significant negative correlation with SO_2 (r range: -0.5to-0.9) except *Ficus religiosa* (r = 0.35) (depicted in table 6.5). The RWC of *Ficus benghalensis* (r = -0.98) and *Ocimum sanctum* (r = -0.91) showed a strong negative correlation with SO_2 followed by *Mangifera indica* (r = -0.87), *Polyalthia longifolia* (r = -0.84) and *Syzygium cumini* (r = -0.85). The RWC

in *Morus alba* (r = -0.72) also showing a significant negative correlation, followed by *Psidium guajava* (r = -0.66) and *Mentha piperita* (r = -0.66), *Murraya koenigii* (r = -0.63) and Moringa oleifera (r = -0.6). The RWC of other plants such as *Alstonia scholaris* (r = -0.57), *Melia azedarach* (r = -0.54) and *Ziziphus mauritiana* (r = -0.53) have comparatively weaker correlation with SO_2, followed by *Yellow oleander* (r = -0.44). Thawale et al. (2010) also reported a negative correlation between SO_2 and RWC. It may be because higher concentration of SO_2 increases the stomatal aperture and decrease stomatal resistance, resulting in a subsequent reduction in the transpiration rate (Ashenden, 1979). It was observed that *Ficus religiosa* and *Ficus benghalensis* exhibited the highest RWC values among all species at all sites. This may be the result of larger leaf area which leads to higher transpiration rates under pollution stress. Thus, it leads to higher RWC level under air pollution stress.

Another possible cause of this phenomenon is the increase in the stomatal density of plant leaves when exposed to air pollution. Similarly, RWC did not have a very significant relationship with NO_2. The current study defies prior research that suggested a negative correlation between chlorophyll and NO_2 (Thawale et al. 2010). Ashenden (1979) has reviewed the plants responses to NO_2, but the concentrations required to exhibit the effects of NO_2 in plants were still not properly defined. In the later studies, it was stated that NO_2 can show ambiguous effects on plants (both toxic and beneficial) (Petitte and Ormod 1992; Siegwolf et al. 2001). The results of the present study are consistent with that of the aforementioned literature.

Table 6.2 pH of plants species from different locations of Punjab

Plants	Jalandhar	Amritsar	Ludhiana	Sec 22	Sec 25	Sec 53
Ficus benghalensis	6.8	6.9	6.8	7.1	7.5	6.8
Ficus religiosa	7.2	8.6	7.5	6.8	7.4	7.5
Polyalthia longifolia	7.2	7.4	7.5	7.1	6.8	7.6
Mangifera indica	5.9	7.4	5.1	7.5	7.1	7.1
Alstonia scholaris	6.1	6.3	7.6	7.3	6.8	7
Moringa oleifera	6.5	7.5	7.7	7	6.9	6.9
Cascabela thevetia	7.4	7.6	6.6	6.9	7.5	6.8
Ocimum sanctum	6.8	7.6	7.7	7.5	7.1	7.9
Ziziphus mauritiana	6.2	7	7	7.1	7	7.6
Mentha piperita	6.1	7.1	6.5	6.8	6.5	6.1
Syzygium cumini	5.03	6.9	6.5	6.5	6.9	6.8

Plants	Jalandhar	Amritsar	Ludhiana	Sec 22	Sec 25	Sec 53
Murraya koenigii	4.9	7.5	7.5	6.5	7.1	6.4
Melia azedarach	6.3	8	7.6	6.7	6.5	7.5
Psidium guajava	6.5	7.8	7.1	7.1	7.5	6.8
Morus alba	7.4	7.9	7.8	7.5	6.9	6.9

6.2.2 pH

Air pollutants adversely affect physiological processes in plants, including pH, and weaken their resistance to other stresses (Rai et al. 2011). The pH of the leaf samples varied in the range of 5-7.9. The samples generally had an acidic pH, as shown in table 6.2. In Jalandhar, the highest pH was observed in *Cascabela thevetia* (7.4) and *Morus alba* (7.4). In Amritsar, pH was found to be the highest in *Morus alba* (7.9) followed by *Psidium guajava* (7.8), *Cascabela thevetia* (7.6), *Ocimum sanctum* (7.6). In Ludhiana, the highest pH was found in *Morus alba* (7.8), followed by *Ocimum sanctum* (7.7), and *Moringa oleifera* (7.7). In sector 22, *Ocimum sanctum* (7.5), had the highest pH followed by *Morus alba* (7.5). In sector 25, the highest pH recorded in *Cascabela thevetia* (7.5) and *Psidium guajava* (7.5). In sector 53, the highest pH was observed in *Ocimum sanctum* (7.9) followed by *Ziziphus mauritiana* (7.6). In the current study, it has been observed that shrubs and herbs exhibited higher pH compared to the trees. A possible explanation of this trend is herbs and shrubs have limited growth and pollutant exposure compared to trees.

It may imply that, the higher the leaf pH, the better the ability of the plants to absorb SO_2 and NO_2 (Zou 2007; Singh et al. 1991). Another possible contributor is the leaf texture. The present study correlates high pH with rough leaf texture. Possibly, due to the rough texture, some plant leaves absorb a lower amount of pollutants compared to the plants with smooth leaf texture.

Table 6.3 AA (mg/g) of plants species from different locations of Punjab

Plants	Jalandhar	Amritsar	Ludhiana	Sec 22	Sec 25	Sec 53
Ficus benghalensis	2.1	1.8	2.3	2	2.1	2.1
Ficus religiosa	1.8	6.5	4.5	2.7	2.1	2.1
Polyalthia longifolia	4.5	4.5	5	2.5	1.8	1.8
Mangifera indica	6.9	4	3	2.1	2.1	2.1
Alstonia scholaris	1.5	4.6	6.5	2.5	1.5	1.5
Moringa oleifera	2.4	6	6	2.6	1.4	1.4
Cascabela thevetia	3.3	3.3	5.5	3.2	3.2	3.2
Ocimum sanctum	2.1	4.3	3	1.7	0.98	0.98

Plants	Jalandhar	Amritsar	Ludhiana	Sec 22	Sec 25	Sec 53
Ziziphus mauritiana	1.8	5	4.5	1.8	1.8	1.8
Mentha piperita	3.9	6	3.1	1.5	1.5	1.5
Syzygium cumini	3	5	5	2.4	2.1	2.1
Murraya koenigii	3.9	6	5	2.5	2.8	2.8
Melia azedarach	3.6	6	3.5	2.6	1.9	1.9
Psidium guajava	2.1	5.5	5.5	2.6	1.6	1.6
Morus alba	4.5	5	6	2.6	2.1	2.1

High pH is effective in increasing the productivity of the conversion of hexose to ascorbic acid (Zhen 2000; Escobedo et al. 2008). In general, most of the species at the sampling sites have an acidic pH. When plants are exposed to SO_2, large amounts of H^+ ions are produced in their intercellular fluid to react with SO_2 This H^+ may produce H_2SO_4 which lowers the pH (Ghafari et al., 2020; Karamakar et al., 2020). However, as shown in table 6.5, the present study, in general, demonstrated a significant correlation between pH and SO_2. The "r" values for pH vary from species to species. The pH of *Ficus benghalensis* leaf extract had a strong negative correlation with SO_2 (r = -0.72). *Morus alba* had the strongest positive correlation with SO_2 (r = 0.81) followed by *Ficus religiosa* (r = 0.66), *Melia azedarach* (r = 0.6) and *Polyalthia longifolia* (r = 0.6). The pH of other plant species was correlated with SO_2 but not significantly. For example, *Alstonia scholaris* (r = -0.36), *Mangifera indica* (r = -0.25), *Mentha piperita* (r = 0.35), *Murraya koenigii* (r = 0.007), *Ocimum sanctum* (r = 0.15), *Psidium guajava* (r = 0.13), *Syzygium cumini* (r = -0.21), *Yellow oleander* (r = 0.14) and *Ziziphus mauritiana* (r = -0.2). In contrast, as shown in table 6.6 pH of leaf extracts of the same plants was positively correlated with the NO_2.

The pH of *Ficus religiosa* (r = 0.8) and *Psidium guajava* (r = 0.83) had the strongest positive correlation with NO_2, followed by *Murraya koenigii* (r = 0.67) and *Syzygium cumini* (r = 0.67). A significant weak correlation was found between the pH of *Yellow oleander* and NO_2 (r = 0.51), followed by *Mentha piperita* (r = 0.51) and *Melia azedarach* (r = 0.58). Air pollutants SO_2 and NO_2 in the ambient air; shift the pH towards the acidic side, which means it reduces the pH. However, in the present study, both NO_2 and SO_2 have a positive correlation with pH. The present results are contradictory to the previous studies as conducted by Paulsamy and Senthilkumar (2009), Chandawat et al. (2011), Govindaraju et al. (2011), Leghari et al. (2011) and Karmakar et al. (2020).

Exposure to NO_2 causes nitrate and nitrite in plants to produce and consume H^+, leading to a decrease in ammonium uptake, and potentially a reduction in H^+ ions. This decrease in acidity in plants exposed to NO_2 may be associated with the reduction of the nitrate and nitrite produced from NO_2 (Qiao and Murray, 1997). Additionally, when plants are exposed to pollutants (particularly, SO_2 and NO_2), they have been shown to have increased stomatal sensitivity to pollutants and rapid closure of stomata in response to stress (Ghafari et al. 2020; Uka and Chukwuka 2014). Therefore, leaf extract pH can be suggested as an indicator of SO_2 and NO_2 pollution in the local atmosphere (Ghafari et al. 2020).

6.2.3 Ascorbic acid content

The Ascorbic acid is involved in the synthesis of cell walls, defensive system, cell divisions, and improves plant tolerance to air pollutants. The ascorbic acid content in the leaf samples varied from 0.9 to 6.9 mg/g as shown in Table 6.3. In Jalandhar, the highest AA was observed in *Mangifera indica* (6.9), followed by *Moringa oleifera* (4.5 mg/g), *Polyalthia longifolia* (4.5 mg/g). In Amritsar, *Ficus benghalensis* (6.9 mg/g) was found to have the highest AA, followed by *Mentha piperita* (6 mg/g), and *Murraya koenigii* (6 mg/g). In Ludhiana, *Alstonia scholaris* (6.5 mg/g) has the highest AA, followed by *Morus alba* (6 mg/g) and *Moringa oleifera* (6 mg/g). In sector 22, *Cascabela thevetia* (3.2 mg/g) has the highest AA, followed by *Ficus religiosa* (2.7 mg/g). In sector 25, the highest AA was recorded in *Cascabela thevetia* (3.2 mg/g) and *Murraya koenigii* (2.8 mg/g). In sector 53, the highest AA was observed in *Cascabela thevetia* (3.2 mg/g) and *Murraya koenigii* (2.8 mg/g).

Table 6.4 TC (mg/g) of plants species from different locations of Punjab

Plants	Jalandhar	Amritsar	Ludhiana	Sec 22	Sec 25	Sec 53
Ficus benghalensis	2.4	0.52	0.67	1.5	1.9	1.1
Ficus religiosa	0.65	0.68	0.22	0.98	1.6	1.5
Polyalthia longifolia	0.22	1.2	0.19	0.76	1.5	1.1
Mangifera indica	0.77	1.86	0.26	1.3	0.9	1
Alstonia scholaris	0.34	0.17	0.12	0.76	1.7	1.5
Moringa oleifera	0.22	0.64	0.37	1.5	1.5	1.7
Cascabela thevetia	0.12	0.64	0.37	0.26	1.6	1.7

Plants	Jalandhar	Amritsar	Ludhiana	Sec 22	Sec 25	Sec 53
Ocimum sanctum	0.21	0.1	0.57	1.9	1.8	2.1
Ziziphus mauritiana	0.13	0.1	0.19	2.1	2.1	0.98
Mentha piperita	0.16	0.61	0.78	1.9	1.9	2
Syzygium cumini	1.9	0.9	0.98	1.7	0.8	0.81
Murraya koenigii	0.27	0.64	0.11	0.23	0.7	1
Melia azedarach	0.18	0.48	0.15	1.6	1	1.5
Psidium guajava	0.21	0.19	0.16	2.1	0.9	0.76
Morus alba	0.33	2.1	0.9	1.8	0.7	0.89

High ascorbic acid indicates high resistance of the plants to pollution stress (Prajapati and Tripathi 2006). In the present study, Jalandhar, Amritsar, and Ludhiana sampling plant species induced an increase in AA content in almost all species and a decrease in AA content in plants sampled from Chandigarh (sectors 22, 25 and 53). So, plants species with higher leaf AA content at Jalandhar, Amritsar and Ludhiana can be considered pollution resistant due to the antioxidant properties of AA, while plant species with lower AA content can be considered pollution-sensitive.

Ghafari et al (2020) found that, out of 18 plant species, pollution led to a decrease in AA content in 16 plant species, and an increase in AA content was observed in only two plants. They considered the first 16 species as sensitive to pollution and the second two species as pollution resistant. Plant species growing in Sector 22, Sector 25, and Sector 53 displayed lower AA content in leaves compared to Jalandhar, Amritsar and Ludhiana. This may be due to the low concentrations of SO_2 at those study locations. In addition to that, current study has revealed that AA has a good positive correlation with SO_2 (Table 6.5). A strong positive correlation was observed in the AA of *Ocimum sanctum* (r = 0.93) and *Mentha piperita* (r = 0.93) followed by *Melia azedarach* (r = 0.89), *Polyalthia longifolia* (r = 0.84), *Murraya koenigii* (r = 0.83) and *Syzygium cumini* (r = 0.81), *Moringa oleifera* (r = 0.78), *Ziziphus mauritiana* (r = 0.76), *Psidium guajava* (r = 0.75), *Ficus religiosa* (r = 0.75) and *Morus alba* (r = 0.75). At higher concentrations of SO_2, a higher production of reactive oxygen species (ROS), such as SO_3^-, HSO_3^-, OH^-, O_2^- etc., occurs by absorbing SO_2 and photo oxidation of SO_3^- to SO_4^- and sulphites. These free radical productions under SO_2 exposure would increase the production of free radical scavengers such as ascorbic acid (Ninave et al. 2001; Rawal et al. 2001; Tripathi and Gautam 2006; Tripathi and Gautam 2007; Paulsamy and Senthilkumar 2009; Elawa et al. 2021). Therefore, higher ascorbic acid content in plants indicates greater tolerance to SO_2 pollution. Similar conclusions have also been drawn by Ghafari et al. 2020; Ghosh et al. 2021).

Atmospheric NO_2 can induce changes in plant growth and photosynthetic activity, leading to changes in antioxidant defense systems as well as oxidative damage (Chen et al. 2010; Takahashil et al. 2011; Li et al. 2007; Miao et al. 2008). This insight supports our results.

6.2.4 Total Chlorophyll

The measurement of chlorophyll is considered a very important tool to evaluate the effect of air pollutants on plants. Since, plant growth is directly proportional to the chlorophyll concentration of plants (Karmakar et al. 2021**).** As shown in the Table 6.4, *Ficus benghalensis* (2.4 mg/g) and *Syzygium cumini* (1.9 mg/g) exhibited the highest chlorophyll in Jalandhar. In Amritsar, *Morus alba* (2.1 mg/g) has the highest TC followed by *Polyalthia longifolia* (1.2mg/g). In Ludhiana, *Morus alba* (0.9 mg/g) was found to be with the highest TC followed by *Syzygium cumini* (0.9 mg/g *Psidium guajava* (2.1 mg/g) and *Ziziphus mauritiana* (2.1 mg/g) recorded the highest chlorophyll in sector 22. In sector 25, the highest chlorophyll was studied in *Psidium guajava* (2.1 mg/g) and *Ficus benghalensis* (1.9 mg/g). In sector 53, the highest chlorophyll was observed in *Ocimum sanctum* (2.1mg/g) and *Mentha piperita* (2 mg/g). Higher chlorophyll content in plants may indicate their tolerance and resistance to air pollution (Singh and Verma 2007). Zhang et al. (2016) also found plants with high TC are more tolerant to SO_2 pollution, and plants with low TC in their leaves are sensitive to SO_2. The results of the present study are consistent with that of the aforementioned literature.

It has been observed that chlorophyll had significant negative correlation with SO_2. *Ziziphus mauritiana* (r = -0.88), *Alstonia scholaris* (r = -0.85), *Ocimum sanctum* (r = -0.85) and *Mentha piperita* (r = -0.8) had the significant negative correlation with SO_2, followed by *Ficus religiosa* (r =-0.75), *Moringa oleifera* (r = -0.74), *Psidium guajava* (r = -0.64) and *Melia azedarach* (r = -0.63) and *Yellow oleander* (r = -0.55). Chlorophyll is the main attack site for air pollutants such as SPM, SO_2 and NO_2 (Tripathi and Gautam 2006; Paulsamy and Senthilkumar 2009; Priyanka and Dibyendu 2009; Kuddus et al. 2011).

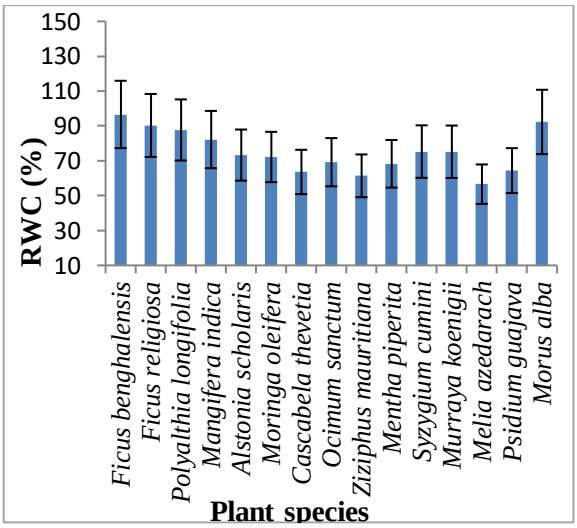
The reduction in chlorophyll content has often been suggested as an indicator of air pollution damage (Rawal et al. 2001; Leghari et al. 2011). On other hand, TC does not show a significant correlation with NO_2 (as shown in table 6.6). Kammerbauer and Dick (2000) studied that NO_2 absorption and nitrogen precursors increased the synthesis of photosynthetically active pigments (PAR) by 15 %. The increase in NO_2 absorption and nitrogen precursors increases chlorophyll content. Patidar et al. (2016) observed that reduction in TC was mainly associated with reactive oxygen species which damage chloroplasts. Muneer et al. (2014) studied high concentrations of CO, SO_2 and NO_2 reduce chlorophyll pigment.

Nitrate and nitrite, are used by the plant during the normal process of nitrate metabolism. But high concentrations of NO_2, result in excessive accumulations of nitrite and cell acidification which further produces reactive oxygen species, inhibiting both Nitrogen assimilation and plant growth. The effects of NO_2 exposure on plants remain highly controversial, and a unified conclusion has not been reached. This insight supports our present results.On the other side, as shown in Figures (6.3-6.8), the variation in biochemical parameters of the sampled species is due to disparities in their ability to tolerate stress conditions. In Jalandhar (Figure 6.3), the total chlorophyll content, ascorbic acid, leaf extract pH, and relative water content ranged from 0.12 to 2.4 mg/g, 1.5 to 6.9mg/g, 4.9 to 7.4 and 56.6 to 96.6% respectively. Highest RWC was observed in *Ficus benghalensis* (96.6 %), *Morus alba* (92.3%) and *Ficus religiosa* (90.3 %).
Low RWC content was observed in *Melia azedarach* (56.6%) followed by *Ziziphus mauritiana* (61.4%), and *Cascabela thevetia* (63.6%). *Murraya koenigii* depicted the lowest pH (4.9) among all plant species, followed by *Syzygium cumini* (5.03). The highest AA was observed in *Mangifera indica* (6.9 mg/g) followed by *Polyalthia longifolia* (4.5 mg/g) and *Morus alba* (4.5 mg/g). In Ludhiana (Figure 6.3), the total chlorophyll content, ascorbic acid, leaf extract pH and relative water content ranged from 0.1 to 0.9 mg/g, 2 to 6.5mg/g, 6 to 7.8 and 31.8 to 93.4% respectively. Highest RWC was observed in *Ficus religiosa* (93.4 %) followed by *Morus alba* (90.8%). The highest amount of AA content was observed in *Alstonia scholaris* (6.5 mg/g) followed by *Morus alba* (6mg/g). Highest pH was recorded in *Morus alba* (7.8). Thus, *Morus alba* can be considered as a tolerant species for the sampling site. In Amritsar (Figure 4), the total chlorophyll content, ascorbic acid, leaf extract pH and relative water content ranged from 0.1 to 2.1 mg/g, 1 to 6.5mg/g, 6 to 8.6 and 53.7% to 91.7% respectively. *Ficus religiosa* exhibited the highest leaf water content among all the 15 plant species in Amritsar (Figure 6.3a). Possibly, this led to the conclusion that *Ficus religiosa* can be considered as one of the plants that can maintain its physiological balance against air pollutants (especially SO_2 and NO_2). In Chandigarh Sector 22, (Figure 6.5), the total chlorophyll content, ascorbic acid, leaf extract pH, and relative water content ranged from 0.2 to 2.1 mg/g, 1 to 3.2mg/g, 6 to 7.5 and to 68% to 98.3% respectively. High RWC was observed in *Ficus benghalensis* (98.3%) followed by *Murraya koenigii* (88.8%). In Chandigarh Sector 25 (Figure 6.6), the total chlorophyll content, ascorbic acid, leaf extract pH, and relative water content ranged from 0.7 to 2.2 mg/g, 1 to 3.2 mg/g, 6 to 7.5 and 70% to 96.6% respectively. Apart from pH and RWC, AA content was also high in both *Ficus benghalensis* (2.1 mg/g) and *Ficus religiosa* (2.1 mg/g). These two plant species can be considered as tolerant species for Sector 25.
Similarly, in Chandigarh Sector 53, the total chlorophyll content, ascorbic acid, leaf extract pH, and relative water content ranged from 0.7 to 2.1 mg/g, 1 to 3.2mg/g, 6 to 7.9 and to 72% to

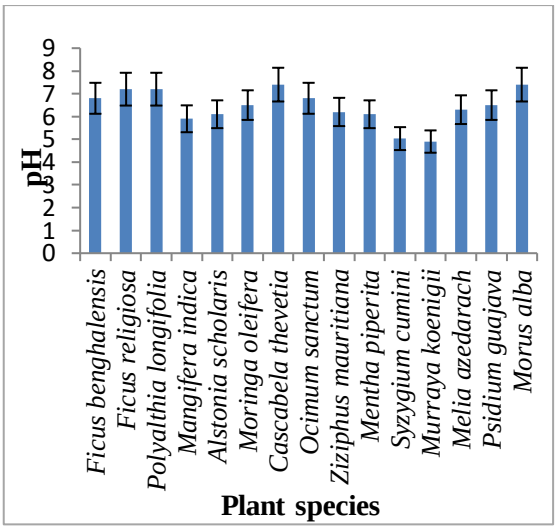
92.3% respectively.

Higher amounts of AA, pH, and RWC were found in *Ficus religiosa*. It can be considered as tolerant for Sector 53. Different plant species responds different. It is evident from tables 6.1-6.6 and Figure 6.3- 6.8 that, under field conditions, no species had maximum values for all four parameters. Each parameter plays a distinctive role in determining the tolerance and sensitivity of plants. Variation in biochemical parameters among the same plant species may be due to genetic differences, different pollutant concentrations in the ambient air, environmental parameters, or any morphological parameters.

It is somewhat difficult to study the role of multiple parameters in plant tolerance to air pollution at the same time. Thus, in the present study, variations in the biochemical parameters were studied under different air pollutant concentrations. Besides, a statistical approach has been used to better understand the relationship between air pollutants and biochemical parameters.



(a)



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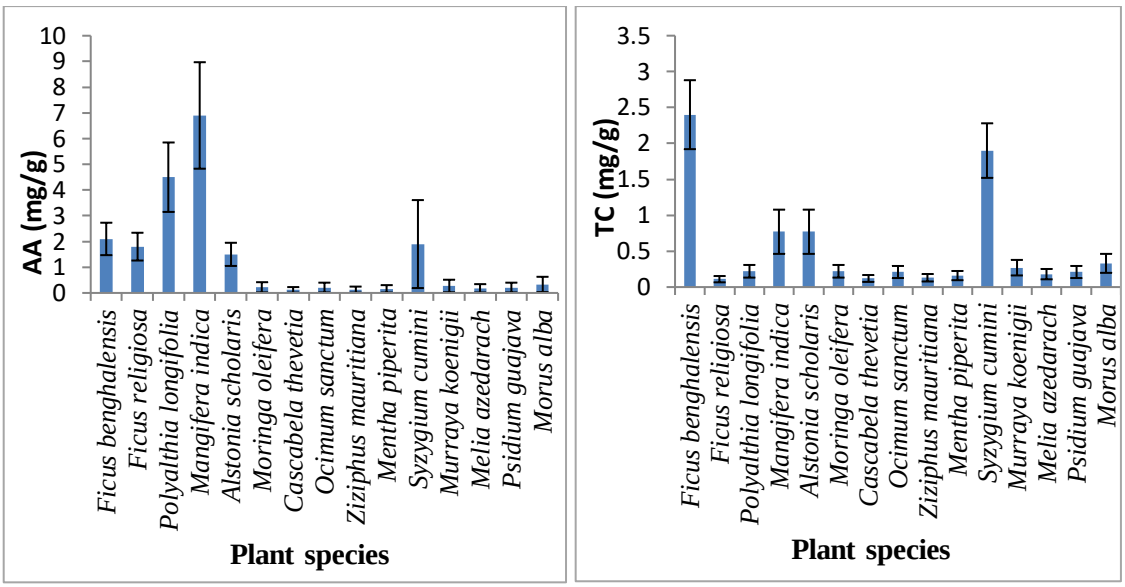
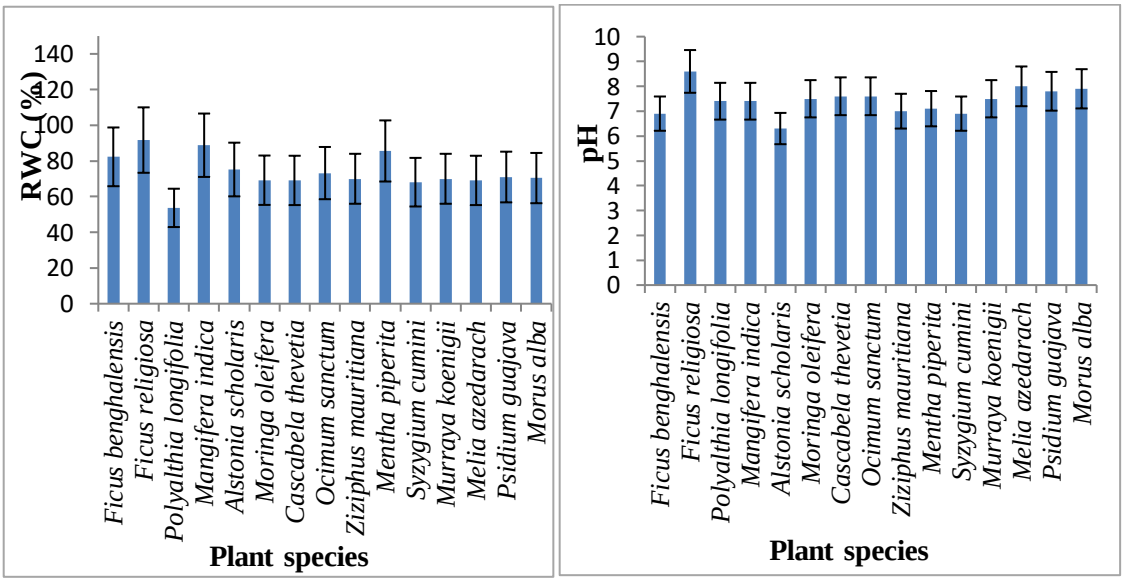


(c) (d)

Figure 6.1 Variation in biochemical parameters of plants species at SO_2 and NO_2 concentration of 14(µg/m^3) and 7 (µg/m^3) respectively in Jalandhar city.



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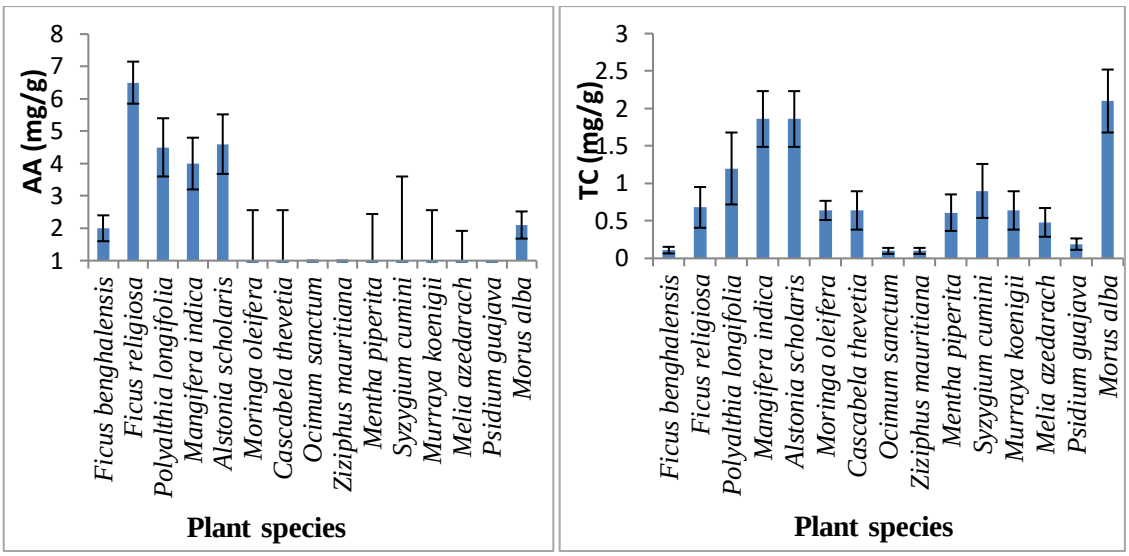
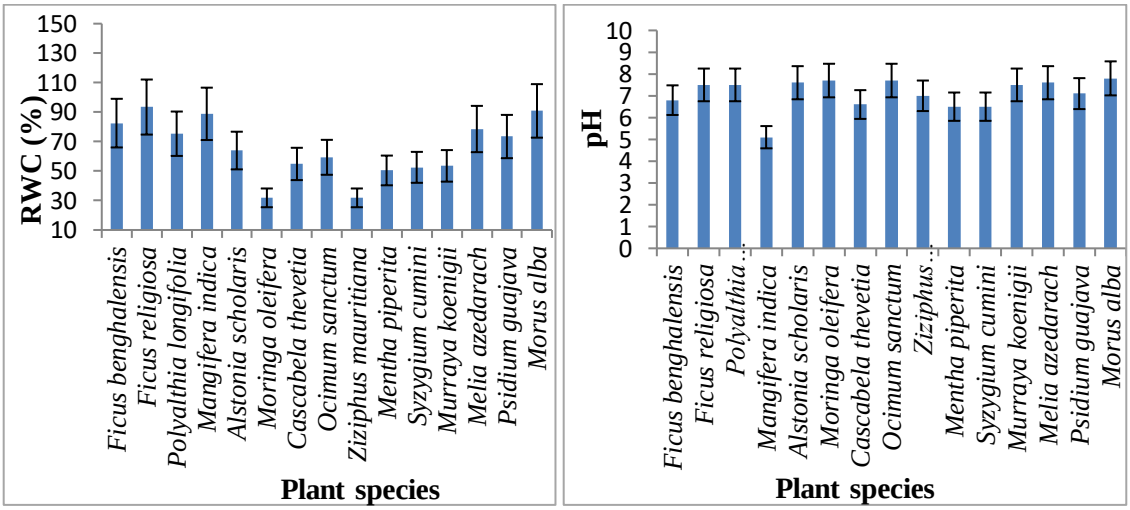


(c) (d)

Figure 6.2 Variation in biochemical parameters of plants species at SO_2 and NO_2 concentration of 20(µg/m^3) and 58 (µg/m3) respectively in Amritsar cities.



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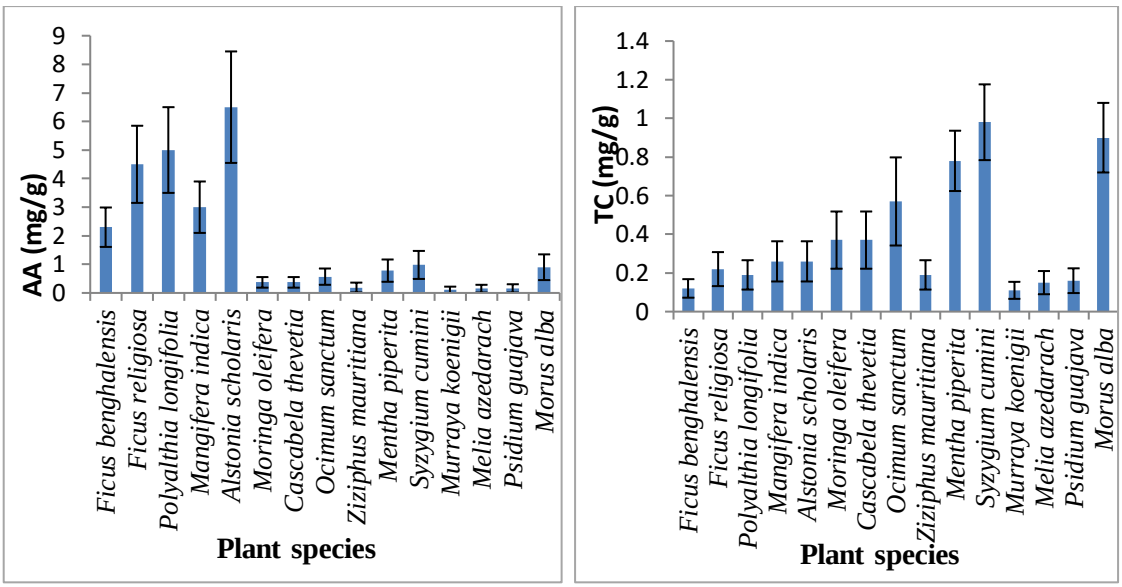
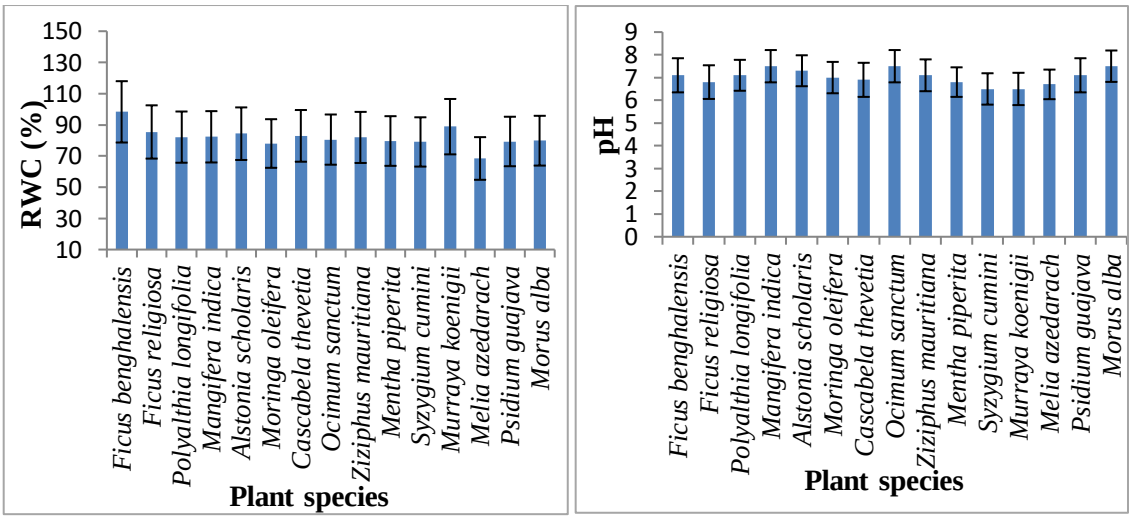


(c) (d)

Figure 6.3 Variation in biochemical parameters of plants species at SO_2 and NO_2 concentration of 14(µg/m^3) and 22 (µg/m^3) respectively in Ludhiana city.



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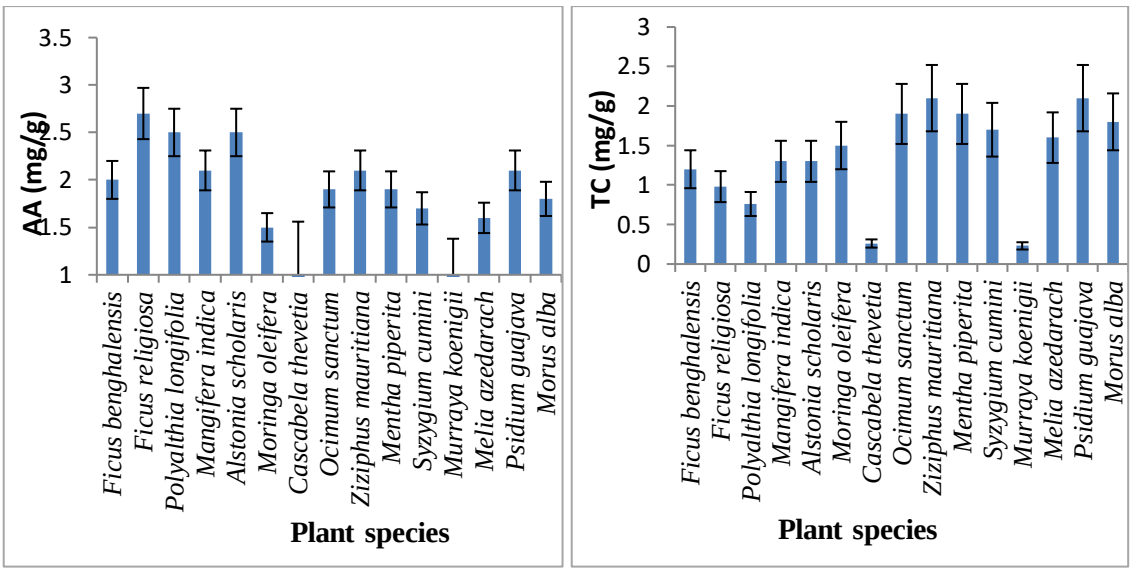
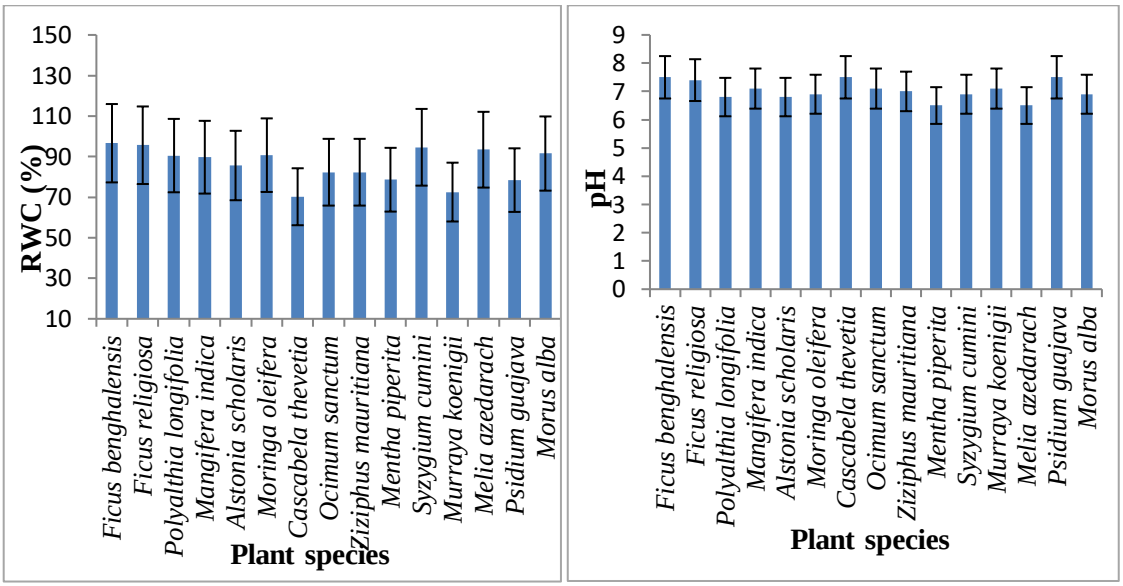


(c) (d)

Figure 6.4 Variation in biochemical parameters of plants species atSO_2 and NO_2 concentration of 8(µg/m^3) and 7 (µg/m^3) respectively in Chandigarh sectors 22



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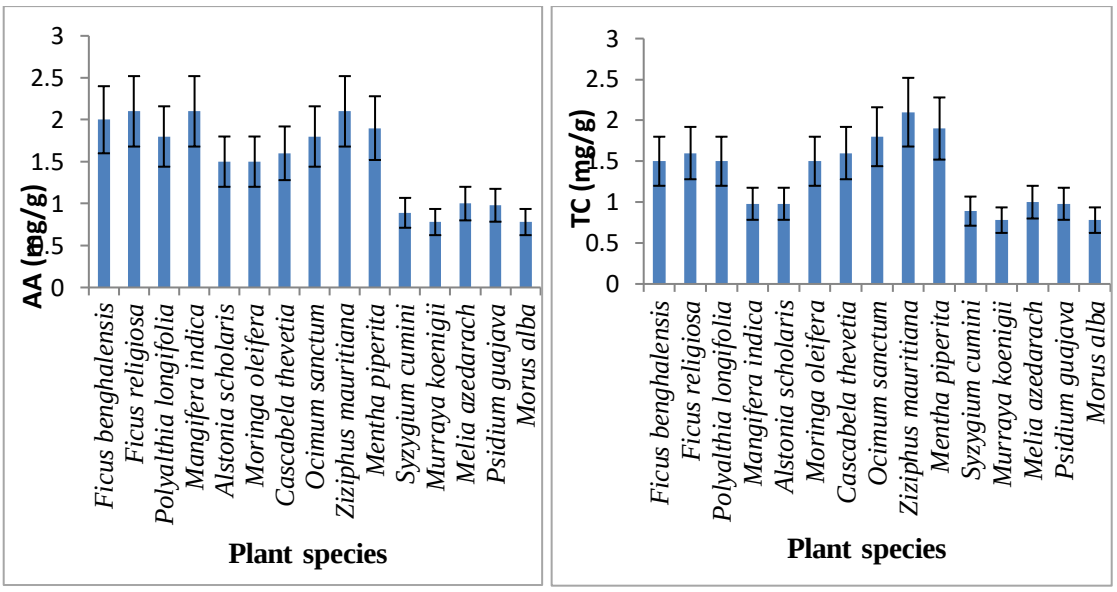
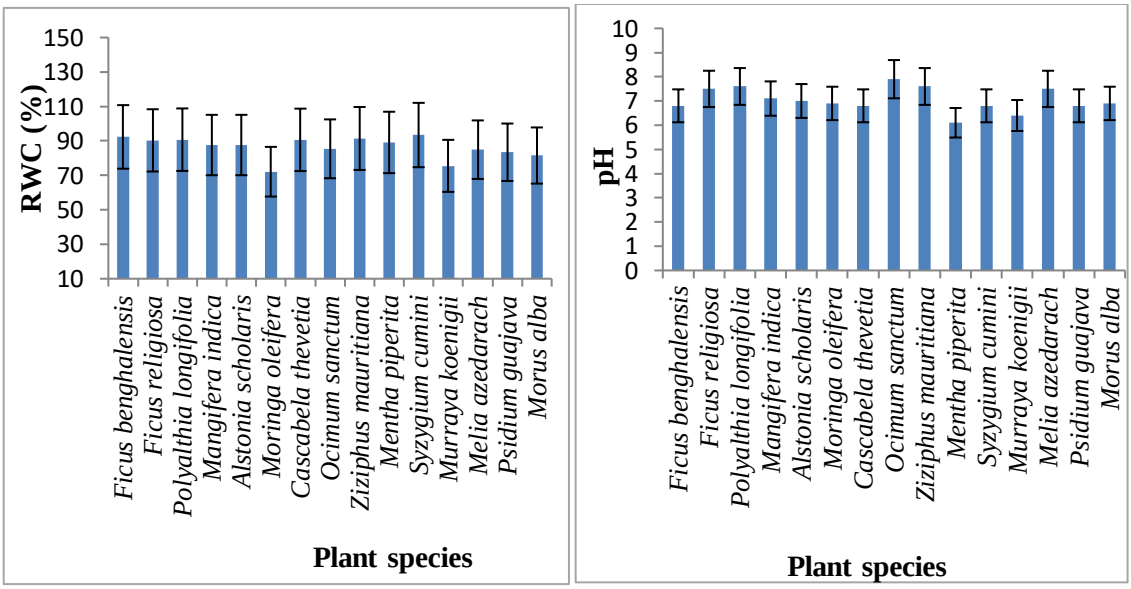


(c) (d)

Figure 6.5 Variation in biochemical parameters of plants species at SO_2 and NO_2 concentration of 3(µg/m^3) and 42 (µg/m^3) respectively in Chandigarh sectors 25



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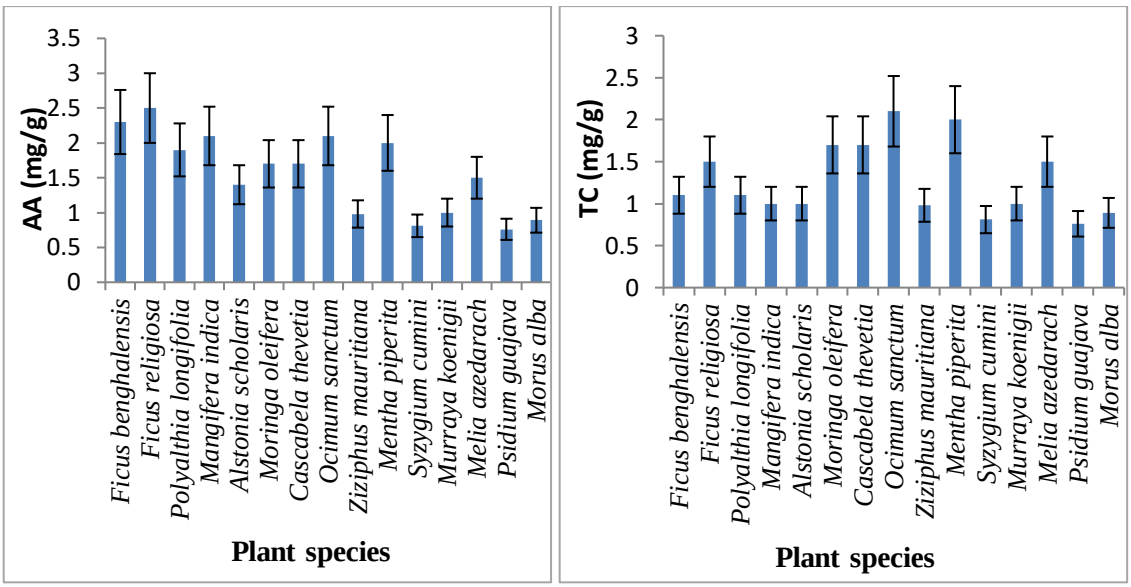

(c) (d)

Figure 6.6 Variation in biochemical parameters of plants species at SO_2 and NO_2 concentration of 9(µg/m^3) and 28 (µg/m^3) respectively in Chandigarh sectors 53.

Table 6.5 Correlation coefficient (r) between biochemical parameters of plants species and SO_2 collected from six different sites of Punjab.

Plant	RWC	pH	AA	TC
Alstonia scholaris	-0.57	-0.36	0.59	-0.85*
Ficus benghalensis	-0.98*	-0.72*	-0.39	-0.49
Ficus religiosa	0.35	0.66*	0.75*	-0.75*
Mangifera indica	-0.87*	-0.25	0.56	0.25
Melia azedarach	-0.54	0.6*	0.89*	-0.63*
Mentha piperita	-0.66*	0.35	0.93*	-0.8*
Moringa oleifera	-0.6	0.45	0.78*	-0.74*
Murraya koenigii	-0.63*	0.07	0.83*	-0.29
Ocimum sanctum	-0.91*	0.15	0.93*	-0.85*
Polyalthia longifolia	-0.84*	0.6*	0.84*	-0.38
Psidium guajava	-0.66*	0.13	0.75*	-0.64*
Syzygium cumini	-0.85*	-0.21	0.81*	0.03
Cascabela thevetia	-0.44	0.14	0.29	-0.55
Ziziphus mauritiana	-0.53	-0.2	0.76*	-0.88*
Morus alba	-0.72*	0.81*	0.75*	0.34

*Correlation is significant at the 0.05 level.

The present findings also highlight that plants with a larger leaf area are showing a negative correlation with SO_2. Possibly, it could be due to the large leaf surface area, leads to higher absorption of gaseous pollutants. Due to which biochemical parameters are affected and the tolerance of the plant varies (Bhart et al. 2018). Thus, morphological parameters can be considered as influential parameter for plant tolerance to air pollutants like SO_2. Apart from this, it has been observed that the degree of damage caused by the same level of SO_2 exposure varies between plant species. The difference in the degree of damage among plant species may be due to the disparity in their capacity to tolerate SO_2 and NO_2. Besides, it may be due to different stomatal resistance to SO_2 or biochemical detoxification of absorbed SO_2, which could be the cause of the variations between plant species (Prasad and Rao 1983). Stomatal closure at high SO_2 is directly linked with CO_2 absorption into sub-stomatal cavities leading to decline photosynthesis; TC, RWC and AA content (Thawale et al. 2010).

Table 6.6 Correlation coefficient (r) between biochemical parameters of plants species and NO_2 collected from six different sites of Punjab.

Plant	RWC	pH	AA	TC
Alstonia scholaris	0.11	-0.26	0.22	0.11
Ficus benghalensis	-0.17	0.25	-0.42	-0.52
Ficus religiosa	0.07	0.8*	0.6*	0.2
Mangifera indica	0.05	0.37	-0.23	0.53
Melia azedarach	0.26	0.58	0.42	-0.1
Mentha piperita	-0.04	0.51	0.46	0.02
Moringa oleifera	0.25	0.45	0.32	0.08
Murraya koenigii	-0.02	0.67*	0.43	0.59
Ocimum sanctum	-0.37	0.25	0.44	-0.16
Polyalthia longifolia	-0.56	0.03	-0.02	0.71*
Psidium guajava	0.09	0.83*	0.34	-0.35
Syzygium cumini	-0.23	0.67*	0.33	-0.78*
Cascabela thevetia	-0.32	0.51	-0.09	0.49
Ziziphus mauritiana	0.08	0.32	0.52	-0.12
Morus alba	-0.7	0.08	0.04	0.41

* Correlation is significant at the 0.05 level.

However, some plant species exhibit strong resistance to air pollutants, which may be due to modifications in their morphological and physiological functions. *Sanseveria* plant has thick leaves that can absorb lethal pollutants such as carbon monoxide (CO), benzene, formaldehyde, and CO_2 released by incomplete combustion in motor vehicles (Permana et al. 2022). Thick wax layer on the leaves is considered one of the factors preventing leaf damage due to air pollution. Additionally, the thick coating retains water content, reduces the loss of nutrients and metabolites, facilitates gas exchange, and protects against reactive pollutants such as CO and O_3.

In the previous experiment of the present study, the effect of environmental factors on biochemical parameters of 15 plants species was studied. To maintain the consistency and flow of the present study the effect of air pollutants on the biochemical parameters of *Mentha piperita* and *Ocimum sanctum* has also been studied separately.

6.3 Effect of ambient air pollutants (SO_2 and NO_2) on biochemical parameters of *Mentha piperita* and *Ocimum sanctum*

The results of the current study revealed that the *Mentha piperita* and *Ocimum sanctum* exhibited significant variations in their biochemical parameters (as shown in the Figures 6.7-6.11). The differences observed in biochemical parameters among the selected plant species can be ascribed to variations in their ability to absorb pollutants, indicative of their tolerance levels. Statistical analysis of the results was conducted utilizing Microsoft Excel and Statistical Package for the Social Sciences (SPSS) software. The relationship between biochemical parameters and air pollutants was examined by using Pearson's correlation and linear multiple regression analysis. R_p and R-square (R^2) values were derived to assess the extent of variability within the investigated data.

6.3.1 Effect of SO_2 and NO_2 on relative water content (%)

The relative water content (RWC) in leaves of *Mentha piperita* ranged from 67.8% to 98.4% (Figure 6.7a and 6.8a) and 62% to 98.6% in the leaves of *Ocimum sanctum* (Figure6.9a and 6.10a) at the study sites. Higher relative water content in the leaves of *Mentha piperita* (98.4%) and *Ocimum sanctum* (98.6%) has been observed at low concentrations of SO_2 (1.2µg/m^3). Conversely, the lowest RWC values are observed at higher concentrations of ambient SO_2 concentrations (14µg/m^3). The low concentration of SO_2 was observed in LPU and highest was observed in Jalandhar and Ludhiana.

Highest RWC was an indicator of the plants' better resistance against water stress and are resistant to air pollution.

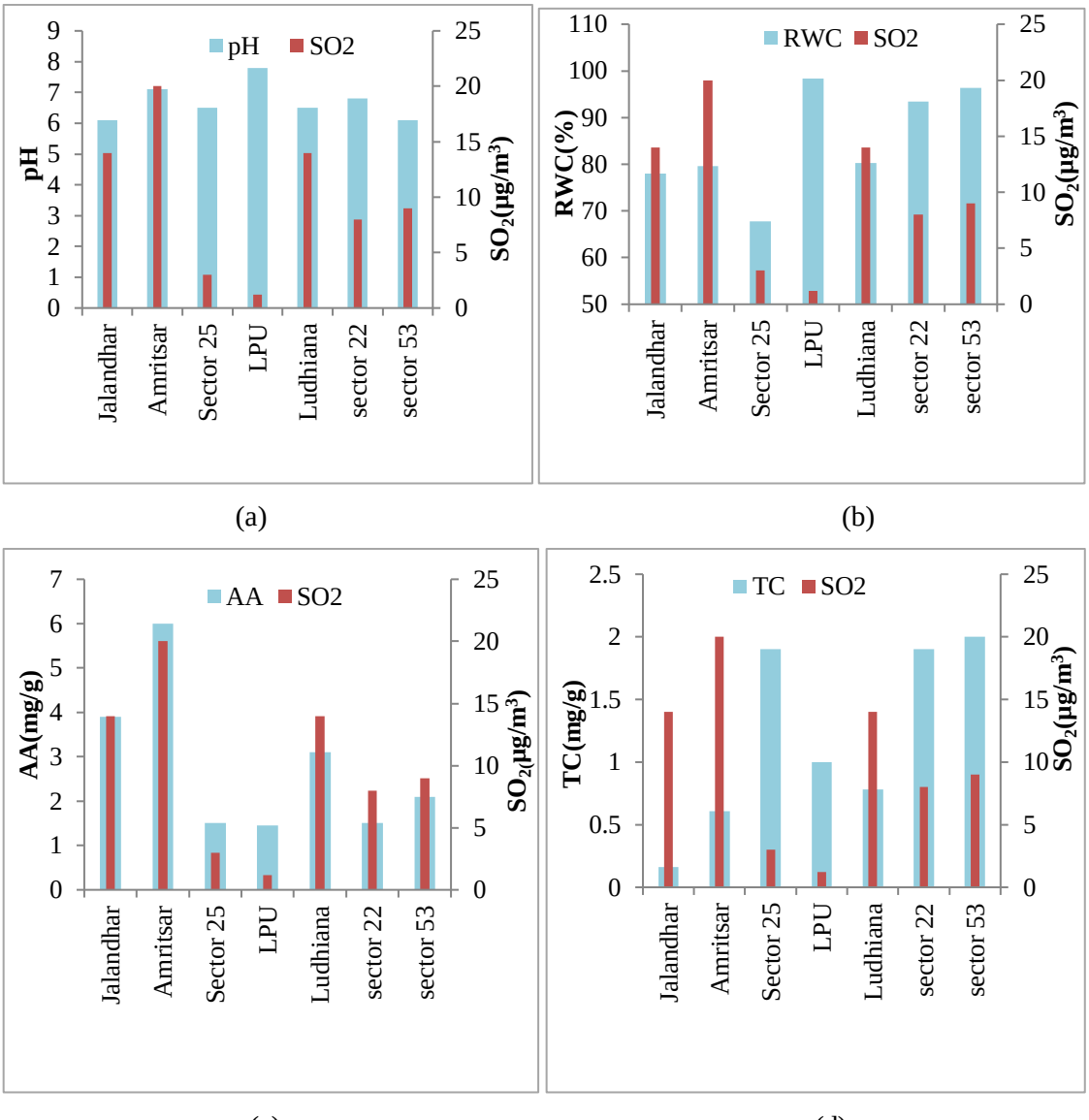

Figure 6.7 Variation in biochemical parameters of *Mentha piperita* at different concentrations of SO_2 (from seven different locations of Punjab)

Kohan et al., (2018) also observed decrease in RWC of spinach leaves in their study was due to prolonged exposure to pollutants reflecting the plant response to pollution stress, closure of stomata and consequent loss of transpiration rates. It may also be possible that at high concentrations of SO_2 increase cell permeability resulting in loss of water and nutrients.

Ashenden, (1979) in his study made similar findings that SO_2 increased the stomatal aperture and reduced the stomatal resistance which subsequent reduced the transpiration rate. Variation

in the leaf water content reflects the impact of air pollution and the sensitivity of the plant (Ghafari et al., 2020; Rashidi et al., 2017). Huang et al., (2004) also observed in their study that the loss of water content in the tissue of sensitive plants and no change in the resistant plants.

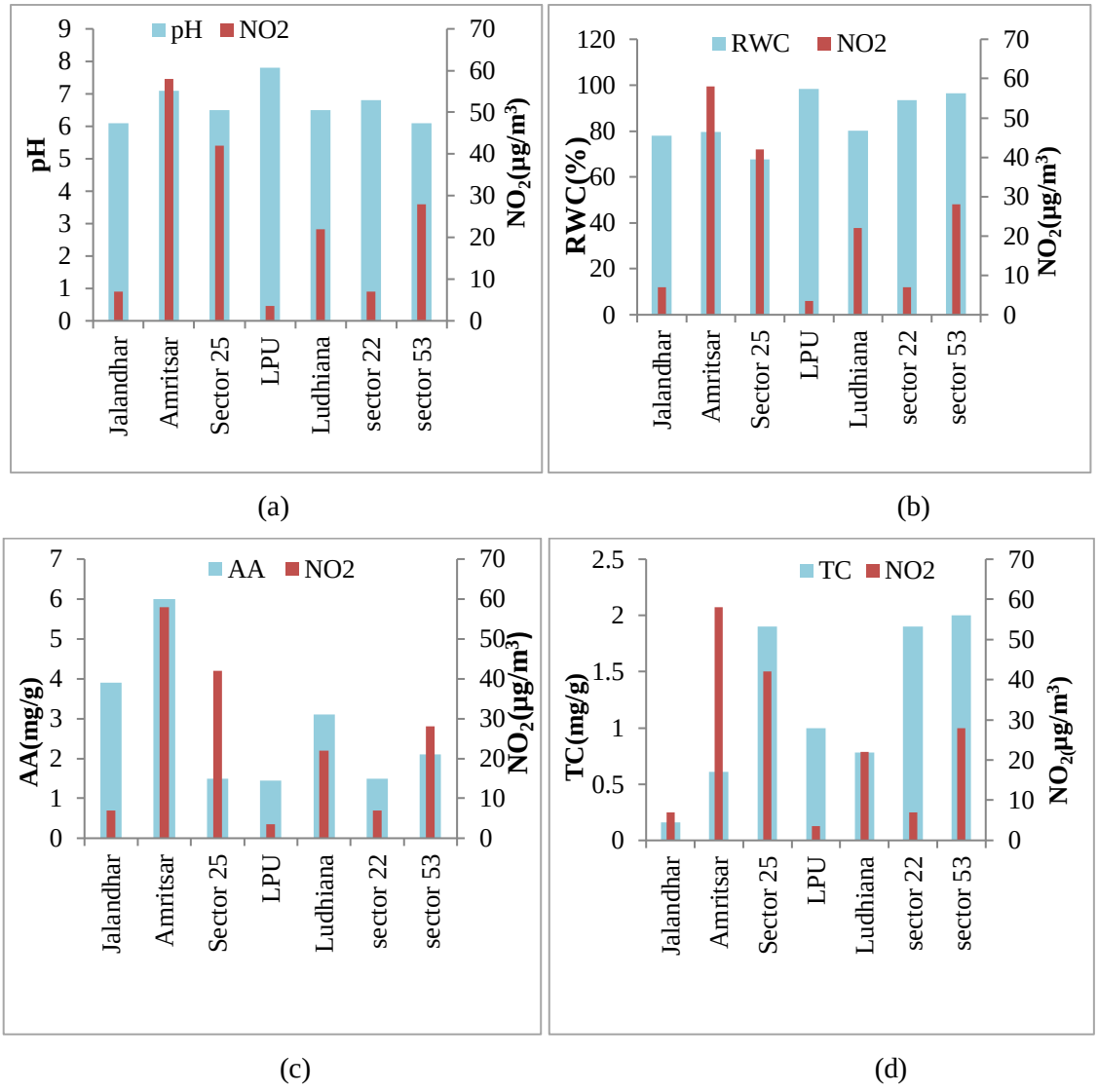

Figure 6.8 Variation in biochemical parameters of *Mentha piperita* at different concentrations of NO_2 (from seven different locations of Punjab).

In support of this fact, several researchers such as Ghafari et al., (2020); Enete et al., (2013) ; Amini et al., (2009) Jyothi and Jaya (2010) have also drawn similar conclusions. NO_2 from seven different locations of Punjab**).** However, response of plants to NO_2 has been reviewed but the concentrations required to plants are still not properly defined (Ashenden, 1979). Later, it was stated that NO_2 can have ambiguous effect on plants (toxic and beneficial) (Petitte and Ormod, 1992; Siegwolf et al., 2001). This aforementioned finding is in consistent with our present results, both plant species (*Mentha piperita* and *Ocimum sanctum*) also showing

variation in RWC and no specific pattern observed with NO_2. Since, NO_2 does not always acts as a pollutant. It might be possible that the observed concentration of NO_2 remain unaffected for the RWC of both *Mentha piperita* and *Ocimum sanctum*. The difference in the degree of damage due to SO_2 and NO_2 among plants species may be due to the disparity in their capacity to tolerate stress conditions.

Pearson correlation analysis (as shown in Table 6.7 and 6.8) was performed to assess the correlation between the biochemical parameter (RWC) and the air pollutants (SO_2 and NO_2). A significant negative correlation at $p < 0.005$ level was observed between RWC (%) of *Mentha piperita* and SO_2 ($R_p = -0.79$) and weak negative correlation with NO_2 ($R_p = -0.37$). *Ocimum sanctum* has also exhibited a significant negative correlation of RWC with SO_2 ($R_p = -0.89$) and NO_2 ($R_p = -0.5$). Some researchers reported negative impact of NO_2 on the relative water content (Chun Yan et al., 2007' Pryimark, 2012). This is in consistent with our results also; *Ocimum sanctum* shows significant negative correlation with NO_2. The results of correlation analysis of the current study have been obtained in consistent with the literature. As, explained in the literature that increase in SO_2 results in a decrease in RWC. Thawale et al. (2010) also reported RWC has negative correlation with SO_2 in their study. Other side, Banerjee et al., (2021) found no correlation of SO_2 and NO_2 with RWC in their study. Multiple linear regressions is a prediction tool for defining the quantitative relationships between multiple independent variables with the help of regression analysis. The results obtained from multiple linear regressions also revealed the significant relationship between the relative water content (dependent variable) with SO_2 (independent variable). The influence of SO_2 and NO_2 on RWC was predicted with great significant p values (< 0.05). Regression coefficients have significant positive R^2 value between SO_2 and RWC of *Mentha piperita* ($R^2 = 0.79$) and low insignificant R^2 value with NO_2 ($R^2 = 0.37$) whereas *Ocimum sanctum* exhibited significant positive R^2 value between RWC and both SO_2 and NO_2 ($R^2 = 0.89$ and $R^2 = 0.55$ respectively).

However, a significant combined effect of both SO_2 and NO_2 is also observed on RWC. The regression coefficient between SO_2 and NO_2 (combined effect of SO_2 and NO_2) with RWC has been found to be significant ($R^2 = 0.79$) at $p < 0.05$. The current findings are consistent with the previous literature. For instance, Petitte and Ormod (1992) observed in their study which was conducted on potato plant, that exposure to NO_2 alter the water status of plants by affecting the osmotic potential and xylem water potential especially in combination with SO_2. Exposure of soybean (Amundson and Weinstein, 1981) and garden bean (Ashenden, 1979) to SO_2 and NO_2 caused parallel decrease in transpiration and photosynthesis rates due to increased stomatal resistance.

6.3.2 Effect of SO_2 and NO_2 on pH

The pH of leaf extracts ranged from 6.1 to 7.8 for *Mentha piperita* (as shown in Figure 6.7b and Figure 6.8b) and 6.8 to 7.9 for *Ocimum sanctum* (as shown in 6.9b and 6.10b*). Ocimum sanctum* has higher range of pH when compared to *Mentha piperita*. The results revealed that *Mentha piperita* exhibited acidic pH at most of the study sites. This might be due to exposure to SO_2 and NO_2 pollutant. Since, when plants exposed to SO_2, large amount of H^+ ions are produced in their intercellular fluid to react with SO_2. This H^+ may produce H_2SO_4 which lower the pH (Heber and Hueve, 1997; Karamakar et al., 2020; Ghafari et al., 2020). Similarly, when plants exposed to NO_2, nitrate and nitrite produced and consumes H^+ which causing decrease in ammonium uptake which may result in decrease in H^+ ions. This decrease in acidity in plants exposed to NO_2 may be associated with the reduction of the nitrate and nitrite produced from NO_2 (Qiao and Murray, 1997). It is also reported in the previous literature that the change in leaf extracts pH towards acidic range occurs due to the presence of SO_2 and NO_2 in the ambient air (Karmakar et al., 2020). This aforementioned finding is consistent with our results as significant reduction in pH of *Mentha piperita* was found in Jalandhar, Ludhiana and Chandigarh (22, 25, 53).

On other side, this is dissimilar to our findings for *Ocimum sanctum.* A significant increase in leaf extract pH of *Ocimum sanctum* was observed in all the studied locations. It has been predicted that this might be response of *Ocimum sanctum* to SO_2 and NO_2. Prajapati and Tripathi, (2008) also concluded that plant species exhibiting an increase in pH in polluted environment should be considered as tolerant species. In current findings, higher pH of *Ocimum sanctum* can be considered a response to counter the effect of SO_2 and NO_2. This suggests the *Ocimum sanctum* can be used to absorb SO_2 and NO_2 in the study sites.

Pearson correlation analysis (as shown in Table 6.7 and 6.8) was performed to assess the correlation between the biochemical parameter (pH) and the air pollutants (SO_2 and NO_2). A weak correlation has been found at $p<0.005$ level between pH of *Mentha piperita* and *Ocimum sanctum* with SO_2 (R_p = -0.29 and R_p = 0.09) and NO_2 (R_p = -0.08 and R_p = 0.20) respectively. The results obtained from multiple linear regressions also revealed the insignificant relationship between the pH (dependent variable) with SO_2 and NO_2 (independent variable). Regression coefficients have insignificant positive R^2 value between SO_2 and pH (R^2 = 0.29 and R^2 = 0.09) of *Mentha piperita* and *Ocimum sanctum* respectively whereas, for NO_2 (R^2 = 0.08 and R^2= 0.2 respectively). The current findings contradict with the previous literature. As, in the previous studies conducted by Leghari et al. 2011; Paulsamy and Senthilkumar 2009; Govindaraju et al. 2011; Chandawat et al. 2011; Karmakar et al. 2020 found significant relationship of pH with air

pollutants. Besides, insignificant impact of combined SO_2 and NO_2 was also observed on pH. The regression coefficient for SO_2 and NO_2 (combined effect of SO_2 and NO_2) with pH has been found to be insignificant ($R^2 = 0.3$ and $R^2 = 0.2$) at $p < 0.05$. This might be because SO_2 and NO_2 have indirect effects on pH and other biochemical parameters dominate the variations and pH remains unaffected.

Interestingly, a significant pattern of pH variation due to high concentration of SO_2 and NO_2 has already been discussed above so that might be due to the higher RWC levels which also counteract the high acidity with its cells sap to control drought conditions. Niami et al., (2023) also found similar findings in their study.

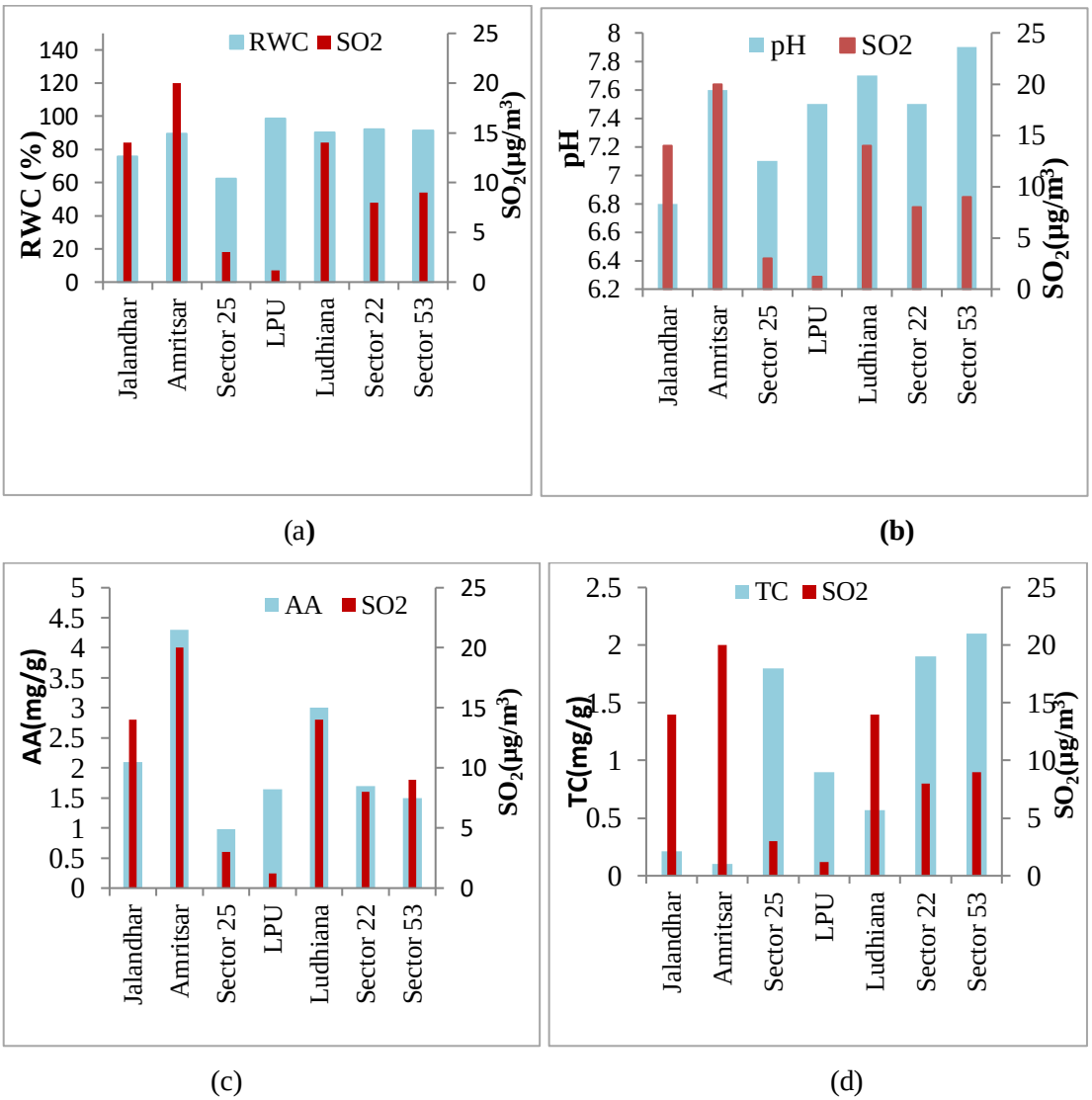

Figure 6.9 Variation in biochemical parameters of *Ocimum sanctum* at different concentrations of SO_2 (from seven different locations of Punjab)

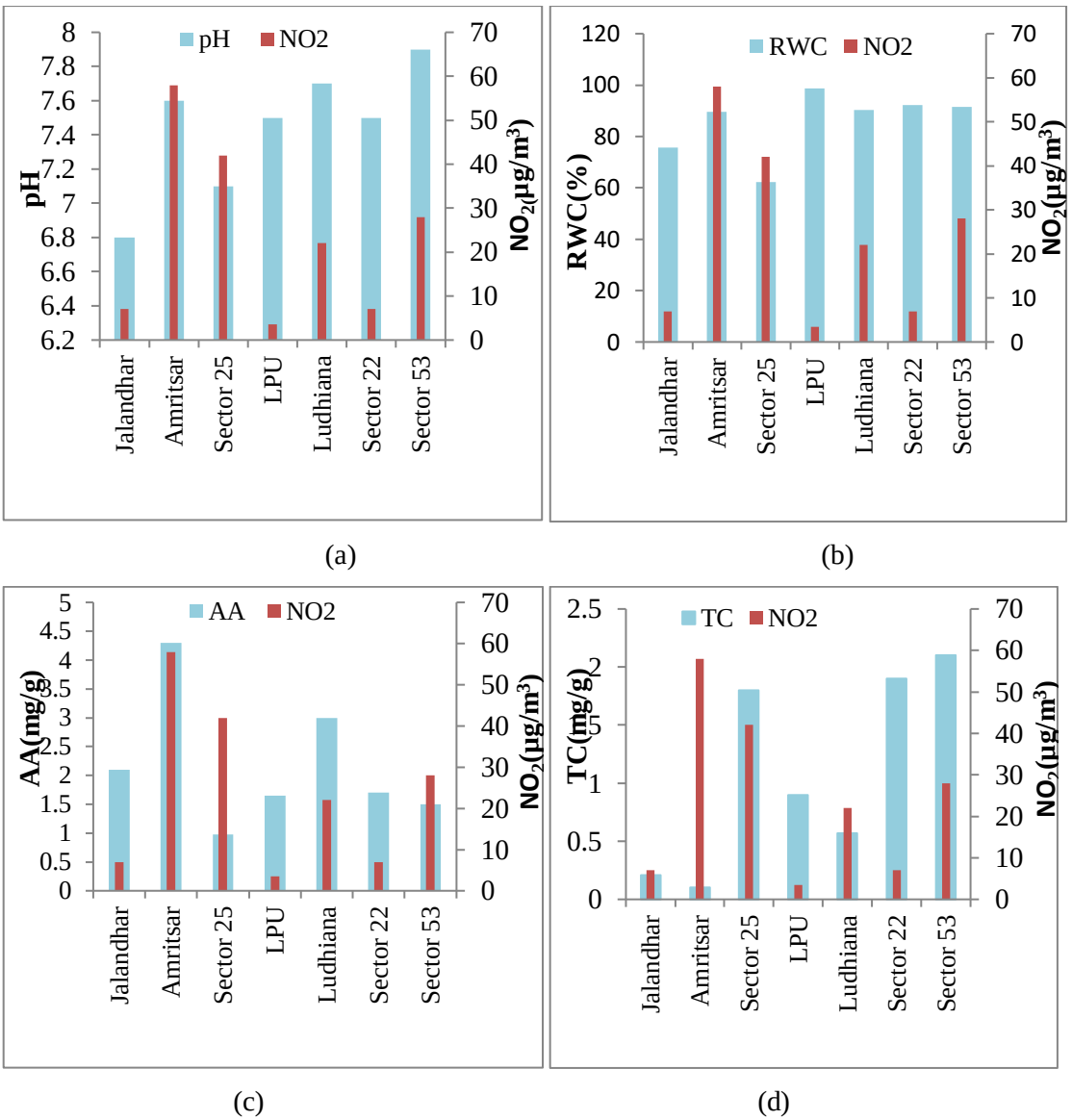

Figure 6.10 Variation in biochemical parameters of *Ocimum sanctum* at different concentrations of NO_2 from seven different locations of Punjab.

6.3.3 Effect of SO_2 and NO_2 on Total chlorophyll (mg/g)

Total chlorophyll content (TC) of leaf samples varied in the ranged of 0.1mg/g to 2mg/g in *Mentha piperita* and 0.1mg/g to 2.1mg/g in *Ocimum sanctum*. The highest chlorophyll content (2mg/g and 2.1mg/g) in the leaves of the *Mentha piperita* (Figure 5.7d and Figure 5.8d) and *Ocimum sanctum* growing was observed in Chandigarh sector 53 followed by Chandigarh sector 25 and 22 respectively (Figure 6.9d and Figure 6.10d). The results of the present study showed a pattern with SO_2 concentrations. The highest chlorophyll was found at the lowest concentrations of SO_2 and lowest TC was found at the highest concentration of SO_2. However, the same pattern

is not observed for NO_2. It might be because when SO_2 enters through stomata, it gets oxidised to sulphur trioxide and reacts with water to form sulphuric acid inside the mesophyll cell and damage the chloroplast. This may also be possible due to the increase in stomatal resistance induced by high concentrations of SO_2. According to Rawal et al. (2001) reduction in chlorophyll content has often been suggested as an indicator of air pollution damage, (mainly from higher absorption of SO_2).

Decrease in total chlorophyll content can be considered an indicator of the increasing rate of SO_2. Chlorophyll is the main attack site for air pollutants such as SPM, SO_2 and NO_2 (Tripathi and Gautam 2006; Priyanka and Dibyendu 2009; Paulsamy and Senthilkumar 2009;Kuddus et al. 2011). It lowers the pH and internal environment of mesophyll cells becomes acidic which results in loss of chlorophyll. This is in consistent with previous literature. Since, the effect of SO_2 on TC has been well explained in previous literature (Bhardwaj et al., 2022).

The another reason could be the same as reported by Malhotra and Khan (1984) in their study that SO_2 can affect photosynthesis by affecting carboxylation reactions and by attacking photosynthetic electron transport and photophosphorylation reaction. Several researchers such as Amini et al., (2009), Olumi et al., (2016); Zhang et al., (2016); Ghafari et al., 2020; Karmakar et al., 2020; Rawal et al. 2001; Leghari et al. 2011 also observed plant species with higher total chlorophyll content due to SO_2 pollution. Other side, the current study did not found any specific trend with NO_2. Kammerbauer and Dick (2000) observed the chlorophyll increase to NO_2 uptake which is agreement with our results of the increased chlorophyll of both the plant species at most of the sampling locations. The nitrate and nitrite are utilized by the plant during the process of nitrate metabolism.

However, high concentration of NO_2 result in accumulations of nitrite and cell acidification, leading to adverse effects including generation of ROS and inhibition of Nitrogen assimilation and plant growth, further causing leaf damage, chlorosis or even death. Since, exposures to NO_2 produce physiological responses across various plant species. The impact of NO_2 exposure on plants remains a subject of considerable debate, with no consensus reached among researchers. Moreover, there is a lack of comprehensive data concerning plant species that exhibit high tolerance to NO_2 and their subsequent recovery mechanisms. The relationship between photosynthesis, stomatal behaviour and chloroplast remains to be systematically explored. Similar conclusions have also been drawn by Sheng and Zhu et al.., 2019; Sheng and Zhu et al., 2018).

Pearson correlation analysis (as shown in Table 6.7 and 6.8) was performed to assess the correlation between the TC (mg/g) and the SO_2 and NO_2. A significant negative correlation at p

<0.005 level was observed between TC (mg/g) of *Mentha piperita* and SO_2 (R_p = -0.58) and insignificant correlation with NO_2 (R_p = 0.007). *Ocimum sanctum* has also exhibited a significant negative correlation between TC (mg/g) and SO_2 (R_p = -0.64) and insignificant correlation with NO_2 (R_p = -0.1). Similar findings were reported by Thawale et al. (2010). The results obtained from multiple linear regressions also revealed the significant relationship between the TC mg/g (dependent variable) and SO_2 only (independent variable) for both the plant species. The influence of SO_2 on TC (mg/g) was predicted with great significant p values (< 0.05). Regression coefficients have significant positive R^2 value between SO_2 with TC of *Mentha piperita* and *Ocimum sanctum* (R^2 = 0.58 and R^2= 0.64) and the low R^2 value with NO_2 and TC (R^2 = 0.37 and R^2= 0.10). However, a significant combined effect of both SO_2 and NO_2 is observed on TC. The regression coefficient between SO_2 and NO_2 (combined effect of SO_2 and NO_2) with TC has been found to be significant (R^2 = 0.68 and R^2 = 0.66) at p <0.05. The current results are consistent with previous literature.

6.3.4 Effect of SO_2 and NO_2 on Ascorbic acid content (mg/g)

Ascorbic acid of leaf samples varied from 1mg/g to 6 mg/g in *Mentha piperita* (as shown in Figure 6.7c and 6.8c) and 0.9mg/g to 4.3mg/g in *Ocimum sanctum* (as shown in Figure 6.9c and 6.10c). The highest ascorbic content in *Mentha piperita* and *Ocimum sanctum* (6mg/g and 4.3mg/g) was observed at higher concentration of SO_2 in Amritsar. Conversely, lowest Ascorbic acid content of both the species has been found at lower concentration of SO_2. The production of Reactive Oxygen Species (ROS), including SO_3^-, HSO_3^-, OH^-, O_2^-, etc., may be attributed to the absorption of SO_2 and the subsequent photo oxidation of SO_3^- to SO_4^-. This process generates sulphites and leads to an increased presence of free radicals under SO_2 exposure. The rise in ascorbic acid levels serving as a defense mechanism against ROS generated by the photosynthetic apparatus. This suggests a potential adaptive response wherein increased ascorbic acid acts as an antioxidant to mitigate the oxidative stress induced by elevated ROS levels during SO_2 exposure. (Kour and Adak, 2021; Kour and Adak, 2023). Similar findings were drawn Rawal et al., 2001; Ninave et al. 2001; Tripathi and Gautam 2006; Paulsamy and Senthilkumar 2009;Elawa et al. 2021; Pandey et al., 2015; Banerjee et al., 2018; Karmakar et al., 2020). In the present findings, high RWC was found in sampled plants.

Under conditions of water stress, there is a tendency for the ascorbic acid content to rise, as a response to protect the thylakoid membrane from oxidative stress. In Prior studies, it was reported that plant species exhibiting higher ascorbic acid content can be considered as tolerant to air pollution (Ghafari et al., 2020, Karmakar et al., 2020; Naimi et al., 2023; Eslamdoust et

al., 2023). Higher ascorbic acid content is a sign of more tolerance of specific SO_2 pollutant (Ghafari et al., 2020). This is consistent with our findings that at higher concentrations of SO_2 both plants species (*Mentha piperita* and *Ocimum sanctum*) exhibited higher ascorbic acid. This suggests both plant species studied can be used as sink for SO_2. Prajapati and Tripathi (2008), Enete et al., 2013 have also drawn the same conclusion. While, NO_2 can induce changes to growth and photosynthetic activity, leading to changes in antioxidant defense systems as well as oxidative damage (Chen et al., 2010; Ma et al., 2007; Takahashil et al., 2011). This is in consistent with our results that it leads changes in antioxidant defense systems. As of, in current findings in some places ascorbic acid content of both the plant species (*Ocimum sanctum* and *Mentha piperita*) exhibited higher values at both higher and lower concentrations of NO_2.

Hence, it is anticipated that plants may exhibit adverse effects following exposure to atmospheric NO_2. This expectation aligns with previous observations reported by Li et al. (2007) and Miao et al. (2008). Additionally, Mustafa and Tierney (1978) along with Pathmanathan et al. (2003) have highlighted in their respective studies that NO_2 acts as an oxidant pollutant, leading to oxidative damage to cell membranes and the subsequent generation of ROS.

Despite these insights from prior literature, the specific mechanisms underlying plant defense against NO_2-induced stress remain relatively understudied. It is acknowledged that plants possess the ability to scavenge excess ROS through the activation of antioxidant defense systems as a protective response to NO_2 stress. However, the precise defense mechanism against NO_2 remains elusive based on the current findings. Further investigation is necessary to elucidate the intricate pathways involved in plant adaptation to NO_2 exposure.

Pearson correlation analysis (as shown in Table 6.7 and 6.8) was performed to assess the correlation between the AA (mg/g) and the SO_2 and NO_2. A significant positive correlation at $p < 0.005$ level was observed between AA (mg/g) of *Mentha piperita* and SO_2 ($R_p = 0.91$) and with NO_2 ($R_p = 0.54$). *Ocimum sanctum* has also exhibited a significant positive correlation of AA (mg/g) with SO_2 ($R_p = 0.86$) and with NO_2 ($R_p = 0.51$). The results obtained from multiple linear regressions also revealed the significant relationship between the AA mg/g (dependent variable) with SO_2 (independent variable). The influence of SO_2 and NO_2 on AA (mg/g) was predicted with great significant p values (< 0.05).

Regression coefficients have significant positive R^2 value between SO_2 with AA (mg/g) of *Mentha piperita* and *Ocimum sanctum* ($R^2 = 0.91$ and $R^2 = 0.86$) and the significant R^2 value with NO_2 and AA (mg/g) ($R^2 = 0.54$ and $R^2 = 0.51$). However, a significant impact of combined SO_2 and NO_2 is observed on AA (mg/g). The regression coefficient between SO_2 and NO_2

(combined effect of SO_2 and NO_2) with AA (mg/g) has been found to be significant ($R^2 = 0.93$ and $R^2 = 0.87$) at $p < 0.05$. The current results are consistent with previous literature.

Table 6.7 Pearson correlation analysis of air pollutants (SO_2 and NO_2) and biochemical parameters of *Mentha piperita* (*Marked correlations between morphological and biochemical parameters are significant at $p < 0.05$

	RWC	pH	AA	TC	SO_2	NO_2
RWC	1					
pH	0.56	1				
AA	-0.68	-0.06	1			
TC	0.49	-0.11	-0.70	1		
SO_2	-0.79	-0.29	0.91	-0.58	1	
NO_2	-0.37	-0.08	0.54	0.071	0.42	1

Table 6.8 Pearson correlation analysis of air pollutants (SO_2 and NO_2) and biochemical parameters of *Ocimum sanctum* (*Marked correlations between morphological and biochemical parameters are significant at $p < 0.05$)

	RWC	pH	AA	TC	SO_2	NO_2
RWC	1					
pH	-0.12	1				
AA	-0.69	0.25	1			
TC	0.35	0.28	-0.75	1		
SO_2	-0.89	0.09	0.86	-0.64	1	
NO_2	-0.55	0.20	0.51	-0.10	0.42	1

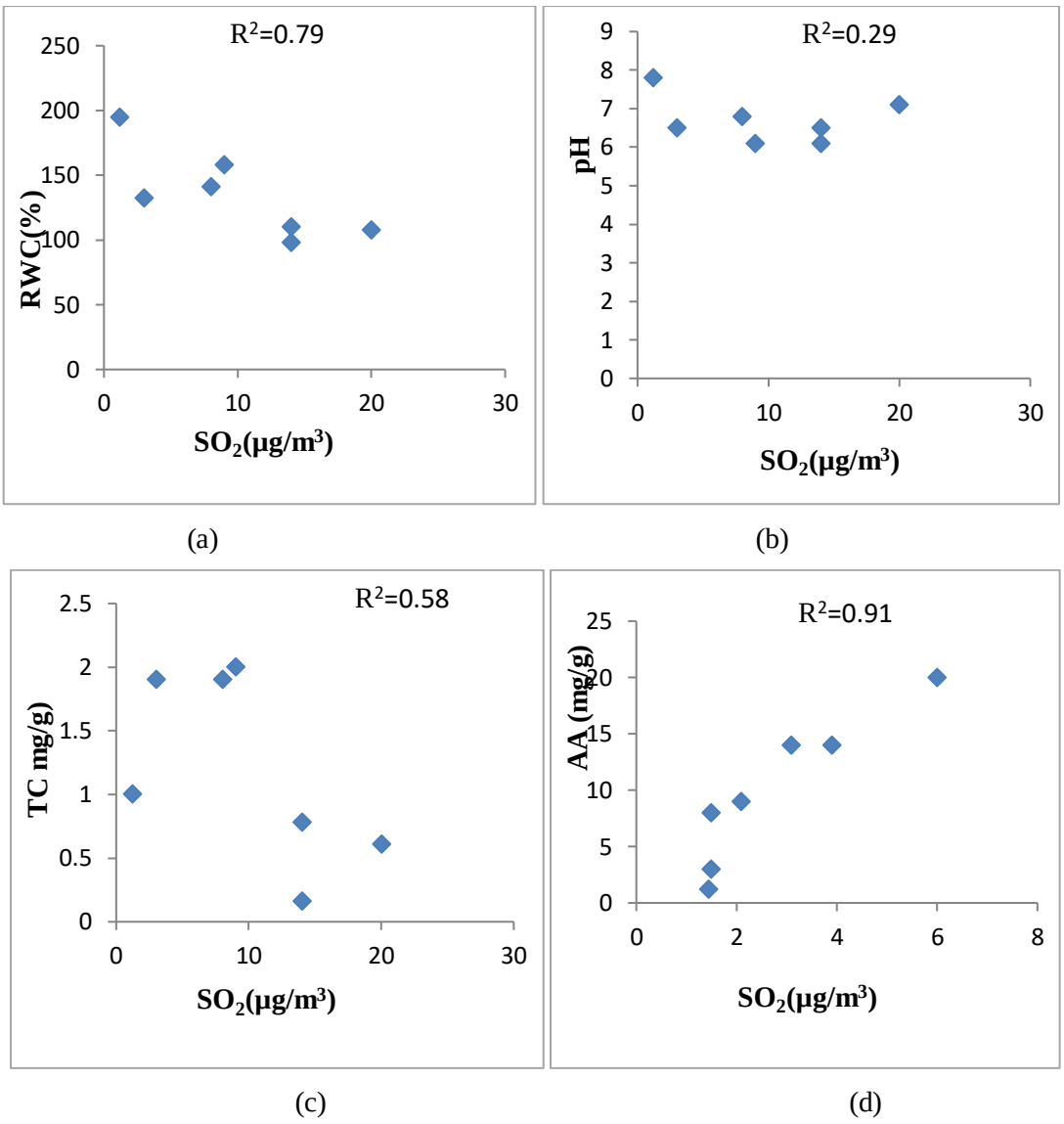

Figure 6.11 Scatter plots of biochemical parameters of the *Mentha piperita* with SO_2 concentrations in ambient air

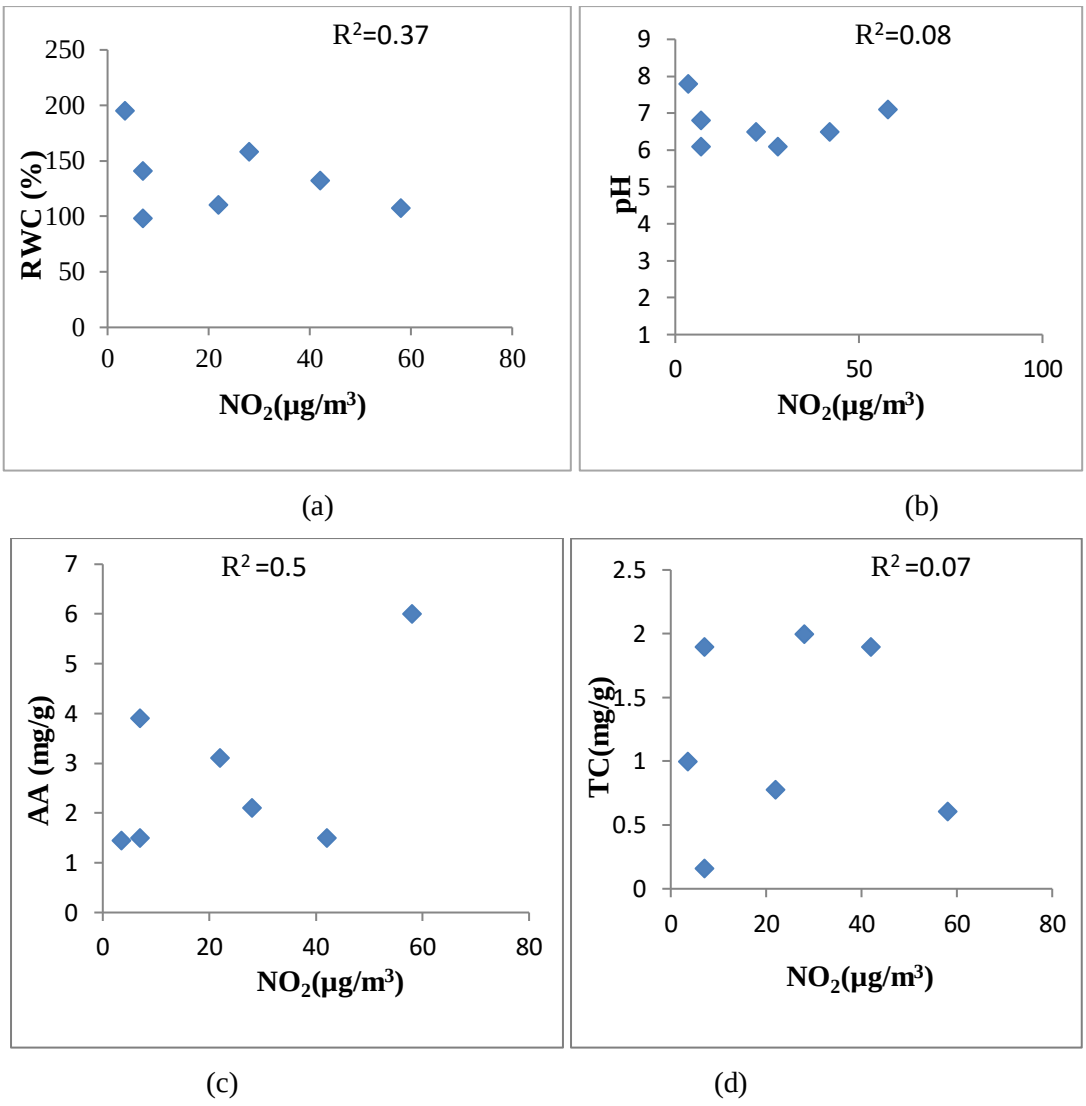

Figure 6.12 Scatter plots of biochemical parameters of the *Mentha piperita* species with NO_2concentrations in ambient air

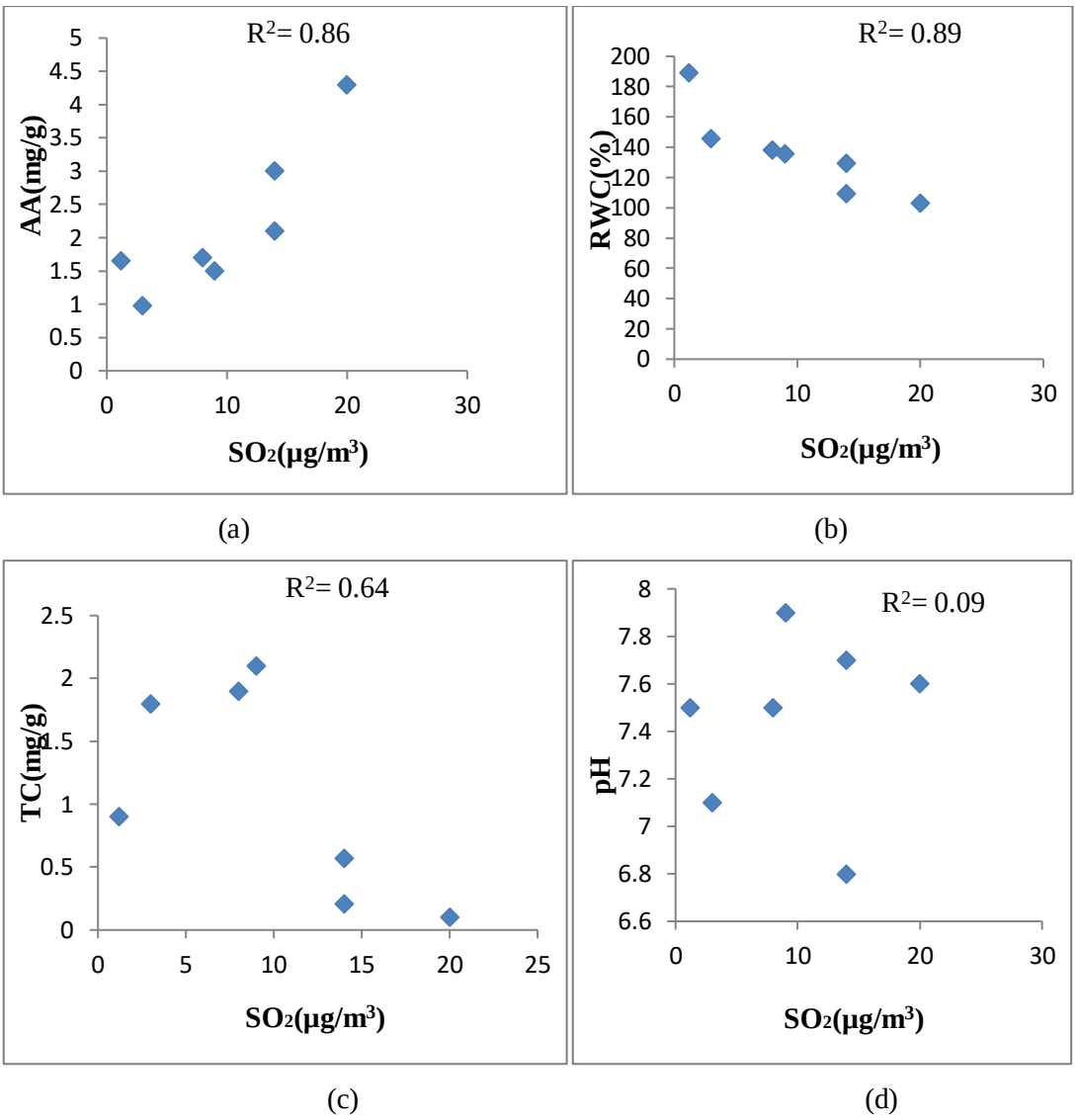

Figure 6.13 Scatter plots of biochemical parameters of the *Ocimum sanctum* with SO_2 concentrations in ambient air

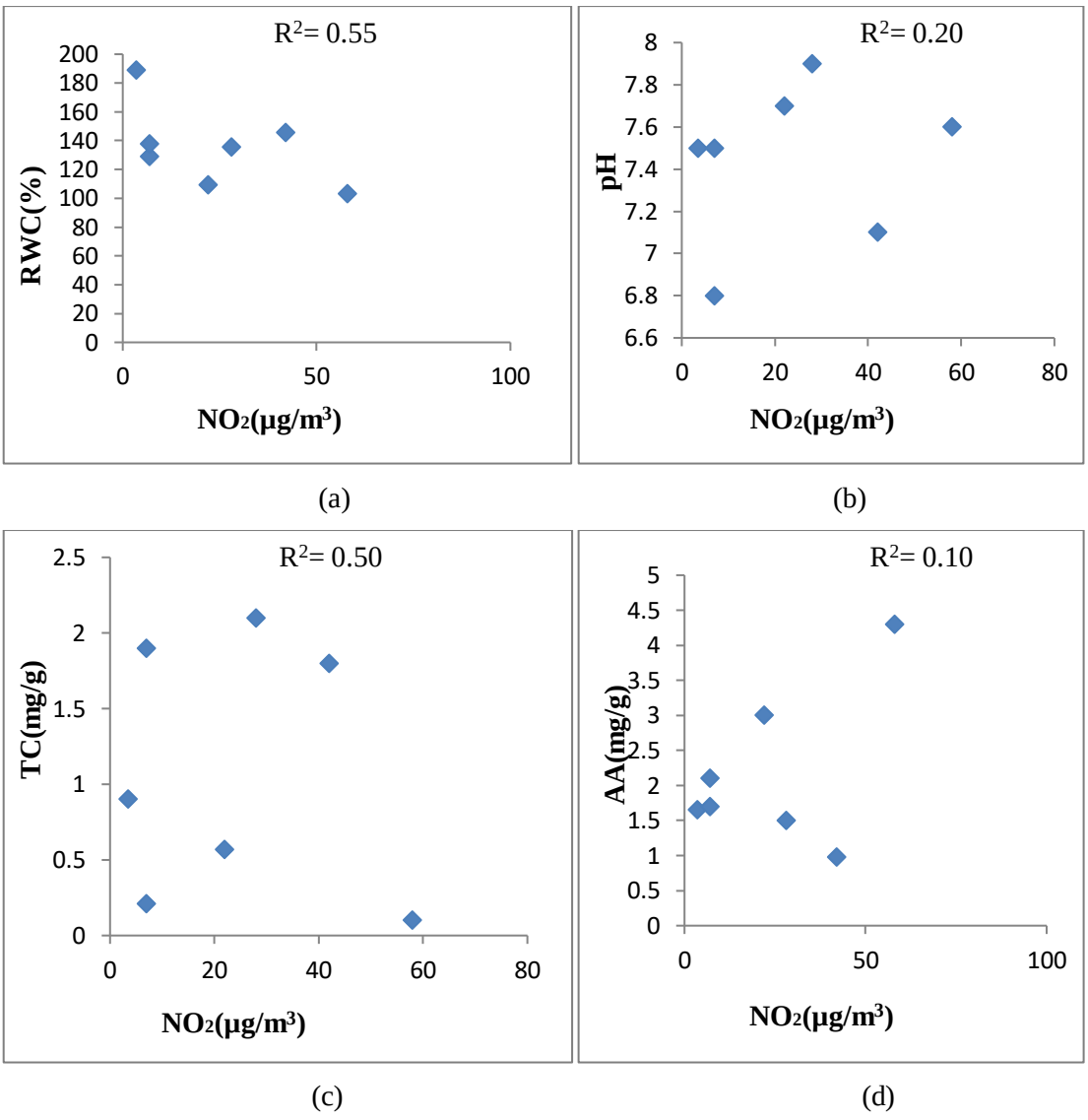

Figure 6.14 Scatter plots showing the relationship between biochemical characteristics of the *Ocimum sanctum* species and the levels of NO_2 in the surrounding air.

CHAPTER 7 INTEGRATED APTI MODEL

7.1 Background

The biochemical parameters such as ascorbic acid, total chlorophyll, pH and relative water content are measured to calculate APTI of various plants. Depending on the APTI values, a plant can be classified as tolerant and sensitive to air pollution. A tolerant plant species can be used for developing green belts to reduce air pollution. Development of green belts in urban areas is one of the effective long term solutions in mitigating air pollution. However, proper selection of plants, and their absorbance ability are not properly evaluated. Plants respond differently to different air pollutants. Perhaps, some plants do not exhibit any physical changes but some plants get harmed and injured. Also, plants exhibit different responses to different environment. Due to which significant variations has been observed in photosynthesis, stomata regulation, respiration and various enzymatic, metabolic and biochemical processes. These variations may also prove significant when estimating air pollution tolerance index of plant. These are the major limitations of APTI method that its formula lacks certain important parameters. The variability of four biochemical parameters determination may not be the only way to classify plant species as tolerant or sensitive to air pollution (Karmakar et al., 2020). Since, many other parameters also participate in changing the biochemical parameter of plants. Previous literature also highlights the other parameters but detailed relationship needs to be explored. In the current study, two most influential parameters (Environmental factors and Air pollutants) affecting biochemical parameter have been studied. It is necessary to add these parameters to the previous APTI model and developed proposed APTI model. It needs to be further used while calculating plant tolerance index for effective screening and identification of plants for effective mitigation of air pollution. Based on previous studies, several researchers modified the models to expand the application of existing model, such as Arora et al., 2001 modified the photosynthetic light response curve model for single leaf to larger scales, some researchers have linked nitrogen content (Arora et al., 2001), chlorophyll content (Xu et al., 2014; Xu et al., 2015), moisture content, leaf temperature and global site factors (Calama et al., 2013; Mayoral et al., 2015) to PLR models. E.M Nederhoff and J.G vegter also modified Acock and Thornley model to enhance the compactness and simplicity. Lin et al., 2015 also build a model for predicting the relationship of photosynthetic rate, photosynthetically active radiation and the relative pollution in a polluted environment. In the

current study, the objective was to modify the APTI model for dependence on environmental factors and air pollutants that can be used in global and large scale tolerance index studies. The general two models have been proposed with correction term (CT) and can be written as Eq 7.1 and Eq 7.2:

$BC_{(monthly)} = BC_{(annual)} + CT_{(biochemical\ parameters,\ environmental\ factors)}$ ..... (Eq.7.1)

$BC_{(pollutant)} = BC_{(control)} + CT_{(biochemical\ parameters,\ air\ pollutants)}$ ..... (Eq. 7.2)

Here, $BC_{(monthly)}$ = Monthly average of biochemical parameter

$BC_{(annual)}$ = Annual average of biochemical parameter

$BC_{(pollutant)}$ = biochemical parameters values at pollutant site

$BC_{(control)}$ = biochemical parameters values at control site

$CT_{(biochemical\ parameters,\ environmental\ parameters)}$ = Correction term for biochemical parameters and environmental parameter.

$CT_{(biochemical\ parameters,\ air\ pollutants)}$ = Correction term for biochemical parameter and air pollutants.

7.2 Results and Discussion

Using 4^{th} order polynomial equation and the annual average environmental factors data, the values of biochemical parameters $BC_{(annual)}$ has been calculated. The value of the biochemical parameters obtained from experimental data was used as $BC_{(monthly)}$. Then the, the value of $BC_{(annual)}$ was subtracted from $BC_{(monthly)}$ to obtained Correction term ($CT_{biochemical\ parameters,\ environmental\ parameters}$) (as shown in the Tables 7.1- 7.8). Further, CT values were validated with six random experimental values (as shown in the tables 7.9-10). Similarly, using linear regression equation $BC_{(pollutant)}$ was calculated. The value of the biochemical parameters obtained from experimental data was used as $BC_{(control)}$. Then, the value of $BC_{(pollutant)}$ was subtracted from $BC_{(control)}$ to obtained Correction term ($CT_{biochemical\ parameters,\ air\ pollutants}$) (as shown in tables 7.11- 7.18). In the current study, CT is based on biochemical parameters as well as environmental parameters and air pollutants of a particular area. So, CT is pollutant concentration and site specific.

Proposed a multiple variable model to describe the annual value of biochemical parameters based on the monthly data and CT (CT is site specific and pollutant concentration specific). One general formulations includes environment factors and biochemical parameters and was derived by computing a non linear multiple regression of individually fitted values of environmental factors (light intensity, temperature and humidity) and biochemical parameters

(TC, RWC, AA, and pH). The other general models include air pollutants and biochemical parameters and were derived by multiple linear regressions of individual fitted air pollutants (SO_2 and NO_2) and biochemical parameters (TC, RWC, AA, and pH). Data sets used to derive $BC_{(annual)}$ and $BC_{(control)}$ is already mentioned in previous chapters.

Table 7.1 Correction term (CT) for RWC of *Mentha piperita* and environmental factors (Temperature, Light intensity, Humidity)

Months	$\mathbf{CT_{(RWC,L,T,H)}}$
January	-22.44
February	-16.84
March	-13.44
April	-13.44
May	-0.99
June	-2.04
July	3.45
August	10.85
September	14.35
October	11.25
November	-17.64
December	-8.60

In the Eq7.3, RWC_a was considered as RWC (annual) and has been calculated from 4th order polynomial equation using annual average data of light intensity, temperature and humidity. Similarly, RWC_m was considered as RWC (monthly) and that has been estimated from 4th order polynomial equation using monthly average data of light intensity, temperature and humidity. The $CT_{(RWC,\ L,T,H)}$ has been calculated from subtracting the RWC (monthly) and RWC (annually). For each month, different $CT_{(RWC,\ L,T,H)}$ have been estimated due to the variations in environmental factors during each month and thus, 12 $CT_{(RWC,\ L,T,H)}$ values have estimated (from Eq. 7.3). Further $CT_{(RWC,\ L,T,H)}$ were validated with the RWC exp data. Further, RWC (monthly) calculated from the Eq 3 and compared with experimental RWC (monthly) data and denoted as RWC (model prediction) and RWC (exp) respectively. It was observed that degree of model prediction error was 0.18 and 0.23 for *Mentha piperita* and *Ocimum sanctum* respectively. The RWC_a, RWC_m and $CT_{(RWC,\ L,T,H)}$ have been calculated for both (*Mentha piperita* and *Ocimum sanctum*) plant species (as shown in the table 7.1 and 7.2).

$RWC_m = RWC_a + CT_{RWC,\ environmental\ factors}$ ..... (Eq 7.3)

Table 7.2 Correction term (CT) for RWC of *Ocimum sanctum* and environmental factors (Temperature, Light intensity, Humidity)

Months	$\mathbf{CT_{(RWC,L,T,H)}}$
April	-5.1
May	-13.65
June	-14.1
July	-20.55
August	-8.55
September	3.85
October	15.6
November	-24.85
December	-21.8

In the Eq 7.4, pH_a was considered as pH (annual) and has been calculated from 4th order polynomial equation using annual average data of light intensity, temperature and humidity. Similarly, pH_m was considered as pH (monthly) and that has been estimated from 4th order polynomial equation using monthly average data of light intensity, temperature and humidity. The $CT_{(pH,\ L,T,H)}$ has been calculated from subtracting the pH (monthly) and pH (annual). For each month, different $CT_{(pH,\ L,T,H)}$ have been estimated due to the variations in environmental factors during each month and thus, 12 $CT_{(pH,\ L,T,H)}$ values have estimated (as Eq 7.4).

$pH_{(m)} = pH_{(a)} + CT_{pH,\ environmental\ factors}$ ..... (Eq 7.4)

Table 7.3 Correction term (CT) for pH of *Mentha piperita* and environmental factors (Temperature, Light intensity, Humidity)

Months	$\mathbf{CT_{(RWC,L,T,H)}}$
January	-0.60
February	-0.88
March	-0.31
April	--0.11
May	-0.11
June	-0.13

Months	$CT_{(RWC,L,T,H)}$
July	-0.14
August	-0.06
September	-0.15
October	-0.43
November	-0.48
December	-0.46

Further $CT_{(pH,\ L,T,H)}$ were validated with the pH exp data. pH (monthly) was calculated from Eq.7.4 and compared with experimental pH (monthly) data and denoted as pH (model prediction) and pH (exp) respectively. It was observed that degree of model prediction error was 0.98 and 0.40 for *Mentha piperita* and *Ocimum sanctum*. The pH_a, pH_m and $CT_{(pH,\ L,T,H)}$ have been calculated for both (*Mentha piperita* and *Ocimum sanctum*) plant species (as shown in the table 7.3 and 7.4).

Table 7.4 Correction term (CT) for pH of *Ocimum sanctum* and environmental factors (Temperature, Light intensity, Humidity)

Months	$CT_{(RWC,L,T,H)}$
April	-0.18
May	-0.39
June	-0.14
July	-0.27
August	-0.49
September	-0.09
October	0.04
November	0.21
December	0.24

In the Eq 7.5, TC_a was considered as TC (annual) and has been calculated from 4^{th} order polynomial equation using annual average data of light intensity, temperature and humidity. Similarly, TC_m was considered as TC (monthly) and that has been estimated from 4^{th} order polynomial equation using monthly average data of light intensity, temperature and humidity. The $CT_{(TC,\ L,T,H)}$ has been calculated from subtracting the TC (monthly) and TC (annually). For each month, different $CT_{(TC,\ L,T,H)}$ have been estimated due to the variations in environmental

factors during each month and thus, 12 $CT_{(TC, L,T,H)}$ values have estimated (as Eq 7.5).

$TC_{(m)} = TC_{(a)} + CT_{TC, \text{environmental factors}}$ ..... (Eq 7.5)

Table 7.5 Correction term (CT) for TC of *Mentha piperita* and environmental factors (Temperature, Light intensity, Humidity)

Months	$CT_{(TC, L,T,H)}$
January	-0.89
February	-0.95
March	-0.12
April	-0.33
May	0.22
June	0.58
July	0.44
August	0.18
September	0.55
October	0.18
November	-0.22
December	-1.08

Further $CT_{(TC, L,T,H)}$ were validated with the TC exp data. TC (monthly) was calculated from the Eq 7.5 and compared with experimental TC (monthly) data and denoted as TC (model prediction) and TC (exp) respectively. It was observed that degree of model prediction error was 0.84 and 0.96 for *Mentha piperita* and *Ocimum sanctum* respectively. The TC_0, TC_m and $CT_{(TC, L,T,H)}$ have been calculated for both (*Mentha piperita* and *Ocimum sanctum*) plant species (as shown in the table 7.5 and 7.6).

Table 7.6 Correction term (CT) for TC of *Ocimum sanctum* and environmental factors (Temperature, Light intensity, Humidity)

Months	$CT_{(TC,L,T,H)}$
April	-0.77
May	0.56
June	0.24
July	0.26
August	0.62

Months	$CT_{(TC,L,T,H)}$
September	0.75
October	0.13
November	-0.62
December	-1.20

In the Eq 7.6, AA_a was considered as AA (annual) and has been calculated from 4th order polynomial equation using annual average data of light intensity, temperature and humidity. Similarly, AA_m was considered as AA (monthly) and that has been estimated from 4th order polynomial equation using monthly average data of light intensity, temperature and humidity. The $CT_{(AA,\ L,T,H)}$ has been calculated from subtracting the AA (monthly) and AA (annually).For each month, different $CT_{(AA,\ L,T,H)}$ have been estimated due to the variations in environmental factors during each month and thus, 12 $CT_{(AA,\ L,T,H)}$ values have estimated (as Eq.7.6).

Table 7.7 Correction term (CT) for AA of *Mentha piperita* and environmental factors (Temperature, Light intensity, Humidity)

Months	$CT_{(AA,L,T,H)}$
January	0.30
February	-0.21
March	-0.30
April	-0.19
May	0.22
June	0.20
July	-0.28
August	0.08
September	0.09
October	-0.10
November	-0.10
December	0.39

Further $CT_{(AA,\ L,T,H)}$ were validated with the AA exp data. As, AA (monthly) was calculated from Eq 7.6 and compared with experimental AA (monthly) data and denoted as AA (model prediction) and AA (exp) respectively. It was observed that degree of model prediction error

was 0.59 and 0.93 for *Mentha piperita* and *Ocimum sanctum* respectively. The AA_0, AA_m and $CT_{(AA, L,T,H)}$ have been calculated for both (*Mentha piperita* and *Ocimum sanctum*) plant species (as shown in the table 7.7 and 7.8).

$AA_{(m)} = AA_{(a)} + AA_{AA, environmental\ factors}$ ..... (Eq 7.6)

Table 7.8 Correction term (CT) for AA of *Ocimum sanctum* and environmental factors (Temperature, Light intensity, Humidity)

Months	$CT_{(AA,L,T,H)}$
April	-0.43
May	0.45
June	0.74
July	0.46
August	0.49
September	0.53
October	-0.00
November	-0.64
December	-1.21

Table 7.9 Biochemical parameters model predicted vs Biochemical parameters experimental data (*Mentha piperita*)

Months	$RWC_{(model\ prediction)}$	$RWC_{(exp)}$	$pH_{(model\ prediction)}$	$pH_{(exp)}$	$TC_{(model\ prediction)}$	$TC_{(exp)}$	$AA_{(model\ prediction)}$	$AA_{(exp)}$
July	83.2	84.9	7.73	7.8	2.0	2.1	2.84	2.9
October	91.2	92.7	7.30	7.2	1.85	2.5	2.43	2.9
March	65	68	7.44	7.5	1.36	1.5	3.18	3
May	79.8	80.4	7.83	7.8	2.09	1.2	2.07	2.5
December	71.2	72.7	6.96	7.1	0.65	0.76	2.36	1.78
September	94.3	95.8	7.82	7.9	2.39	2.6	2.25	2.9

Table 7.10 Biochemical parameters model predicted vs Biochemical parameters experimental data (*Ocimum sanctum*)

Months	$RWC_{(model\ prediction)}$	$RWC_{(exp)}$	$pH_{(model\ prediction)}$	$pH_{(exp)}$	$TC_{(model\ prediction)}$	$TC_{(exp)}$	$AA_{(model\ prediction)}$	$AA_{(exp)}$
July	61	62	6.98	7.54	1.88	1.8	3.13	3.1
October	97.1	98.2	7.31	7.21	1.75	2	2.66	2.8

Months	$RWC_{(model\ prediction)}$	$RWC_{(exp)}$	$pH_{(model\ prediction)}$	$pH_{(exp)}$	$TC_{(model\ prediction)}$	$TC_{(exp)}$	$AA_{(model\ prediction)}$	$AA_{(exp)}$
April	76.4	77.5	7.12	7.39	0.85	0.89	2.24	2.6
May	67.2	68.9	6.86	7.65	2.19	1.8	3.12	3.4
December	58	60.8	7.50	7.01	0.41	0.64	1.46	1.9
September	85.2	86.4	7.17	7.35	2.37	2.1	3.21	3

Standard error was measured with the help of Index of agreement method, and all the degree of model prediction error varies between 0 to 1, that indicates the agreement or perfect match between them.

Table 7.11 Correction term (CT) for RWC of *Ocimum sanctum* and air pollutants (SO_2, NO_2) using linear regression model

$RWC_{(polluted)}$	$CT_{(RWC, SO2,NO2)}$
Jalandhar	-6.2
Amritsar	-16.2
Ludhiana	-10.1
Sector 22	-9.3
Sector 25	-21.1
Sector 53	-14.3

In the Eq 7.7, $RWC_{(polluted)}$ was considered as RWC at polluted sites and has been calculated from linear equation using different concentrations of air pollutant concentrations. Similarly, $RWC_{(control)}$ was considered as RWC at control site, and that has been estimated from linear equation using control area pollutant concentrations.

The $CT_{(RWC, SO2,NO2)}$ has been calculated from subtracting the $RWC_{(polluted)}$ and $RWC_{(control)}$. For each site, different $CT_{(RWC, SO2,NO2)}$ have been estimated due to the variations in air pollutants concentrations at each site. Thus, 6 $CT_{(RWC, SO2,NO2)}$ values have estimated. Further $CT_{(RWC, SO2,NO2)}$ were validated from the RWC experimental data.

Table 7.12 Correction term (CT) for RWC of *Mentha piperita* and air pollutants (SO_2, NO_2) using linear regression model

$RWC_{(polluted)}$	$CT_{(RWC, SO2,NO2)}$
Jalandhar	-9.3
Amritsar	-24.2

Ludhiana	-13.4
Sector 22	-8.6
Sector 25	-17.8
Sector 53	-14.6

From the proposed model, $RWC_{(polluted)}$ calculated and compared with experimental RWC analyzed from each site and denoted as RWC (model prediction) and RWC (exp) respectively. It was observed that degree of model prediction error was 0.47and 0.47 for *Mentha piperita* and *Ocimum sanctum* respectively. The $RWC_{(polluted)}$, $RWC_{(control)}$ and $CT_{(RWC, SO2\ NO2)}$ have been calculated for both (*Mentha piperita* and *Ocimum sanctum*) plant species (as shown in the table 7.11 and 7.12).

$$RWC_{(polluted)} = RWC_{(control)} + CT_{(RWC, SO2,NO2)} \ldots\ldots\ldots \text{(Eq. 7.8)}$$

Table 7.13 Correction term (CT) for pH of *Ocimum sanctum* and air pollutants (SO_2, NO_2) using linear regression model

$pH_{(polluted)}$	$pH_{(RWC, SO2,NO2)}$
Jalandhar	-0.12
Amritsar	0.08
Ludhiana	0.01
Sector 22	-0.06
Sector 25	-0.12
Sector 53	-0.03

In the Eq 7.9, $pH_{(polluted)}$ was considered as pH at polluted sites and has been calculated from linear equation using different concentrations of air pollutant concentrations. Similarly, $pH_{(control)}$ was considered as pH at control site, and that has been estimated from linear equation using control area pollutant concentrations. The $CT_{(pH, SO2,NO2)}$ has been calculated from subtracting the $pH_{(polluted)}$ and $pH_{(control)}$. For each site, different $CT_{(pH, SO2,NO2)}$ have been estimated due to the variations in air pollutants concentrations at each site. Thus, 6 $CT_{(pH, SO2,NO2)}$ values have estimated. Further $CT_{(pH, SO2,NO2)}$ were validated from the RWC experimental data.

Table 7.14 Correction term (CT) for pH of *Mentha piperita* and air pollutants (SO_2, NO_2) using Linear regression model.

$pH_{(polluted)}$	$pH_{(RWC, SO2,NO2)}$
Jalandhar	-1.25
Amritsar	-1.32
Ludhiana	-0.86
Sector 22	-1.22
Sector 25	-1.07
Sector 53	-1.06

From the proposed model (Eq.7.9), $pH_{(polluted)}$ calculated and compared with experimental pH analyzed from each site and denoted as pH (model prediction) and pH(exp) respectively. It was observed that degree of model prediction error was 0.14 and 0.41 for *Mentha piperita* and *Ocimum sanctum* respectively. The $pH_{(polluted)}$, $pH_{(control)}$ and $CT_{(pH, SO2 NO2)}$ have been calculated for both (*Mentha piperita* and *Ocimum sanctum*) plant species (as shown in the table 7.13 and 7.14).

$$pH_{(polluted)} = pH_{(control)} + CT_{(pH, SO2,NO2)} \ldots\ldots\ldots \text{(Eq. 7.9)}$$

Table 7.15 Correction term (CT) for TC of *Ocimum sanctum* and air pollutants (SO_2, NO_2) using linear regression model

$TC_{(polluted)}$	$TC_{(RWC, SO2,NO2)}$
Jalandhar	-0.34
Amritsar	-0.44
Ludhiana	0.98
Sector 22	-0.21
Sector 25	0.20
Sector 53	0.30

In the Eq 7.10, $TC_{(polluted)}$ was considered as TC at polluted sites and has been calculated from linear equation using different concentrations of air pollutant concentrations. Similarly, $TC_{(control)}$ was considered as TC at control site, and that has been estimated from linear equation using control area pollutant concentrations. The $CT_{(TC, SO2,NO2)}$ has been calculated

from subtracting the $TC_{(polluted)}$ and $TC_{(control)}$. For each site, different $CT_{(TC, SO2,NO2)}$ have been estimated due to the variations in air pollutants concentrations at each site. Thus, 6 $CT_{(TC, SO2,NO2)}$ values have estimated. Further $CT_{(TC, SO2,NO2)}$ were validated from the TC experimental data.

Table 7.16 Correction term (CT) for TC of *Mentha piperita* and air pollutants (SO_2, NO_2) using Linear regression model

$TC_{(polluted)}$	$TC_{(RWC, SO2,NO2)}$
Jalandhar	-0.60
Amritsar	-1.82
Ludhiana	-0.17
Sector 22	-0.81
Sector 25	-0.1
Sector 53	-0.47

From the proposed model, $TC_{(polluted)}$ calculated and compared with experimental TC analyzed from each site and denoted as TC (model prediction) and TC (exp) respectively. It was observed that degree of model prediction error was 0.23 and 0.3 for *Mentha piperita* and *Ocimum sanctum* respectively. The $TC_{(polluted)}$, $TC_{(control)}$ and $CT_{(TC, SO2 NO2)}$ have been calculated for both (*Mentha piperita* and *Ocimum sanctum*) plant species (as shown in the table 7.15 and 7.16).

$$TC_{(polluted)} = TC_{(control)} + CT_{(TC, SO2,NO2)} \text{ …….. (Eq. 7.10)}$$

Table 7.17 Correction term (CT) for AA of *Ocimum sanctum* and air pollutants (SO_2, NO_2) using linear regression model

$AA_{(polluted)}$	$AA_{(RWC, SO2,NO2)}$
Jalandhar	0.84
Amritsar	1.98
Ludhiana	-0.33
Sector 22	0.95
Sector 25	0.06
Sector 53	0.34

In the Eq 7.11, $AA_{(polluted)}$ was considered as AA at polluted sites and has been calculated from linear equation using different concentrations of air pollutant concentrations. Similarly, $AA_{(control)}$ was considered as AA at control site, and that has been estimated from linear equation using control area pollutant concentrations. The $CT_{(AA,\ SO2,NO2)}$ has been calculated from subtracting the $AA_{(polluted)}$ and AA $_{(control)}$. For each site, different $CT_{(AA,\ SO2,NO2)}$ have been estimated due to the variations in air pollutants concentrations at each site. Thus, 6 $CT_{(AA,\ SO2,NO2)}$ values have estimated. Further $CT_{(AA,\ SO2,NO2)}$ were validated from the AA experimental data.

Table 7.18 Correction term (CT) for AA of *Mentha piperita* and air pollutants (SO_2, NO_2) using linear regression model

$AA_{(polluted)}$	**$AA_{(RWC,\ SO2,NO2)}$**
Jalandhar	1.96
Amritsar	4.02
Ludhiana	0.12
Sector 22	2.19
Sector 25	0.67
Sector 53	1.20

From the proposed model, $AA_{(polluted)}$ calculated and compared with experimental AA analyzed from each site and denoted as AA (model prediction) and AA (exp) respectively. It was observed that degree of model prediction error was 0.62 and 0.68 for *Mentha piperita* and *Ocimum sanctum* respectively. The $AA_{(polluted)}$, $AA_{(control)}$ and $CT_{(AA,\ SO2\ NO2)}$ have been calculated for both (*Mentha piperita* and *Ocimum sanctum*) plant species (as shown in the table 7.17 and 7.18).

$$AA_{(polluted)} = AA_{(control)} + CT_{(AA,\ SO2,NO2)} \ldots\ldots\ldots \text{(Eq. 7.11)}$$

Standard error was measured with the help of Index of agreement method, and all the degree of model prediction error varies between 0 to 1, that indicates the agreement or perfect match between them. However, once CT was calculated from the developed integrated model. $BC_{(a)}$ can be easily calculated from the CT and $BC_{(m)}$.

Suppose if $BC_{(m)}$ has been calculated experimentally for a month, we can calculate $BC_{(a)}$ with the help of CT and $BC_{(m)}$ without doing laboratory experiment. All these biochemical parameters are combined together into a proposed APTI model which can be written as follows in Eq.7.12 and Eq.7.13

$$APTI = \frac{AA_m + CT_{AA,EF} + (TC_m + CT_{TC,EF} + pH_m + CT_{pH,EF}) + RWC_m + CT_{RWC,EF}}{10} \ldots\ldots\ldots \text{(Eq.7.12)}$$

Here, CT is site specific and can be include environmental factors data from a particular area.

$$APTI = \frac{AA_m + CT_{AA,AP} + (TC_m + CT_{TC,AP} + pH_m + CT_{pH,AP}) + RWC_m + CT_{RWC,AP}}{10} \ldots\ldots\ldots\ldots \text{(Eq.7.13)}$$

Here, CT is pollutant concentration specific and can be include any air pollutants concentrations data from a particular area.

Table 7.19 Comparison of APTI values for *Mentha piperita* using existing and proposed APTI models

APTI (Existing Model)	**APTI (Proposed Model)**
7.51	8.61
8.82	7.92
9.38	9.27
8.64	9.85
10.48	10.96
10.37	10.12
10.94	10.94
11.16	10.91
12.11	10.83
12.08	10.51
8.14	10.04
8.61	7.69

Table 7.20 Comparison of APTI values for *Ocimum sanctum* using existing and Proposed APTI models

APTI (Existing Model)	APTI (Proposed Model)
9.61	9.66
9.83	10.10
9.78	10.22
8.89	9.49
10.42	9.91
11.63	12.17
12.45	10.81
7.31	8.44
7.35	6.80

Table 7.21 Validation of proposed model for plants from different regions

Plants	Location	RWC (exp)	RWC(model prediction)	pH (exp)	pH(model prediction)	TC (exp)	TC(model prediction)	AA (exp)	AA(model prediction)
Ocimum sanctum	Tamil Nadu	69	92.6	6.5	4.5	14	**12.44***	5	0.8
	Bengaluru	59.45	93.55	7.34	**7.66***	519.4	**517.9***	178.6	**171***
	Varanasi	81	92	6.2	5	5.6	**4.08***	3.4	0.94
	West Bengal	60.1	**75.5**	7.6	0.4	7.4	2.4	4.6	2.8
	Uttar Pradesh	69	84.4	8	0.54	20	13.76	2.9	5.7
Mentha piperita	Iran	88.7	**83.5***	7	2.6	4.12	1.8	25.97	**21.23***

(exp = experimental values). * Model prediction values were found to be close to the experimental values.

Table 7.22 Comparison of APTI values for plants from different regions using existing and Proposed APTI models

Plants	**Location**	**APTI (Existing Model)**	**APTI (Proposed Model)**
Ocimum sanctum	West Bengal	12.9	9.1
	Varanasi	12.1	**10***
	Bengaluru	9.4	**10.7***
	Uttar Pradesh	9.4	**10.7***
	Tamil Nadu	9.6	**9.3***
Mentha piperita	Iran	38	17.86

* Model prediction values were found to be close to the experimental values.

CHAPTER 8 SUMMARY AND CONCLUSION

In the present study, an attempt has been made to **study the effects of environmental factors on plants tolerance to air pollutants.** The study has been conducted during 2019-2024. The present study is based on the traditional method (APTI) to mitigate air pollution of a particular area. It is a biological and economical method to evaluate the plant species affected by air pollution. Based on APTI method, biochemical parameters could be used to evaluate the tolerant and sensitive plants. Variation in biochemical parameters affects plant tolerance. However, literature studies have shown that various parameters affect biochemical parameters under stress conditions, thus various parameters also affects the tolerance. Previous literature (as discussed in **chapter 2**) also highlights the other parameters too but detailed relationship is needed to be explored. This needs to be addressed when calculating plant tolerance index for effective screening and identification of plants and for effective mitigation measures against air pollution. Based on these assumptions, APTI of the plant species have been estimated from different regions (as discussed in **chapter 4**). As a result, the same plant species exhibited variation in biochemical parameters at different sites. Besides, plants exhibited higher APTI values at the control sites compared to the polluted sites. It was due to the different environmental factors, pollutant concentrations, morphological parameters and soil type etc. Further, APTI, biochemical parameters and morphological parameters did not showed any specific pattern with each other. However statistically, it was observed that they had correlation with each other. Hence, it provides an insight that morphological parameters including other parameters can also prove to be important in investigation the ability of plant to cope with air pollution and calculating tolerance index.

Multiple parameter analysis increases the likelihood of identifying air pollutant tolerant plant species compared to single parameter analysis. Further, current study explored the effect of Environmental factors on biochemical parameters of plants (as discussed in detailed in **chapter 5**). The multiple linear and non linear regression models was developed for precisely predicting the effect of collective environmental parameters on the biochemical parameters of selected plant species with best fitted results (R_{nL} = 0.7). Results showed best fit with non linear multiple regression R_{nL}= 0.75, 0.42, 0.7, 0.55 for Ascorbic acid, Relative water content, Total chlorophyll and pH respectively in *Ocimum sanctum.* While in *Mentha piperita* results showed best fit with non linear multiple regression R_{nL}= 0.76, 0.56 for Total chlorophyll and Relative water content respectively. The current study emphasize the significance of considering multiple environmental factors collectively rather than a focusing solely on

individual parameters when assessing their impact on biochemical parameters in plants.

These findings deepen understanding the complex relationship between environmental factors and physiological processes in plant. On other side, in **chapter 6** findings of current study underscore the substantial influence of air pollutants on the biochemical parameters of plants. Using a proposed multiple linear regression model, we were able to predict the impact of air pollutants on selected plant species, yielding impressive fit results (R^2 = 0.9). The scatter plots revealed compelling linear relationships, with R^2 values of 0.91, 0.86, and 0.66 for relative water content, ascorbic acid, and total chlorophyll, respectively, in *Ocimum sanctum*. Similarly, in *Mentha piperita*, significant correlations were observed, with R^2 values of 0.93, 0.79, and 0.68 for ascorbic acid, relative water content, and total chlorophyll, respectively.

Moreover, the current study underscores the importance of considering multiple air pollutants collectively, rather than focusing solely on individual parameters, when evaluating their impact on plant biochemical parameters. Besides, the current study provides deeper insights into the intricate relationship between air pollutants and physiological processes in plants.

These findings contribute to a more comprehensive understanding of the complex interplay between environmental factors and plant health. Thus, based on the present findings there was a need to modify the traditional APTI model to obtain better results. Hence, it will help in extenuating air pollution in a better way. The proposed models have been validated and more parameters were added to the existing APTI model as integrated APTI model (as discussed in **chapter 7**). The degree of model prediction error was varied between 0 and 1. All the measured values were close to 1which indicates the agreement and perfect match between them. A better agreement was observed in non linear models compared to linear models due to the multivariate parameters considered.

The present study confirmed the assumption that biochemical parameters are influenced by various parameters such as environmental factors and air pollutants. By using secondary environmental and air pollutants data of a particular area, biochemical parameters of a plant can be calculated with the help of modified model minimizing the need of laboratory experiment and resources. Using the modified model, tolerant and sensitive plant species can be identified more precisely. Tolerant plants can be used for plantations to develop green belts and green microclimates in urban landscaping. Government has also started number initiatives for developing green belts in urban areas. Thus, the selection of appropriate plant species for green belts and phyto remediation to improve air quality in urban areas helps to achieve environmental sustainability.

Limitations of the current study

In the present study, two air pollutants were considered. Like other air pollutants such as Ozone, particulate matter has also detrimental effects on plants that should also be important to consider. Additionally, detailed information on the pollutant sources (type of the pollutant source, distance from the receptor, emission profile etc.) was not considered in the present study. Two morphological parameters were studied, to study the relationship with biochemical parameters and morphological parameters. Other parameters, such as stomatal frequency, trichomes, shape, surface area, petiole size, arrangement and cuticular texture, edaphic parameters etc., may play important roles in the alteration of plant biochemistry. These parameters can also be considered to better understand the relationship with biochemical parameters and morphological parameters. Several factors influencing tolerance, including soil type in addition to environmental factors and pollutant concentrations were reported in previous literature. However, soil parameter was not considered in present study.

Suggestions and Future work

In the present study, two morphological parameters were studied. Several other morphological parameters (leaf length, leaf breadth, petiole length, midrib width etc) would also be considered. A more detailed study encompassing the effects of the aforementioned factors would be helpful in exploring the general relationship between the plant morphology and the plant tolerance against air pollution. Similar studies in different geographical regions may also reveal a more clear insight into this correlation. The proposed non linear model is climate specific, thus more climate data inventory would be developed to estimate plant tolerance of different regions without Laboratory experimentation. A large climate specific APTI database of different or the same plant species around the world can be prepared without damaging the plants (as leaves are collected as samples, which could be avoided). This can be a more economical, time saving and sustainable approach in the long run. Moreover, this data inventory could help the urban planners, industrialists and researchers to suggest plants that could help to combat environmental stress.

On other side, the proposed integrated linear model is pollutant specific. The effects of various other air pollutants (eg. Ozone, particulate matter, heavy metals, Fluorine etc) including SO_2 and NO_2 on plants would be predicted. This could suggests the plant tolerance to specific pollutants if other pollutants data would also included

An extensive database of biochemical parameters and their corresponding ambient SO_2 and NO_2 concentrations would be more reliable for establishing the relationship between air pollutants and biochemical parameters. This limitation opens up the scope of extensive future work involving a larger dataset, robust statistical methods, and artificial intelligence. Effective tolerance model would act as a sustainable strategy to reduce air pollution in urban area.

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LIST OF PUBLICATIONS

- A review on the effects of environmental factors on plants tolerance to air pollution" (Published in *Journal of Environmental Treatment Techniques in 2021) (*SCI, Impact factor;1*).*
- "Role of air pollution tolerance index (APTI) method for green belt development: A review" (published in *Environment Monitoring and Assessment*) (SCI, Scopus, Q2, Impact factor ;3.1).
- "Assessing the relationship between morphological and biochemical parameters of industrial and roadside plants'' (Published in *Environment Monitoring and Assessment)* (SCI, Scopus, Q2, Impact factor;3.1)
- "The Effects of Atmospheric Nitrogen Dioxide and Sulphur Dioxide on the Biochemical Factors of Plant Tolerance" (Published in *Ecotoxicology,* SCI, Scopus, Q2, Impact factor;2.1).

LIST OF CONFERENCES AND WORKSHOPS

- Oral Presentation in "5th International Conference on Advances in Agriculture Technology and Allied Sciences (ICAATAS 2022)" Centurion University of Technology and Management, Paralakhemundi, Odisha on June 4-5 , 2022.

- Oral Presentation in " Second 3S International Virtual Conference "Sustainability, Spirituality and Simplicity (3S): Environmental sustainability redefined through simple life practices and integrated spirituality" ISS Delhi June 4th and 5th, 2022.

- Oral Presentation in "International Web Conference on Perspective on Agricultural and Applied Sciences (PAAS-2020) "October 4-6, 2020.

- Participated in "India Clean Air Summit "(ICAS) 2021 on 26 and 27 August 2021.

- Participated in a virtual workshop on "National Workshop on Research Methodology and Data Analysis using SPSS" Lovely Professional University, 2021.

- Participated in 5 day National workshop on "Technology emergence for clean water and Air" during 29th may -2 June 2023, organized by National institute of technology (NIT) Rourkela, India.