ASSESSMENT OF AIR POLLUTION TOLERANCE INDEX AND LEAF STRUCTURE OF SELECTED PLANT SPECIES AROUND UDAYAPUR CEMENT FACTORY, NEPAL

A THESIS

SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER'S DEGREE IN BOTANY

BY

POOJA PRASAI

Symbol No. 445/073

(T.U. Registration No: 5-2-37-78-2011)

DEPARTMENT OF BOTANY

AMRIT CAMPUS

TRIBHUVAN UNIVERSITY

KATHMANDU, NEPAL

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DECLARATION

I, "Pooja Prasai", hereby declare that the work enclosed here is entirely my own, except where states otherwise by reference or acknowledgement, and has not been published or submitted elsewhere, in whole or in part, for the requirement for any other degree or professional qualification. Any literature, data or works done by others and cited within this thesis has been given due acknowledgement and listed in the reference section.

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RECOMMENDATION

This is to recommend that the Master's thesis entitled "**Assessment of Air Pollution Tolerance Index of Selected Plant Species Around Udayapur Cement Factory, Nepal**" is carried out by "Pooja Prasai" under my supervision. The entire work is based on original scientific investigations and has not been submitted for any other degree in any institutions. I therefore, recommend this thesis work to be accepted for the partial fulfilment of M.Sc. Degree in Botany.

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APPROVAL

The thesis work submitted by "Pooja Prasai" entitled "**Assessment of Air Pollution Tolerance Index of Selected Plant Species Around Udayapur Cement Factory, Nepal**" submitted to Department of Botany, Amrit Campus, Tribhuvan University by "Pooja Prasai", "5-2-37-78-2011 " has been accepted for the partial fulfilment of the requirement for Master's Degree in Botany.

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ACRONYMS AND ABBREVIATIONS

APTI	Air pollution tolerance index
AA	Ascorbic acid content
cm	Centimeter
DMSO	Dimethyl sulfoxide
E	East
g	Gram
mg	Milligram
ml	Milliliters
N	North
nm	Nanometer
pH	Potential of Hydrogen
PM	Particulate matter
ROS	Reactive oxygen species
RWC	Relative water content
SLA	Specific Leaf Area
S	South
Tchl	Total chlorophyll content
UNEP	United Nations Environmental Programme
W	West
WHO	World Health Organization
µm	Micrometer

ABSTRACT

Present study investigate the effect of cement dust on plants physiology and morphology around Udayapur Cement Factory. Plants can be both bio-indicator and tolerant in order to control the air pollution in urban and industrial areas. Eight commonly available plant species of tree and shrub were selected from study area. Plant species were collected from four directions and four different radial distances from the factory *viz.* (0-250 m), (250-500 m), (500-750 m) and (750-1000 m) from the Udayapur Cement Factory. To study the relative tolerance of plants towards air pollutants, four parameters namely ascorbic acid, leaf extract pH, total chlorophyll and relative water content were measured and computed together to calculate air pollution tolerance index (APTI). The characteristics of leaf structure (stomatal length, breadth and density and specific leaf area) were also studied. The APTI values of most of the plant species increased significantly at distance near to the factory at 0-250 m distance. All the tree species showed high APTI values in eastern direction and among the shrub species studied *Colebrookea oppositifolia* and *Clerodendrum viscosum* had high APTI values at northern direction, *Melastoma melabathricum* showed high APTI values in eastern direction and *Phoenix acaulis* had high APTI values at western direction. Most of the species showed high APTI values in east direction which indicates high air pollution in this direction, which may be due to the presence of road between the forests in order to transport cement from the factory as well as the dust emission from the factory and also the direction of westerly wind. Based on APTI values three tree species (*Casearia graveolens, Cassia fistula* and *Shorea robusta*) and two shrub species (*Colebrookea oppositifolia and Phoenix acaulis*) were found to be tolerant species with APTI values ranging above seventeen. *Derris elliptica* was considered as intermediate species with APTI value ranging between twelve and sixteen. Likewise, shrub species namely *Melastoma melabathricum* and *Clerodendrum viscosum* measured APTI value less than eleven and were kept under sensitive category. The data of stomatal density showed increasing trend and stomatal length and breadth showed decreasing trend while moving near to the cement factory (0-250 m). SLA of all plant species decreased near the cement factory.

Key words: *Ascorbic acid (AA), Total chlorophyll content, Leaf extract pH, Cement dust, Specific leaf area.*

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CHAPTER 1: INTRODUCTION

1.1. Background

A physical, chemical or biological alteration to the quality of air in the atmosphere can be termed as pollution. Air pollution is majorly caused due to the release of various chemicals into the atmosphere. The sustainment of all the living beings is due to a combination of gases that collectively form the atmosphere and any imbalance can be harmful to survival. Air pollution is one of the major problem created due to rapid industrialization and urbanization. The major airborne pollutants in urban areas are sulphur dioxide (SO_2), nitrogen oxides (NO_X), carbon monoxide (CO), particulate matter (PM 10 with a diameter of 10μm or less and PM 2.5 with a diameter of 2.5 μm or less), non-methane volatile organic compounds (NMVOCs), ozone, and ammonia (NH_3) (Pradhan *et al*., 2012). Some pollutants are emitted directly (primary pollutants), while others are formed in the atmosphere from emitted precursors (secondary pollutants such as ozone and secondary particles). Air pollutants either gaseous or particulates both are known to produce harmful effects on plants (Singh, 2005). Pollutants deposition occurs only either through wet or dry deposition. Wet deposition is less important since the pollutants present in the atmosphere is washed away and even from the leaf surface. But the dry deposition process makes the expose to the ambient condition for the longer time (Reinert, 1984). In dry climates, air quality becomes even worse due to various gaseous and particulate materials suspended in air (Ahmad *et al*., 2019).

Cement industries are considered as a major sources of greenhouse gases emission and contributors of extremely large amounts of dust (Ghada and Ahmed, 2016; Magiera *et al.*, 2013). The processes of cement production like calcinations of limestone, combustion of fossil fuel and electricity consumption causes CO_2 emission. The cement production requires massive amount of energy and during storage, milling, packing and transportation huge amount of fly ash and dust is generated (Dubey, 2013). The production of cements has developed as a potential threat to the natural environment and living organisms (Magiera *et al*., 2013). Since plants are stationary and continuously exposed to chemical pollutants from the surrounding atmosphere, air pollution injury to plants is proportional to the intensity of the pollution. The cement dust strongly influence the physiology and morphology including plant height, leaf area, reductions in chlorophyll and carotenoid, non-reducing and total sugars, protein and total lipid contents of the plant (Salama *et al*., 2011). Thus the plants surrounding the cement industry with high pollution exposure can not only be recommended as dust tolerant but can also be used for efficient bio-monitoring (Radhapriya *et al*., 2012). Plants are an integral part of our ecosystem, and trees and shrubs, being long-lived components of the landscape, play a significant role in improving the environmental conditions. Trees and shrubs are more affected by pollutants because they are perennial, and plants exhibit different responses and absorption/resistance to various pollutants (Ahmad *et al*., 2019).

Morphology and internal structure of leaves is altered by heavy load of dust pollutants (Gostin, 2009). It was found that dust deposition affect photosynthesis, stomatal functioning and productivity (Prajapati and Tripathi, 2008). Ade-Ademilua *et al*., (2008) reported a significant reduction in shoot length, total leaf area and dry weight of plants affected by cement dust pollution. There is decrease in size and increase in stomatal density in the leaves of polluted sites (Gostin, 2009). Reduction in growth parameters are due to the cumulative effects of the causal factors on the physiological processes necessary for plant growth and its development (Schutzki and Cregg, 2007). It has also been reported that when exposed to air pollutants, most plant experience physiological changes before exhibiting visible damage to leaves (Dohmen *et al*., 1990). On the other hand, pollutants can cause leaf injury, stomata damage, premature senescence, decreased photosynthetic activity, disturb membrane permeability and reduce growth and yield in sensitive plant species (Tiwari *et al.*, 2006). Air pollution stress leads to stomatal closure, which reduces CO_2 availability in leaves and inhibits carbon fixation (Robinson *et al.,* 1998). Various strategies exist for controlling atmospheric pollution, but vegetation provides one of the natural ways of cleansing the atmosphere by absorption of gaseous and some particulate matter through leaves (Varshney, 1985). Despite of detrimental effects of cement dusts on plants, some still remain tolerant to cement dust pollution. This may be due to the genetic make-up or some biochemical/anatomical modifications during the stress periods (Erdal and Demirtas, 2010). The response of plants to pollutant absorption can be used as an economical and sustainable tool to estimate air quality (Rai *et al.,* 2013).

Plants are highly suitable for the detection, monitoring and mitigation of air pollution effects (Singh, 2005). The degree of sensitivity and tolerance varies among plant species (Khan and Abbasi 2001). Bio-monitoring of air pollution has been found to be extremely useful in detecting the kind and level of pollutants in the air with or without measurements (Prusty *et al*., 2005). Studies have shown that plants can reduce the number of particles in the air by one-third as compared to areas with no vegetation (Setala *et al*., 2013). The plant species which are more sensitive act as biological indicators of air pollution (Lakshmi *et al.,* 2009). To understand if the plant is sensitive or tolerant to air pollution, it can be studied by calculating an index known as Air Pollution Tolerance Index (APTI). In this method four factors namely ascorbic acid, total chlorophyll content, leaf extract pH and relative water content are used to calculate the index (Singh, 1983). Species having higher APTI value are more tolerant to air pollution than those having lower APTI value. Species having lower APTI value may act as bio-indicators of pollution (Lohe *et al*., 2015). Air pollution tolerant shows the plants capabilities to tolerate air pollution (Hamal, 2017). On the basis of APTI values, the plants are categorized under tolerant, intermediate, sensitive, and most sensitive species. Previous studies also showed the impact of air pollution on ascorbic acid content (Agbaire and Esiefarienrhe, 2009), chlorophyll content (Liu and Ding, 2008), leaf extract pH (Klumpp *et al*., 2000) and relative water content (Rao, 1977). Such statistics may be used in a plantation of trees at different polluted sites to withstand air pollutants (Jyoti and Surinder, 2017). APTI determines the reaction of plants towards air pollution and the ability of plants to fight against air pollution (Pathak *et al*., 2011).

With an area of 147,181sq km, Nepal occupies the central part of the Himalayas standing between the Palaeartic (Holartic) and Plaeotropical (Indo-Malayan) regions. Nepal's biodiversity is a reflection of its unique geographic position and variations in altitude and climate. Nepal is one of the developing country and urbanization pattern does not seem geographically uniform across Nepal. In the absence of a strong policy for the regulation and management of rapid urbanization and haphazard developmental projects, people are being victimized with serious airborne diseases. According to a report of World Health Organization (WHO), the maximum status of fine Particulate Matter (PM2.5) in urban areas of Nepal was noted to be 140 $\mu g/m^3$ which is 10 times higher than the desirable value (WHO, 2016). In Nepal over the past few decades, rural-urban migration has led to an increase in the number of people living in urban areas. In 1990, less than nine per cent of Nepalese people lived in cities, by 2050 it is estimated that almost half of the population will be urban (Pradhan *et al*., 2012). Udayapur district of Nepal lies in the eastern part of Nepal. It is surrounded by Mahabharat hills from north and Shiwalik from south, whereas both hills meet together by west which forms the region a valley Udayapur valley. Udayapur cement factory was established in 14th June, 1987 with the capacity of 800 ton per day (Shrestha *et al.,* 2016). The impact of cement dust on local vegetation have not been conducted yet. So this study is mainly focused on the assessment of air pollution tolerance index of plants surrounding Udayapur cement factory.

1.2. Justification

The quality of ecological attribute are in declining stage due to unmanaged developmental activities. Air quality is severely affected by rapid urbanization and industrialization. Hence, in the latest years, urban vegetation have become increasingly important not only for social reasons but also for mitigating air quality.

Udayapur Cement Factory was established in 14th June, 1987 with the capacity of 800 ton per day. There are school, settlement areas and market around 1km from the factory. These areas are exposed to dust pollution and both human and vegetation around are affected with it. Till the date no work has been conducted to understand the effects of dust on plants within area. The APTI determination provides a reliable method for screening large number of plants with respect to their susceptibility to air pollution and it is been used for long time. In case of Nepal, the APTI research are done but very few in number and also in case of Udayapur Cement Factory such research work hasn't been conducted yet. So, APTI analysis can be a step forward for the study of the vegetation and pollution status of this area.

1.3. Research questions

Do all plant species existing in the area have similar response to air pollutant?

1.4. Objectives

1.4.1. General objective

To measure the responses of plants to air pollutants around the Udayapur Cement Factory.

1.4.2. Specific objectives

1. To determine the tolerant, intermediately tolerant and sensitive plant species by measuring different biochemical parameters of APTI.
2. To measure changes in leaf structure of plants exposed to air pollutants.

1.5. Limitation

APTI work is more evident during winter months because of more dust pollution. So, this study was done only during winter season.

CHAPTER 2: LITERATURE REVIEW

The APTI determination provides a reliable method for screening large number of plants with respect to their susceptibility to air pollutants. The methods is simple and easy to adopt and can be used successfully in identifying the tolerant plants (Singh *et al.*, 1991). Limestone and cement dusts, with pH values of 9 or higher, may cause direct injury to leaf tissues or indirect injury through alteration of soil pH (Vardaka *et al.*, 1995; Auerbach *et al.*, 1997). The toxicity of cement dust on the growth of some tree seedlings was also observed by Iqbal and Shafiq (1998). The study concluded that by using APTI it would be possible to recommend plants for screening and improving air quality. Pal *et al.*, 2002 suggested that those plants which were continuously exposed to air pollution have significant changes in the leaf surface structure. It was also observed that epidermal cells were collapsed, cell boundaries were irregularly fused and a two folded increase in stomatal frequency and trichrome length in the micromorphology of leaves of polluted sites.

The use of plants as a bio-indicators is inexpensively easy technique and the analysis of biochemical parameters like chlorophyll, protein, ascorbic acid can assess air quality (Tripathi and Gautam, 2007). The study of physiological responses of some tree species along the roadside of Hariduwar city, India concluded that plant species grown along the roadside act as an absorbent of the various pollutants (Joshi and Swami, 2007). They also suggested that this type of analysis may give the level of pollutant in a particular area of interest. Yan-Ju liu *et al.*, (2008) summarized that combining different parameters can give a more reliable result than only based on a single biochemical parameter. So APTI can help to identify the species which can tolerate air pollution. The study concluded that the air pollution tolerance is also affected by natural climate conditions such as temperature and humidity. Air pollution in urban and industrial areas may get adsorbed, absorbed, accumulated or integrated in the plant body and if toxic, may injure plants in various ways as suggested by Lakshmi *et al.*, (2009). The level of injury can be high in sensitive species and low in tolerant species.

In the study of active monitoring using transplanted mosses Shakya (2012) found that the concentrations of air borne metals (except Mn) were mostly high in all transplanted site. The use of mosses was found to be quite useful in monitoring the intensity and trend of air pollution. In an assessment of APTI of selected plants around cement industry, Coimbatore, India, Radhapriya *et al.*, (2012) showed that plants surrounding the cement industry are indicative of high pollution exposure. The generation of new plants were also less in polluted area than in non-polluted area. The study concluded that the highly tolerant, moderately tolerant and intermediately tolerant species will be suitable for the establishment of an effective "green belt" around the cement factory. The cultivation of those species could be encouraged in large numbers to abate the problem of particulate pollution. Gharge and Menon (2012) suggested that tolerant plant species can be used as indicators of pollution there by acting as a sink to all air pollutants. The study done by Kumar and Thambavani (2012) indicates that exposure to particulate deposition may alter plant growth without physical damage to the plant.

APTI is an important index to mitigate the pollution created by rapid urbanization and industrialization as suggested by Enete *et al.,* (2012). The study showed that plants are more tolerant to air pollution than ornamental shrubs; and such should be preferred for landscaping. Different plants respond differently to air pollution and in the selected study site of Khureshi (2013), plants growing in the polluted environment showed higher APTI than those plants growing in less polluted environment. Hence, the study concluded that those plants species with higher APTI value can be useful for mitigating air pollution. On The comparative assessment of APTI in both industrial and non-industrial area by Rai *et al.,* (2013) it was reflected that tolerance of plants towards air pollution may be site-specific. An overview of the entire result obtained from the study reveals that industrial site have higher APTI values as compared to non-industrial site. Furthermore, Babu *et al.,* (2013) also observed that with increase in air pollution there will be an increase in damage to flora and shrubs like *Cassia auriculata* and *Bougain villea spectabilis* and trees such as *Aegle marmelosa* can be used as sink towards air pollutants. The study on the effect of stone crusher dust on *Butea monosperma* (Lam.) showed that air pollution brought significant changes in foliar morphology of the studied plant (Rahul, 2013). The study also concluded that dust accumulation altered the chlorophyll and carotenoid contents in all plants in the polluted location compared with plants far from the factory in control site. Interception capacity and alteration of various biometric and biochemical attributes in cultivated population of *Ficus carica* was studied by Younis *et al.,* (2013). During the study it was observed that the dust accumulation has caused a significant effect on almost all foliage and biochemical attributes of *Ficus carica*.

Cement dust is one of the major pollutant in surrounding area and would affect human health and other living communities (Oran *et al.,* 2014). The study also concluded that it can also block stomata of leaf surface, might affect photosynthesis, respiration, transpiration, and may causes leaf injury symptoms. The study conducted by Krishnaveni and Lavanya 2014 suggested that plants that are continuously exposed to pollutants leads to accumulation of pollutants, integration of pollutants in to their own systems, thereby altering the nature of leaf and make them more sensitive. The evaluation of plant responses based on single criteria cannot be feasible but a combination of variety of parameters can give a more reliable result (Tsega and Prasad, 2014). Thus APTI determination provides a reliable method for screening large number of plant species with respect to their susceptibility to air pollution. APTI of some terrestrial plants around an industrial area in the Cuddalore District in the Indian state of Tamil Nadu was studied by Bakiyaraj and Ayyappan (2014). According to the study, APTI determination of plants is important in recent century of urbanization and industrialization. It provides information regarding to the tolerance capacity of crop plants which can be recommended to farmers of industrial areas. Plants with higher APTI value can be used as pollution tolerant plants and plants with lower APTI value are considered as bio-indicators (Uka and Chukwuka, 2014). On their assessment of pollution tolerance index of sixteen plants in abakaliki metropolis, south-eastern, Nigeria, they observed that *Magnifera indica* and *Carcia papaya* can be deduced as tolerant to air pollution. Pandey *et al.,* 2015 assessed that climbers along with other higher plants can act as a great sink of air pollutants.

On estimation of tolerance levels of different species when affected by particulate pollution Anoob *et al.,* (2016) found that *Butea monosperma* and *Cassia fistula* were more tolerant which were grown more extensively in the form of plantation in their study area. While species like *Bombax ceiba* and *Terminalia catappa* were found to be sensitive to pollutant. From the study they concluded that selection of trees based on such analysis helps in identifying the best suitable trees for a given location. Planting of tolerant species of economic or ecological value also confirms its chances of survival as well as remediation of the region. APTI of selected plant species along roadside at Karwi, India was studied by Chaurasia and Karan (2016) and suggested that plants have potential to serve as excellent quantitative and qualitative indices of pollution.

APTI of some selected gymnosperm species of Kathmandu was studied by Hamal (2017) and concluded that *Pinus roxburghii, Thuja orientiales, Cedrus deodara* and *Araucaria bidwillii* have high APTI and hence they can withstand air pollution along road side of Kathmandu. Estimation of APTI and API can be reliable method for the selection of appropriate species for green area development in and around traffic congested points, and commercial and industrial area (Kaur and Nagpal, 2017). Likewise, Tak and Kakade (2017) also concluded that tolerant tree species can serve as a sink and sensitive tree species can act as an indicator for air pollution mitigation.

The effect of dust pollution on foliage physiology of urban trees was studied by Chaudhary and Rathore (2019). The result of the study suggested that dust deposition causes impact on leaf area, biomass, pigments and membrane permeability of selected trees species. The study also concluded that *Ficus virens* as a multi benefiting tree for sustainable urban plantation planning due to its high air pollution tolerance index and moderating dust capturing capacity. Pollution tolerance assessment of temperate woody vegetation in Himachal Pradesh was analyzed by Sharma *et al*., 2019 and evaluated APTI and API values of six commonly growing plant species viz., *Quercus leucotrichophora, Rubus ellipticus, Debregeasia saeneb, Hypericum oblongifolium, Punia granatum* and *Grevillea robusta*. On the study, the highest value of APTI and API was observed for *Grevillea robusta* (12.89) and second highest value was observed for *Punica granatum* (10.87). Considering the APTI and API value, the study suggested that *Punica granatum* for plantation among native species. It was also concluded that a combination of APTI and API provides more reliable results to figure out the most suitable plant species for green belt development. Ahmad *et al.*, (2019) concluded that those plant species which are grown at industrial site have higher APTI value indicating higher level of pollutants and a greater efficiency of plants to absorb those pollutants. Likewise, Simran *et al*., (2019) also studied APTI and API of ten plant species growing on college area in New Delhi and concluded that tolerant species can give maximum protection against pollution. Particulate matter tolerance of plants in a biodiversity hotspot located in a tropical region was analyzed by Rai, (2019). According to the study air pollution tolerance indices are of paramount importance as they help to study the interaction of air pollution on plants, with possible eco-control implication for environmental management.

2.1. Reviews on different parameters of APTI

2.1.1. Chlorophyll

Chlorophyll content in all the plants varies with pollutant status of the area i.e. higher the pollution level in the form of vehicular exhausts lower is the chlorophyll content (Jyothi and Jaya, 2009). Effect of air pollution on chlorophyll content of leaves was studied by Giri *et al.*,2013 and concluded that the photosynthetic pigments are must likely to be damaged by air pollution. The result of the study also indicated that the reduction in chlorophyll content is due to degradation of chlorophyll into phaeophytin by the loss of magnesium ions. Similarly presence of heavy metals in the environment was found to be highly toxic to plants (Pant and Tripathi, 2014). Chlorosis, decrease in the biomass and total chlorophyll content was shown by the plants in presence of heavy metals. The effect of cement dust on certain physical and biological parameters of *Sessamum indicum* plants was analyzed by Kumar *et al.,* (2015) and found decrease in the amount of total chlorophyll content in the polluted site in comparison to that of the control site. The study concluded that the chloroplast is the primary site of attack by sir pollutants which make their entrance into the tissues through the stomata and cause partial denaturation of the chloroplast and decreases pigment content in the cells of polluted leaves.

Leaf surface of the plants gets covered with pollutant in areas with high pollution which lowers the total chlorophyll content and inhibit chlorophyll formation (Ahmad *et al.,* 2019). In an investigation done by Chaudhary and Rathore (2019), it was observed that reduction in photosynthetic pigments varies with the nature of foliage of selected plants species as well as plant sensitivity. Dhyani *et al.,* (2019) also concluded that total chlorophyll content may degrade due to the accumulation of dust on the plant leaves that prevent the gaseous exchange or the intensity of light that affect photosynthesis and metabolism.

2.1.2. Ascorbic acid

Increase in ascorbic acid content in the plants of polluted areas is due to the increased rate of production of reactive oxygen species (ROS) during photo-oxidation process (Tripathi and Gautam, 2007). In a study done by Jyoti and Jaya, (2009) an increased trend of ascorbic acid was observed in the plants of polluted areas. Ascorbic acid is found large amount in all growing plant parts and regarded as an antioxidant that influence resistance to adverse environmental condition including air pollution (Bhattacharya, 2011; Kuddus, 2011; Rai, 2016). Dust pollution, its removal and effect on foliage physiology of urban trees was studied by Chaudhary and Rathore (2019) and observed that ascorbic acid content significantly increased in the leaves of experimental (polluted) site at all season.

2.1.3. Relative Water Content (RWC)

Relative water content is reduced due to the impact of air pollutants on the transpiration rate in leaves (Chouhan *et al.,* 2012).It plays a considerable role in maintaining physiological balance among plants under air pollution stress (Babu *et al.,* 2013). In a study done by

Rathore *et al.,* (2018) it was suggested that the plants having higher RWC under polluted condition may be tolerant to pollution. Several studies have revealed that RWC varies with air pollution level and rise in pollution causes increase in relative content of plants (Sharma *et al.,* 2019).

2.1.4. Leaf extract PH

An alteration in pH of the leaf sap towards acidic is the result of the presence of SO_2, NO_2 or other acidic particulates from the industrial emission in the air (Swami *et al.,* 2004; Chauhan, 2010; Rai *et al.,* 2019). Pandey *et al.,* (2015) also reported that the leaf extract pH is lowered in the presence of an acidic pollutant. Higher the level of leaf extract pH of plants higher is the tolerance level to acidic pollutants (Go-vindaraju *et al.,* 2012; Sharma *et al.,* 2019).

2.1.5. Leaf structure (Stomatal density, length, breadth and Specific leaf area)

Negative effects of air pollutant on stomatal densities and opening have also been found in different plant species growing in polluted areas (Verma *et al.,* 2006). Gostin (2009), found that pollution stress altered the structure of the leaves of *Plantago lanceolata.* Nevertheless this species is quite resistant to the air pollutant actions and despite the observed modification they continue to grow and reach maturity (flowering stage). The amount of pollutants taken up from the air by the plants can affect photosynthesis, respiration, leaf conductance and leaf longevity (Tripathi *et al.,* 2009). All of these factors in trees adversely affect canopy carbon fixation and net accumulation of chlorophyll. The influence of cement factory dust pollution on anatomical features of leaves of two plants viz., *Pennisetum purpureum* and *Sida acuta* was analyzed by Ogunkunle *et al.,* (2013). According to the study, the significant modification of stomatal index in the forms of reduced stomatal size and increased stomatal index in the leaves of cement polluted area could be favorable anatomical adaptations to a polluted environment. Impact of cement industry pollution on physio-morphological attributes of apricot tree around industrial belt was studied by Rafiq and Kumawat (2016). On the study it was observed that the cement dust pollutants causes invisible injuries in plants like progressive decline in photosynthetic ability, closure of stomata and thus affect the growth and productivity. In the study of impact of cement dust pollution on the leaf anatomical features of *Lantana camara* and *Calotropis procera* by Tiwari and Pandey (2014), observed discloses modification in epidermal and stomatal features in both species exposed to cement dust area. From the study of effects of air pollution on micromorphological structure of some Broad leave trees Hamal *et al*., (2018) revealed significant alteration in the foliar morphological character of plants grown in polluted area than in non-polluted area. The study also revealed decrease in stomatal size in polluted area. Similarly the thickness of epidermal layer, cuticle and specific leaf area were also found to be decreased in the polluted area. These changes were the adaptive nature of plant towards air pollution stress. In a study done by Amulya *et al.,* (2015) it was observed that plants growing in polluted area have higher number of stomata and clogged stomata, higher stomatal index and decreased stomatal breadth and pores length in comparison to the plants growing in control area. The reduction of leaf size compared to plants growing in control area was also observed. The alterations in anatomical features are mainly an adaptations to resist against cement dust for continual survival. Thus acting as a bio-monitoring species for assessing air pollutant in the environment (Tiwari *et al.*, 2006; Tiwari and Pandey 2014; Zarinkamar *et al.,* 2013). The impact of cement dust pollution on leaf anatomical features of *Lantana camara* and *Calotropis procera* was analyzed by Tiwari and Pandey (2017) and suggested that both species shows remarkable impact on epidermal and stomatal characteristics. The study also concluded that the alterations in anatomical features in the polluted site are mainly adaptation to resist against cement dust and for continuous survival, thus acting as a bio-monitoring species for assessing air pollutant in the environment.

Most of the research work using APTI index are done comparing polluted site and control site or roadside plants in case of Nepal. But distance wise and direction wise study around cement factory are not found in Nepal. Therefore, this study can help to understand the status of pollution around cement factory and also provide information about the susceptible and resistant plant species within the study area.

CHAPTER 3: MATERIALS AND METHODS

3.1. Study area

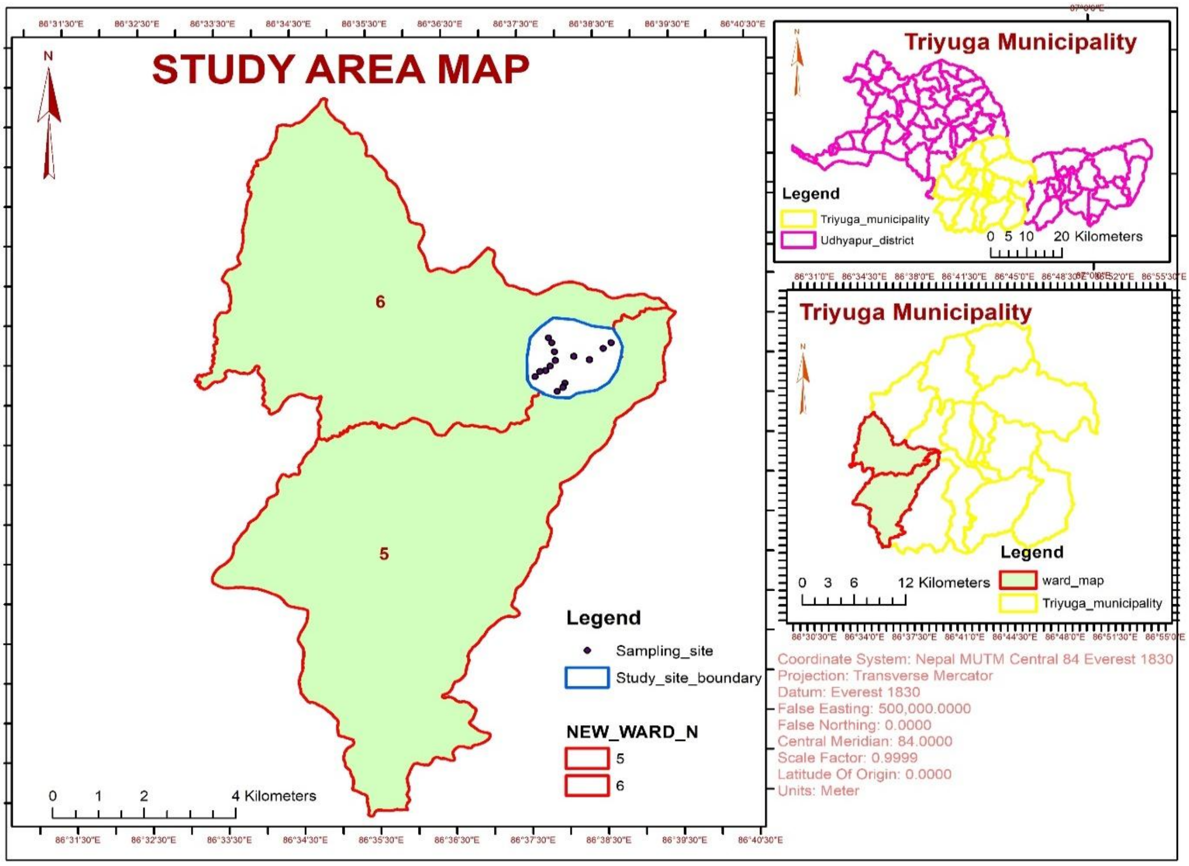


Figure 3. 1. Map of study area with study site boundary and sampling site around Udayapur Cement Factory, Jaljale, Udayapur, Nepal.

Udayapur district is surrounded by Mahabharat hills from north and Shiwalik from south, whereas both hills meet together by west which forms the region a valley Udayapur valley. It lies 26° 55' N and 86° 40' E and is about 30 km long and between 2 km to 4 km wide. It is drained by the Triyuga river flowing east to join the Koshi river. Udayapur Cement Industry limited is a fully Government owned industry located at Jaljale, Udayapur district, Nepal, established on 14th June, 1987 with capacity 800 tpd (Shrestha *et al.*, 2016). The industry uses about 330,000 MT Lime stone, 4,000 MT Iron ore, 57,000-82,500 MT Clay, 10,500 MT Gypsum and up to 21,00 MT Silica Sand per year as raw materials, and 50,000 MT Coal, 12,000 kL Furnace Oil per year and 1500 m3/day of water, and 8,000 kW electricity as input fuels. It was estimated that the carbon dioxide generation per MT of limestone based cement is 277.33kg and that of clinker based cement is 8.74kg. In this assumption, the carbon dioxide emission was about 207,890 MT per year till 2011 (Shrestha *et al.*, 2016).

3.2. Study species

Eight commonly available plant species four of each tree and shrub were collected from study area. They were selected from four different distance and four different direction. The range of four different radial distance are namely (0-250 m), (250-500 m), (500-750 m) and (750-1000 m) from the factory in all directions (east, west, north and south). Plant species were identified according to Bhatt and Khatri (2016) and Shrestha (1998) comparing local name and scientific names. Plant species were selected on the basis of availability of species at all the sites.

Table 3. 1. List of plant species selected for the study

Category	**Scientific name**	**Local name**
Trees	*Casearia graveolens*	Pipiri
	Cassia fistula L.	Rajbriksya
	Derris elliptica (Wall.) Benth.	Deri
	Shorea robusta Gaertn.	Sal
Shrubs	*Clerodendrum viscosum* Vent.	Bhait
	Colebrookea oppositifolia Sm.	Dhusure
	Melastoma melabathricum L.	Angeri
	Phoenix acaulis Roxb. ex Buch.-Ham.	Thakal

3.3. Methods

3.3.1. Research Design

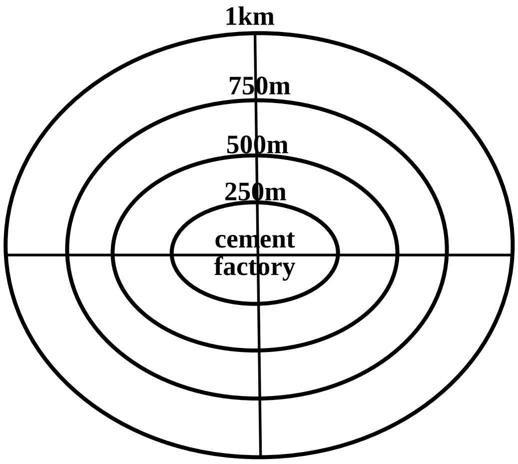


Figure 3. 2. Diagrammatic representation of study area showing different distance

The study area was divided into 4 different distance ranging from 0-250 m, 250-500 m, 500-750 m radius from the Udayapur Cement Factory. Plant samples (trees and shrubs) were collected from each distance and APTI value and study of leaf structure was done. The distance wise comparison of various analytical parameters (pH, RWC, AA, TC, SLA, Stomatal density, length and breadth) were done. The statistical analysis of APTI was done using SPSS version 25. ANOVA followed by Duncan's Multiple range test to understand significant differences (at P = 0.05) among different distance.

3.3.2. Climate and Hydrology

Udayapur Gadhi of Udayapur district is the nearest weather station from the Udayapur Cement Factory, Jaljale. On the basis of data, the average maximum temperature was high during July (39.17℃) and low during January (22.67℃). Likewise, the average minimum temperature was high during August (24.9℃) and low during January (8.9℃). The precipitation was high during July (603.84 mm) and low during November (0 mm).

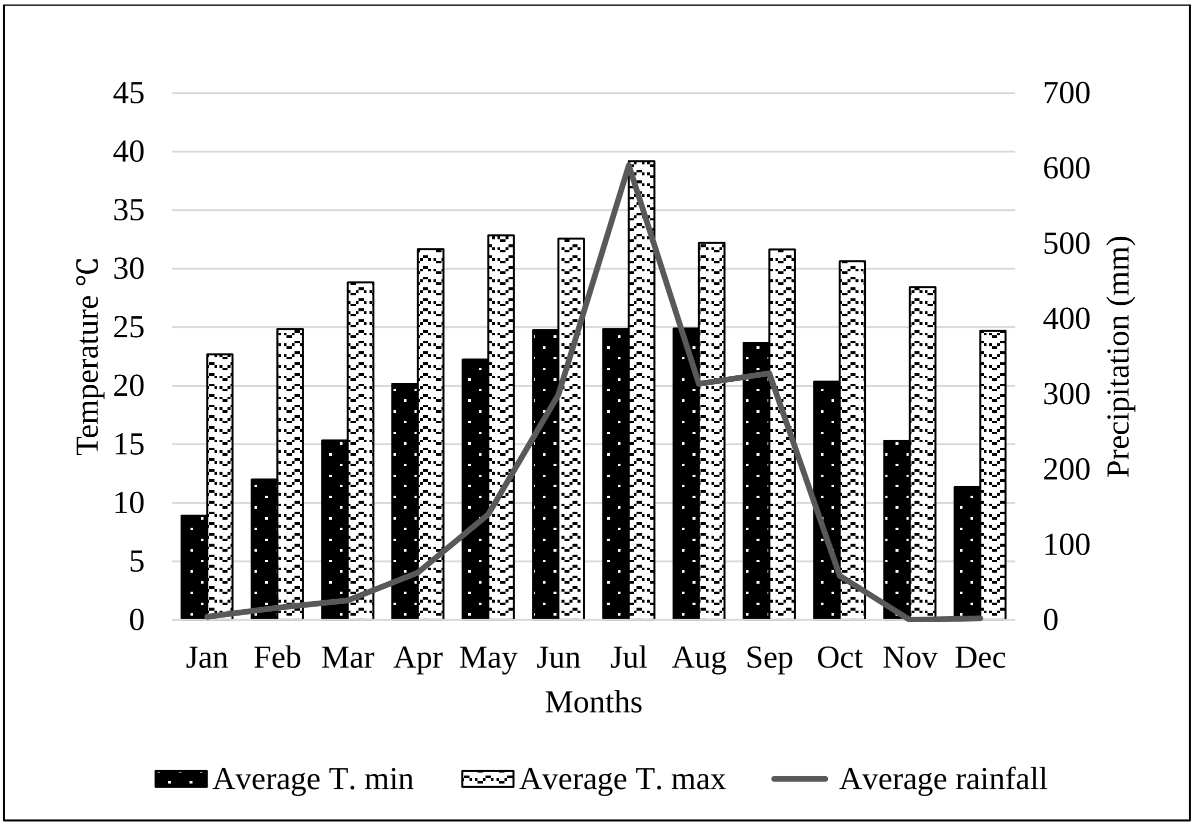


Figure 3. 3. Six years (2015 -2020) climatic graph showing average monthly temperature and precipitation of Udayapur Gadhi, Udayapur (Source: Department of Hydrology and Meteorology, Babarmahal, Government of Nepal).

3.3.3. Sample collection

Leaf samples were collected only during winter season from the study area. Fully mature leaves of each species were collected in the morning hours from almost same diameter at breast height (DBH) and from the shrubs of almost same height. Laboratory work for RWC were done in the laboratory of Udayapur Cement Factory and for rest of the parameters samples were collected and transported to the laboratory of Amrit Campus as soon as possible in a heatproof container. In the laboratory, first of all samples were cleaned using fine brush and initial weight were taken. After the measurement of initial weight, the samples were used to analyze different parameters of APTI. All the experiments were performed in five replicates.

3.3.4. Analysis of various parameters

Air pollution tolerance index (APTI) was calculated by analyzing four different parameters namely, relative water content (RWC), total chlorophyll content (Tchl), ascorbic acid (AA) and leaf extract pH.

3.3.4.1. Relative water content (RWC)

RWC was estimated according to Barrs and Weatherly (1962). First composite sample of leaves were taken and dust particles were removed using fine brush. Fresh weight of leaves were measured and then leaves were immersed in water for up to 24 h. The turgid weight were then recorded, and the leaves were subsequently oven-dried to a constant weight at about 85°C for 24 h.

RWC was calculated by using the following formula:

$$\text{RWC (\%)} = \frac{\text{(Fresh weight - dry weight)}}{\text{(Turgid weight - dry weight)}} \times 100$$

3.3.4.2. Total chlorophyll content

Total chlorophyll content was estimated according to Barnes *et al*., 1992. 0.5 g of leaves was taken and cut into smaller pieces and placed in test tubes containing 5 ml dimethyl sulfo-oxide solvent. Test tubes were then incubated in a water bath at 60-65ºC for an hour. After water bath there was full decolorization of tissues. Cooling at room temperature was followed for 30 minute and filtration was done. After filtration, the filtrated sample was taken and absorbance was measured at 665 nm and 648 nm being the final stages. Blank determination was carried out with dimethyl sulfo-oxide. Absorption measurement was carried out with a Spectrophotometer (Model No. 31).

Chlorophyll a (mg/g F.W) = $(14.85 * A_{665} - 5.14 * A_{648})$ (1)

Chlorophyll b (mg/g F.W) = $(25.48 * A_{648} - 7.36 * A_{665})$ (2)

Total chlorophyll (mg/g F.W) = $(7.49 * A_{665} + 20.34 * A_{648})$ (3)

Where: A_{665} = absorption value at 665 nm

A_{648}= absorption value at 648 nm

3.3.4.3. Leaf extract pH

Five gram of a leaf sample was crushed, and 50 ml deionized water was added, the obtained suspension was measured with a pH meter (Apriyantono *et al*., 1989).

3.3.4.4. Ascorbic acid (AA)

Ascorbic acid content (expressed in mg/g) was calculated using spectrophotometric method (Bajaj and Kaur 1981). One gram of the fresh leaf samples was taken in a test tube and after that 4 ml oxalic acid EDTA extracting solution, 1 ml of Orthophospheric, 1 ml of sulfuric acid, 2 ml of ammonium molybdate and 3 ml of distilled water was added to it. Then the solution was allowed to stand for 15 minutes at room temperature after which the absorbance at 760 nm was measured with a spectrophotometer (Model No.31). Blank determination was carried out with ascorbic acid. The concentrations of ascorbic acid in the sample then extrapolated from a standard ascorbic acid curve.

3.3.4.5. Air Pollution Tolerance Index (APTI)

APTI was calculated according to Singh and Rao (1983).

APTI = [A (T+P) + R]/10

Where A = Ascorbic acid content (mg/g),

T = total chlorophyll (mg/g),

P = pH of leaf extract and

R = relative water content of leaf.

3.3.5. Categorization of plant species

On the basis of APTI values the plants were conveniently grouped as follows (Padmavathi *et al.,* 2013).

Table 3. 2. Categorization of plant species on the basis of APTI score (Padmavathi *et al.,* 2013).

Category of plant species	**APTI score**
Sensitive	Less than 11
Intermediate	Between 12 and 16
Tolerant	Above 17

3.3.6. Leaf Structure

For Specific leaf area (SLA) study, photographs of fresh leaves were taken (five replicate samples) along with a scale and its area was calculated using Image J software. Then leaves were dried in oven till its constant weight and then their dry weight was measured. Finally the leaf area was divided by dry weight to obtain specific leaf area (Zobel *et al.,*1987).

For the study of stomatal anatomy fresh sample of leaves were rinsed with clean water and slides were prepared as per lasting impression method for counting of stomata Wu and Zhao (2017). In this method, nearly one square cm of leaf was painted by a thick patch of transparent nail polish. The nail polish was allowed to dry completely and then nail polish was peeled out with the help of a clear cellophane tape. The leaf impression was taped on slide and will be examined under 15x X 45x magnifications by light microscopy. Number of stomata was counted from five microscopic field was counted for the density.

3.3.7. Data analysis

The data obtained from the above experiments was statistically analyzed using SPSS version 25. ANOVA followed by Duncan's Multiple range test to understand APTI value and to study leaf structure at different distance and direction.

CHAPTER 4: RESULTS

4.1. APTI study

4.1.1. Relative Water Content

The result obtained from the analysis showed high relative water content (RWC) mean value of most of the tree species at the nearest distance from the factory (Figure 4.1). Among the tree species studied, species like *Derris elliptica*, *Casearia graveolens* and *Shorea robusta* have high RWC towards east direction ranging from (0-250 m) from the factory but *Cassia fistula* have high RWC towards south direction at the same distance from the factory. While moving from (750-1000 m) range toward (0-250 m) range, *Derris elliptica* showed significant increase in RWC of east, west and south direction and insignificant in north direction; *Casearia graveolens* showed significant increase in RWC of west and north direction; *Cassia fistula* showed significant increase in all three direction except in east direction and *Shorea robusta* showed significant increase in RWC towards west and south direction (Annex 1).

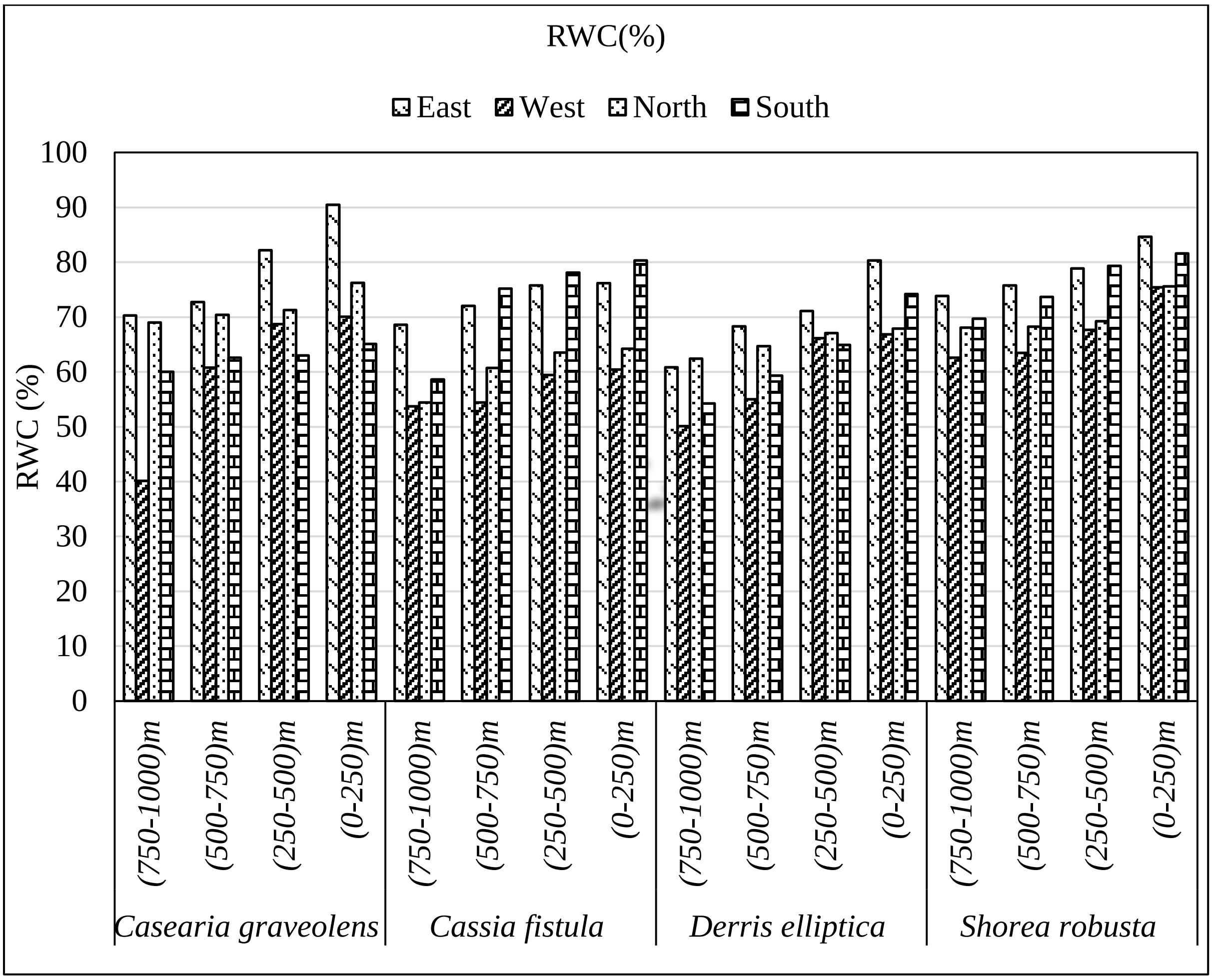


Figure 4. 1. RWC (%) of different tree species in four different distance in each direction.

From the result of shrub species it was observed that, species like *Melastoma melabathricum*, *Colebrookea oppositifolia* and *Clerodendrum viscosum* have high RWC in east direction at the distance of (0-250 m) from the factory but *Phoenix acaulis* showed high RWC in south direction at the same distance (Figure 4.2). Here, all the shrub species showed high RWC mean value at the range (0-250 m) which is nearest distance from the factory. While moving from (750-1000) meters towards nearer to the factory i.e. (0-250) meters, RWC of *Melastoma melabathricum* increased significantly in all three (east , west and north) direction except in southern direction (Annex 2). In case of *Colebrookea oppositifolia* RWC increased significantly in east, west and south direction but was insignificant in north direction (Annex 2). Likewise, RWC of *Phoenix acaulis* have no significant difference in any direction but *Clerodendrum viscosum* showed significant increase in RWC of all direction while moving nearer to the factory (Annex 2).

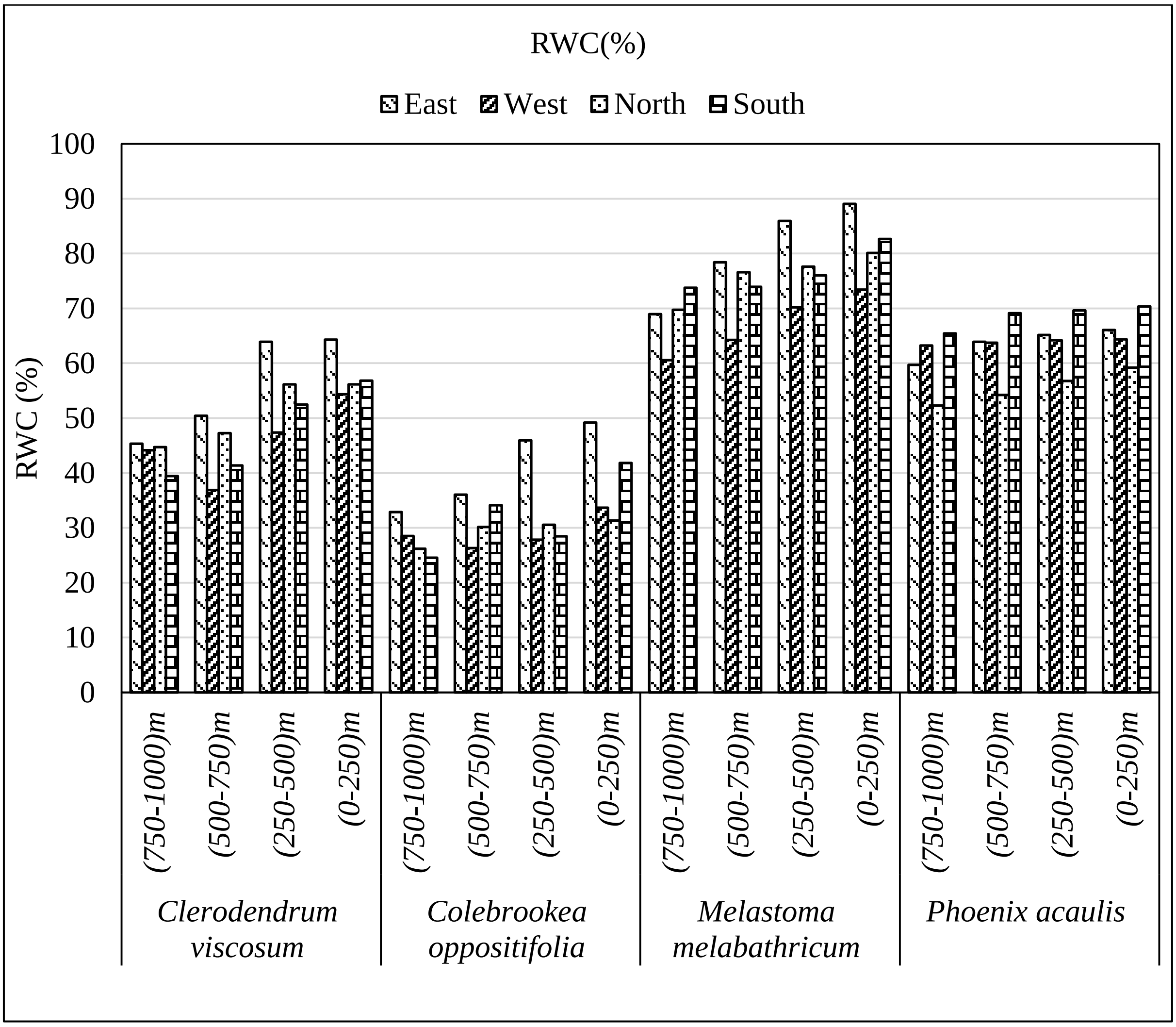


Figure 4. 2. RWC (%) of different shrub species in four different distance in each direction.

4.1.2. Total chlorophyll content

The data of total chlorophyll content showed that, species like *Derris elliptica*, *Casearia graveolens* and *Shorea robusta* have lower total chlorophyll content in western direction at the distance of (0-250 m) but *Cassia fistula* have lower total chlorophyll content in northern direction at the same range (Figure 4.3). Here, all the tree species studied have lower total chlorophyll content mean value at the distance of (0-250 m), which is the nearest distance from the factory (Figure 4.3). While moving from (750-1000 m) towards nearer to the factory i.e. (0-250 m), all the tree species except *Casearia graveolens* showed significantly lower value of total chlorophyll content in all direction (east, west, north and south) (Annex 3). In case of *Casearia graveolens* also except in east direction there was significant decrease in total chlorophyll content of other three direction (Annex 3).

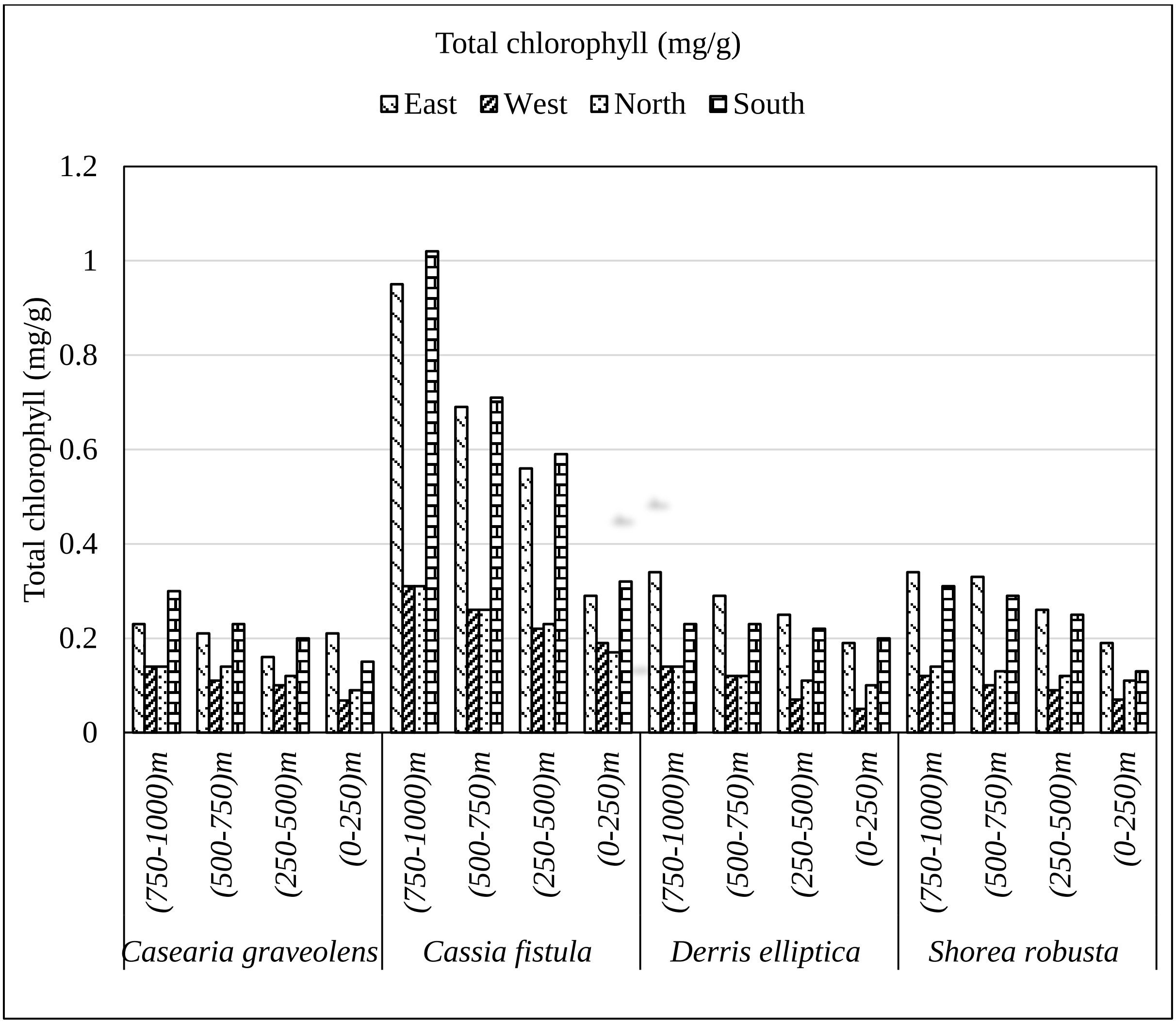


Figure 4. 3. Total chlorophyll content (mg/g) of different tree species in four different distance in each direction.

The result obtained from the analysis showed that, there was lower total chlorophyll content mean value at the nearest distance of (0-250 m) from the factory (Figure 4.4). *Melastoma melabathricum* and *Phoenix acaulis* have lower total chlorophyll content in western direction; *Colebrookea oppositifolia* have lower value in northern direction and *Clerodendrum viscosum* have lower value in eastern and southern direction. Here, all the shrub species have significantly lower total chlorophyll content while moving from (750-1000 m) towards nearer to the factory i.e. (0-250 m) (Annex 4).

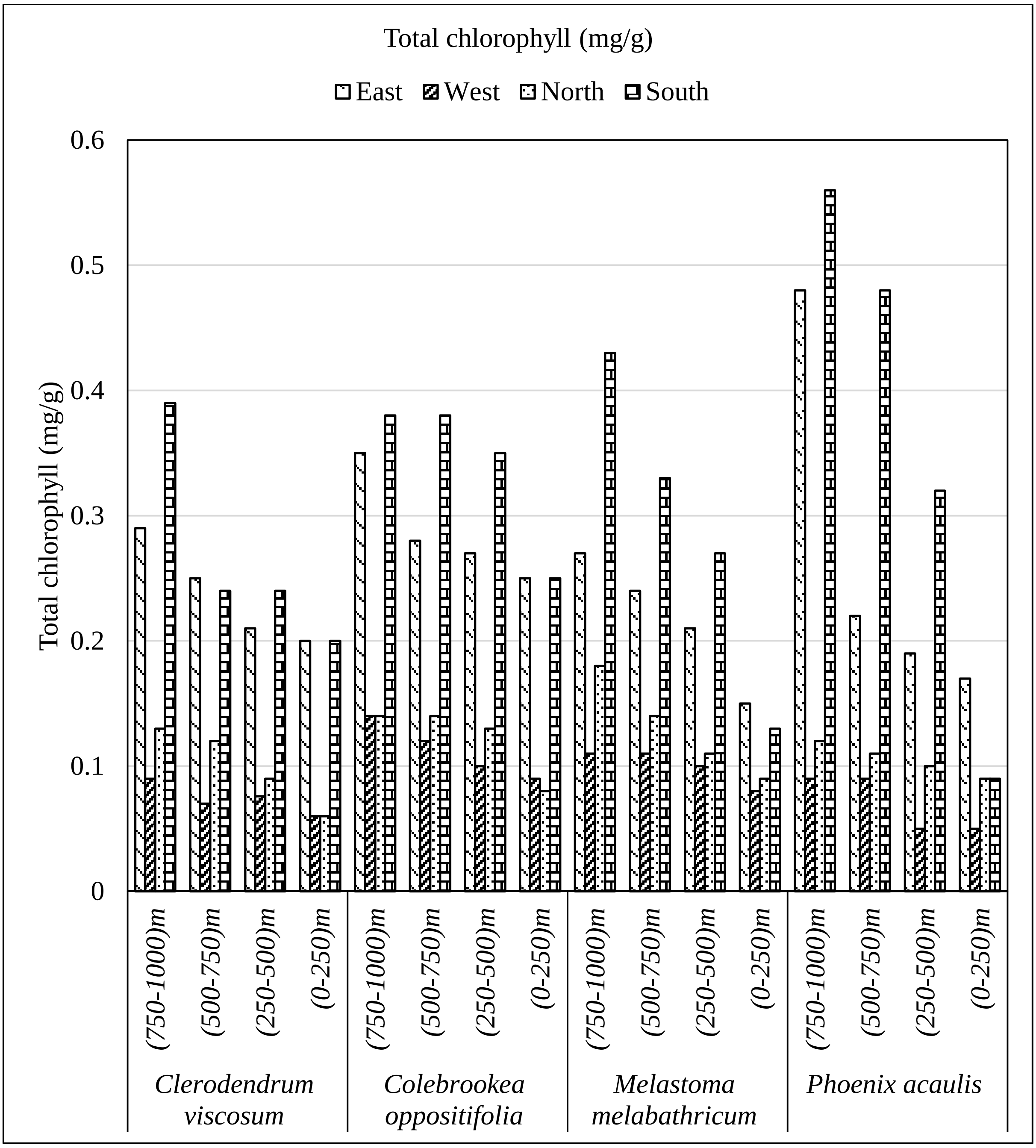


Figure 4. 4. Total chlorophyll content (mg/g) of different shrub species in four different distance in each direction.

4.1.3. Leaf extract pH

The result obtained from the analysis of leaf pH mean value showed decreasing trend in most of the species while moving nearer to the factory (Figure 4.5). Species like *Derris elliptica*, *Casearia graveolens*, *Cassia fistula* have lower pH value in north direction while *Shorea robusta* have lower pH value in south direction (Annex 5). The lower pH value of *Derris elliptica*, *Casearia graveolens* and *Shorea robusta* were obtained from (0-250 m) and *Cassia fistula* from (250-500 m). Here, while moving from (750-1000 m) towards nearer to the factory i.e. (0-250 m), *Derris elliptica* showed significant decrease in pH value in all three direction except south; *Casearia graveolens* showed significant decrease in all direction; *Cassia fistula* showed significant decrease in all three direction except west direction and Sal showed significant decrease in leaf pH in east and south direction (Annex 5).

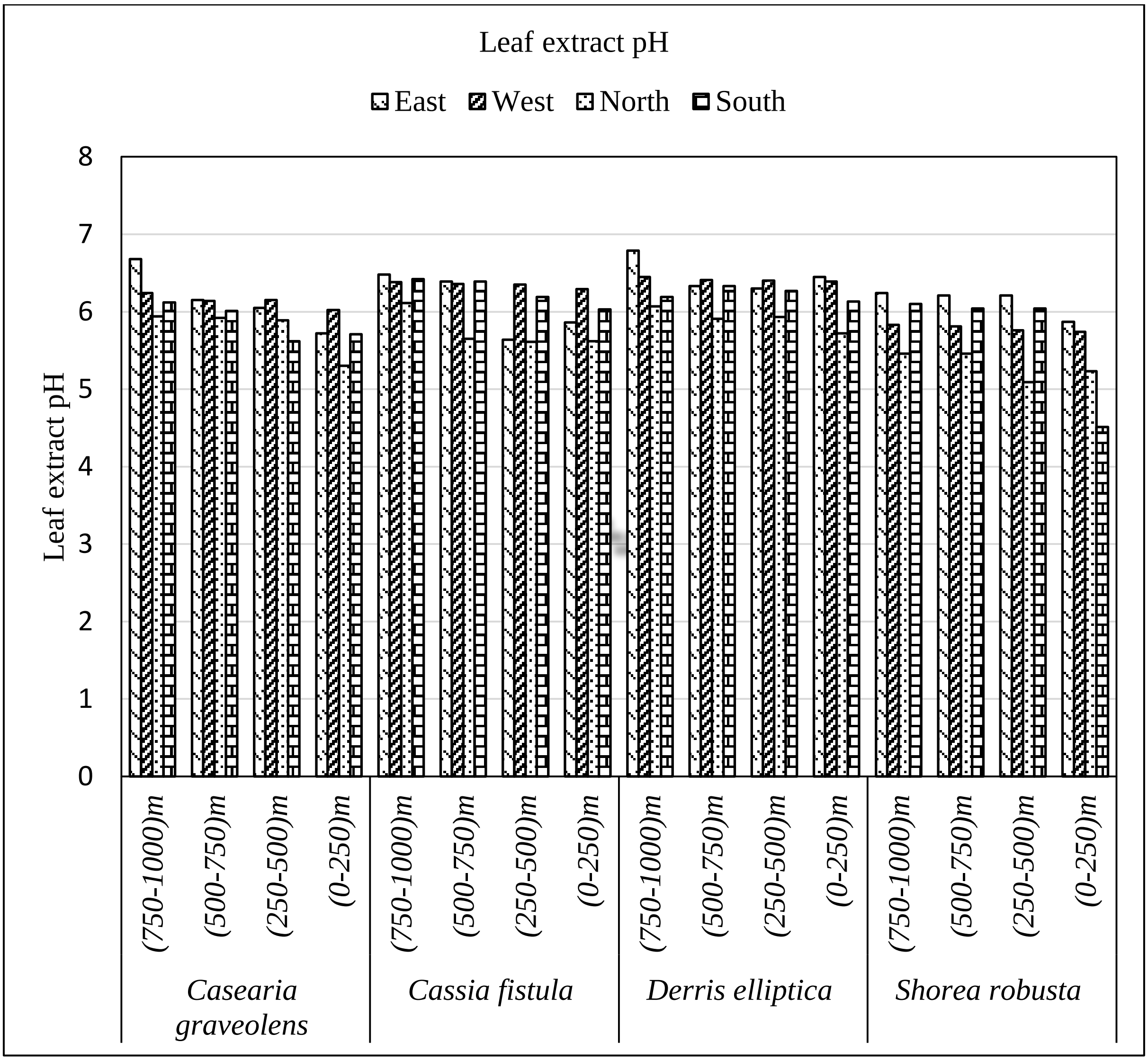


Figure 4. 5. Leaf extract pH of different tree species in four different distance in each direction.

Among the shrub species, *Melastoma melabathricum*, *Colebrookea oppositifolia* and *Clerodendrum viscosum* have lower leaf pH mean value at (250-500 m) distance in southern direction while *Phoenix acaulis* have lower leaf pH in northern direction at the distance of (0-250 m) (Figure 4.6). Here, moving from (750-1000 m) towards nearer to the factory i.e. (0-250 m), among the shrub species studied *Melastoma melabathricum* showed significant decrease in leaf pH of all direction while *Colebrookea oppositifolia* showed significant decrease in east and west direction. Likewise, *Phoenix acaulis* showed significant decrease in leaf pH of all three direction except south direction and *Clerodendrum viscosum* showed significant decrease in all direction except north direction (Annex 6).

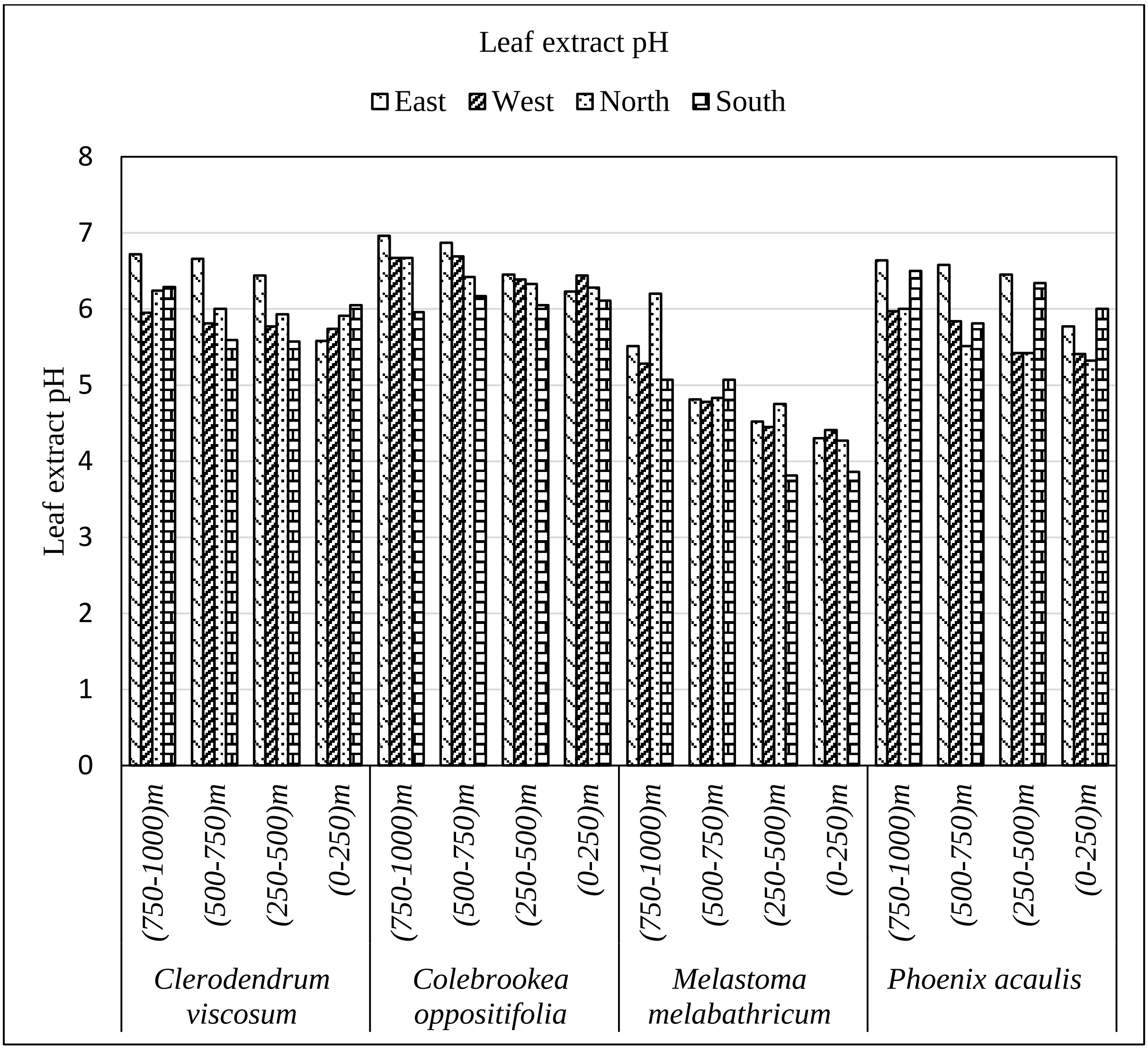


Figure 4. 6. Leaf extract pH of different shrub species in four different distance in each direction.

4.1.4. Ascorbic acid

Among the tree species studied, species like *Derris elliptica*, *Cassia fistula* and *Shorea robusta* showed high ascorbic acid mean value in western direction at the distance of (0-250 m) and at the same distance ascorbic acid of *Casearia graveolens* was found to be high in northern direction (Figure 4.7). Here, all three tree species except *Cassia fistula* showed significant increase in ascorbic acid in all the direction while moving nearer to the factory (Annex 7). In case of *Cassia fistula* also ascorbic acid was found to be insignificant only in eastern direction (Annex 7).

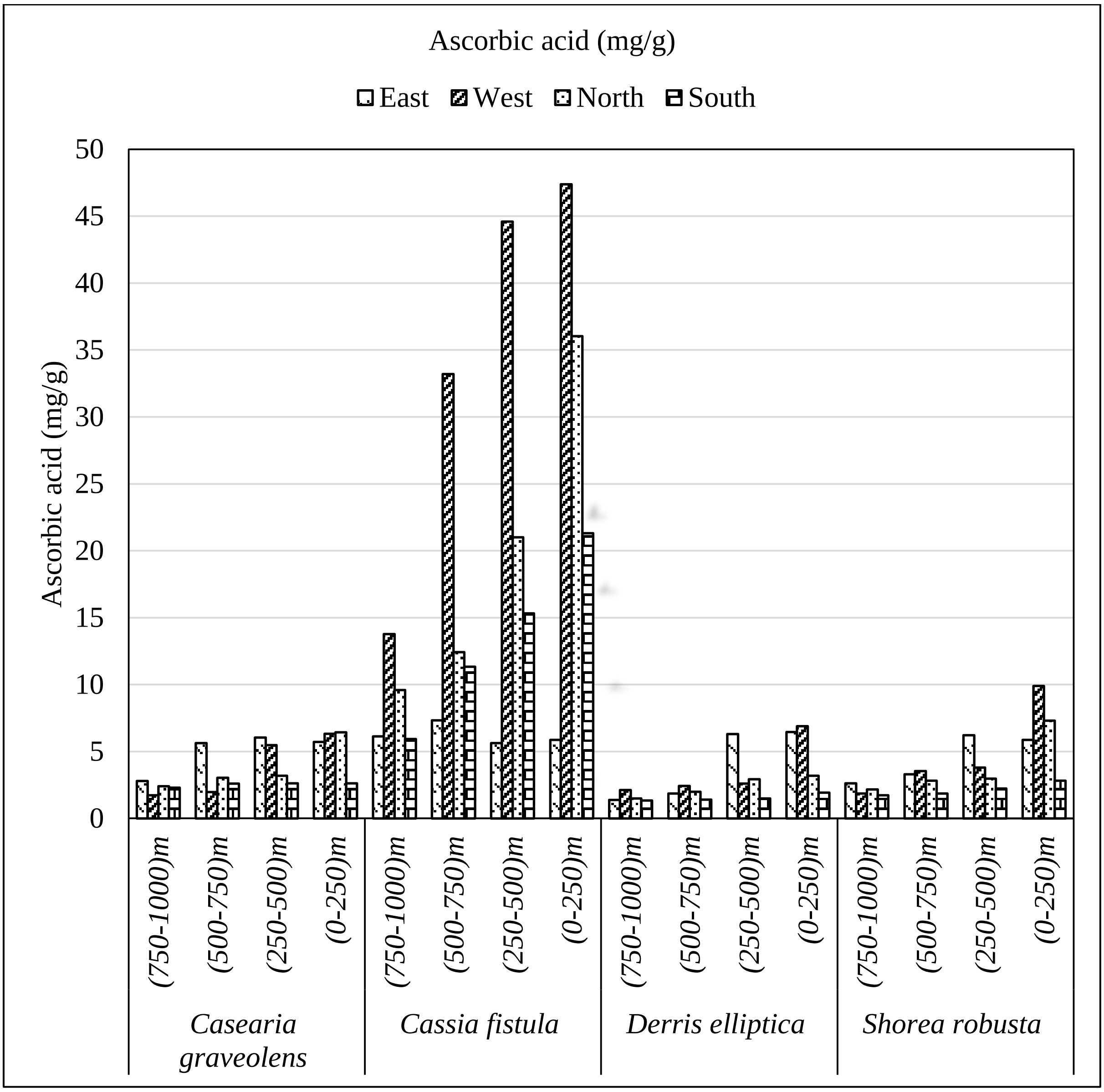


Figure 4. 7. Ascorbic acid (mg/g) of different tree species in four different distance in each direction.

All the shrub species studied showed high ascorbic acid mean value at the range of (0-250 m) from the factory (Figure 4.8). Among the shrub species studied, *Melastoma melabathricum* and *Phoenix acaulis* have high ascorbic acid in western direction and *Colebrookea oppositifolia* and *Clerodendrum viscosum* have high ascorbic acid in eastern direction (Figure 4.8). While moving from (750-1000 m) towards nearer to the factory i.e. (0-250 m) distance, all the shrub species showed significant increase in ascorbic acid at all direction (Annex 8).

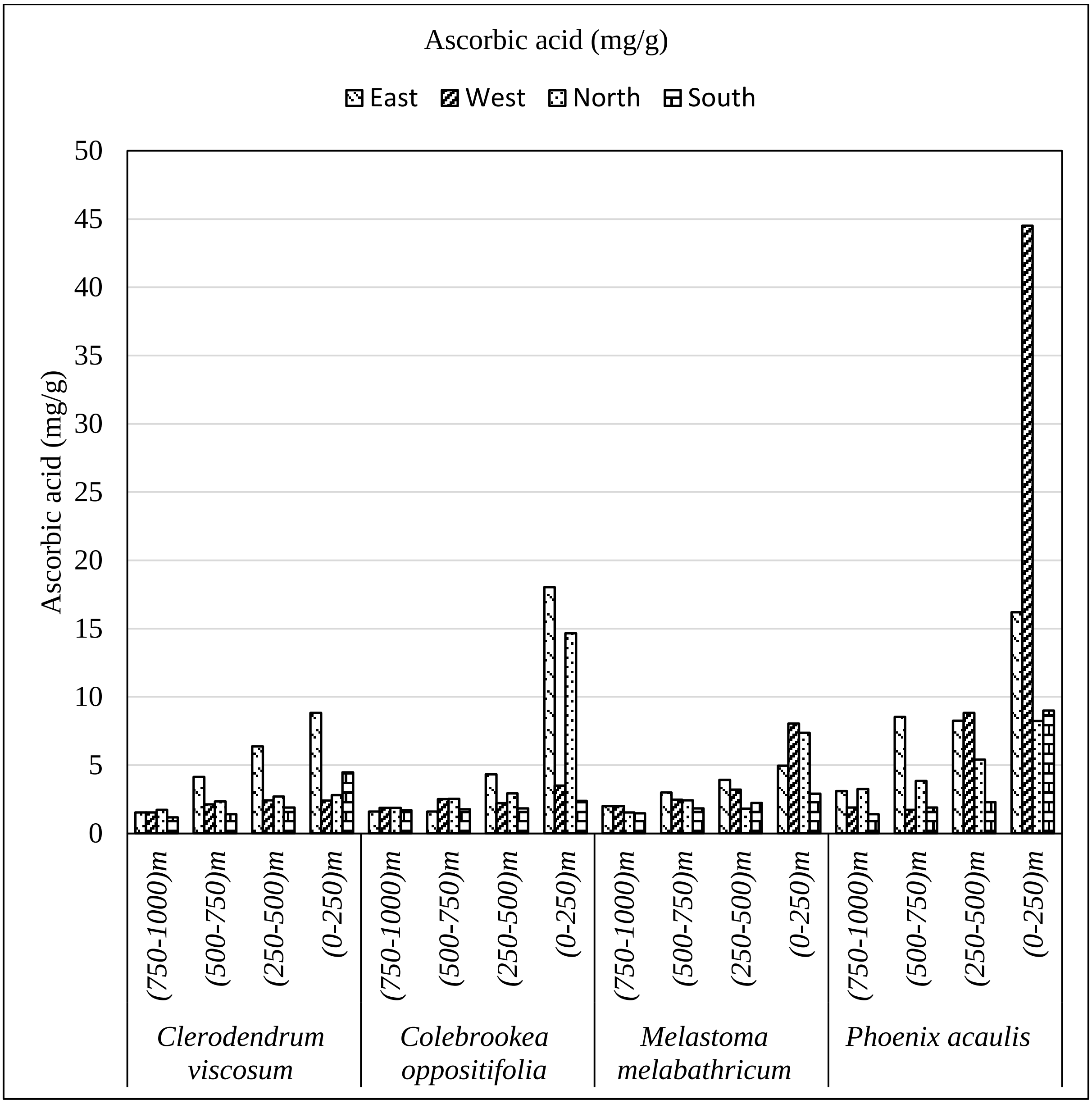


Figure 4. 8. Ascorbic acid (mg/g) of different shrub species in four different distance in each direction.

4.1.5. Air Pollution Tolerance Index (APTI)

From the graph (Figure 4.9) of APTI of tree species it was observed that, the mean value of APTI have been increased while moving nearer to the factory. All the tree species studied showed high APTI value in eastern direction at the nearest range from the factory i.e. (0-250 m) (Figure 4.9). The second highest value of APTI was observed in western direction at the same range except in *Derris elliptica* and *Casearia graveolens*. Both the species, *Derris elliptica* and *Casearia sp* have second highest APTI value in eastern direction at the range of (250-500 m).

Here, APTI value of most of the tree species except *Casearia graveolens* have increased significantly while moving from (750-1000 m) towards nearer to the factory i.e. (0-250 m) distance (Annex 9). In case of *Casearia graveolens* also, it was observed that APTI value have been significantly increased in all three direction (east, west, north) except in southern direction (Annex 9).

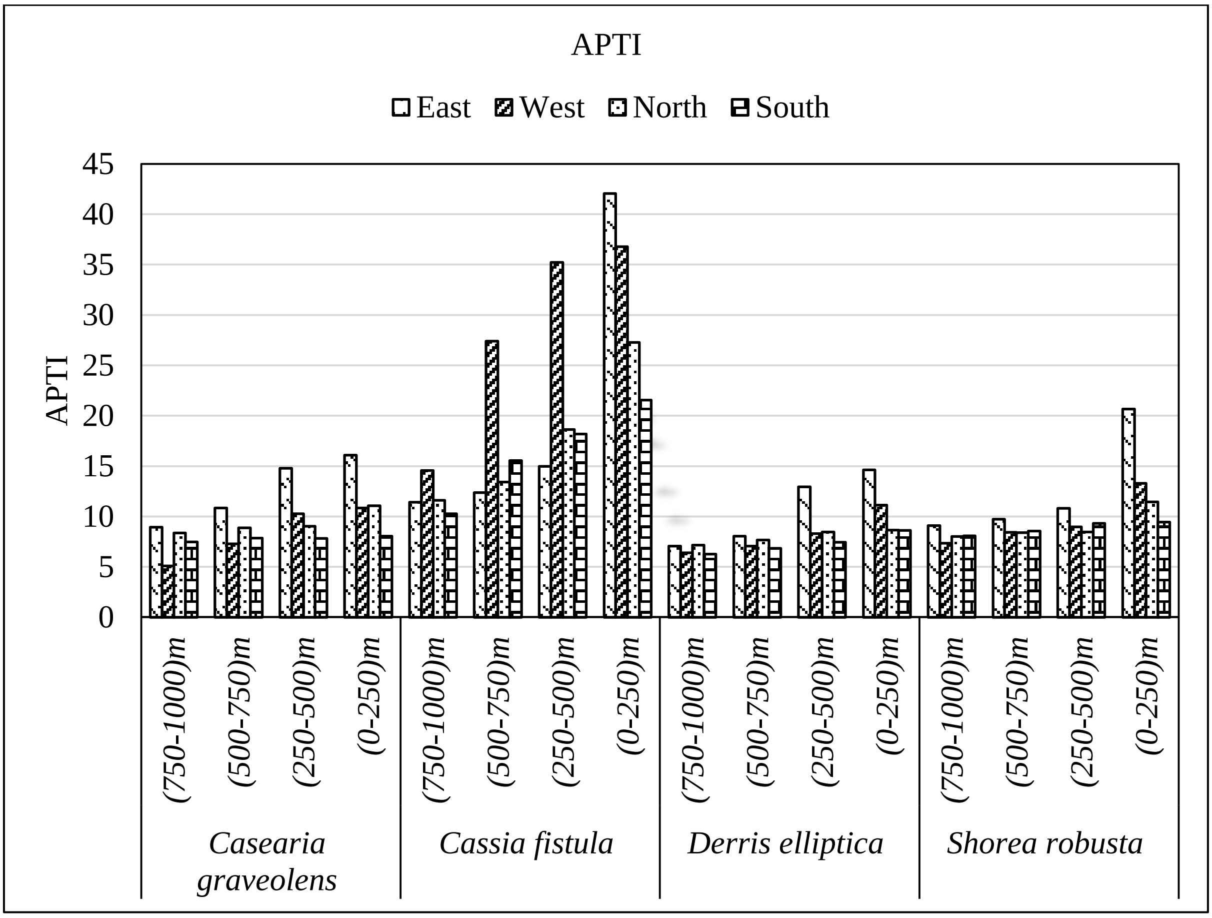


Figure 4. 9. APTI of different tree species in four different distance in each direction.

Studying the APTI value of shrub species (Figure 4.10), it was observed that all the shrub species have high APTI mean value at the range of (0-250 m). *Colebrookea oppositifolia* and

Clerodendrum viscosum have high APTI value at northern direction, *Melastoma melabathricum* showed high APTI values in eastern direction and *Phoenix acaulis* have high APTI value in western direction. While observing the second highest value of APTI, *Melastoma melabathricum*, *Phoenix acaulis* and *Clerodendrum viscosum* have second highest APTI in eastern direction and *Colebrookea oppositifolia* have second highest APTI value in northern direction. APTI value of all the shrub species except *Melastoma melabathricum* have significantly increased in all direction while moving nearer to the factory (Annex 10). In case of *Melastoma melabathricum* also, it was observed that APTI value have increased significantly in all three direction except in southern direction (Annex 10).

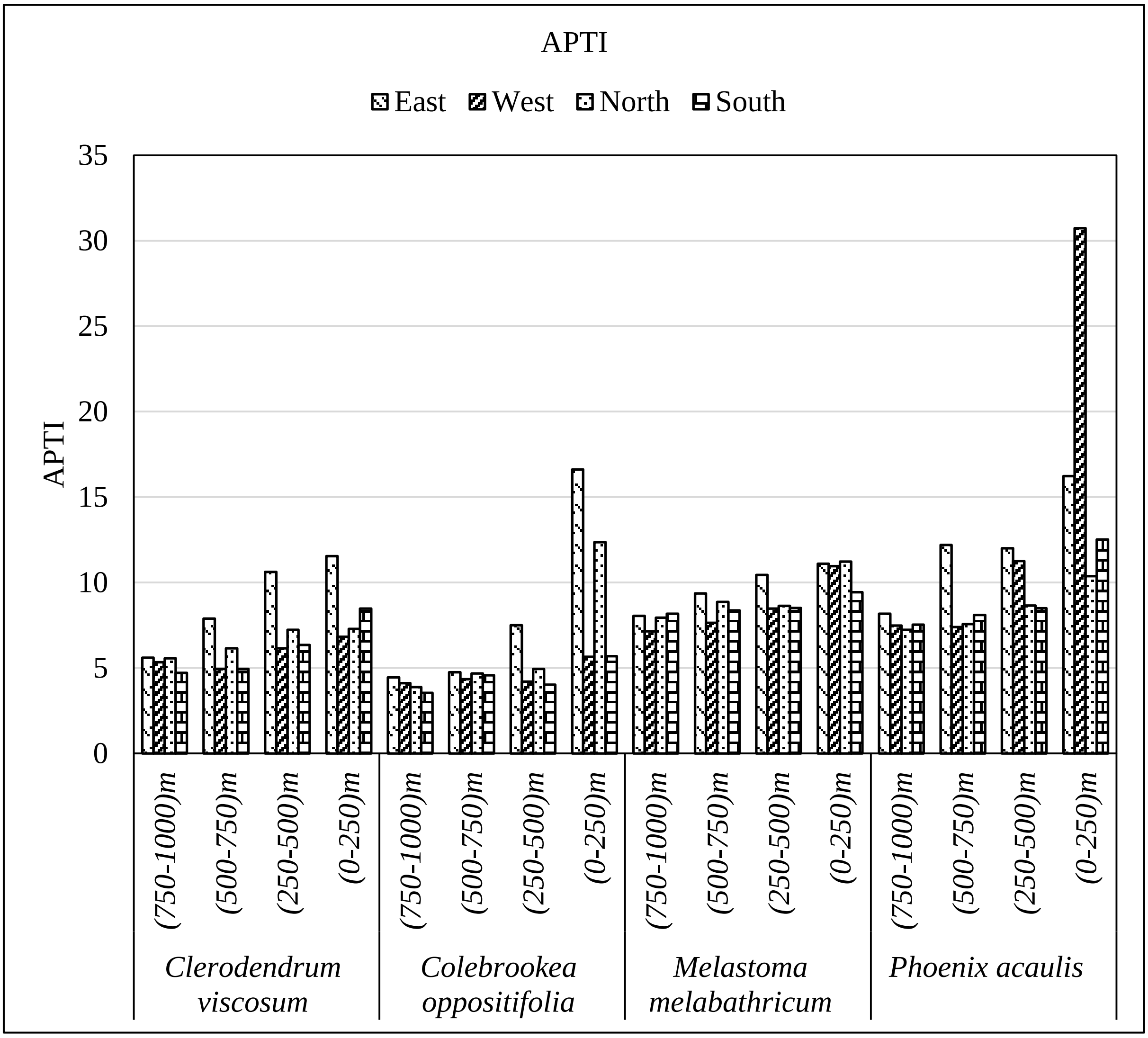


Figure 4. 10. APTI of different shrub species in four different distance in each direction.

4.1.6. Plant Categorization

On the basis of APTI mean value pants were grouped into three different categories *viz.* sensitive, intermediate and tolerant (Padmavathi *et al.*, 2013). The result obtained from the present study showed that out of eight species studied five species such as *Casearia graveolens*, *Cassia fistula*, *Shorea robusta*, *Colebrookea oppositifolia* and *Phoenix acaulis* were tolerant species with APTI value above seventeen. Likewise, plant species *Derris elliptica* showed intermediate tolerance against air pollution with APTI value between twelve and sixteen and two species *viz*. *Melastoma melabathricum* and *Clerodendrum viscosum* were sensitive species with APTI values less than twelve. Here, tolerant plant species showed higher APTI value while moving nearer towards factory (0-250) meters range.

Table 4. 1. Plants categorization on the basis of APTI values (Padmavathi *et al.*, 2013)

Category of plants	**Trees**	**Shrubs**
Sensitive		*Melastoma melabathricum*, *Clerodendrum viscosum*
Intermediate	*Derris elliptica*	
Tolerant	*Casearia graveolens*, *Cassia fistula*, *Shorea robusta*,	*Colebrookea oppositifolia*, *Phoenix acaulis*

4.2. Leaf structure

4.1.7. Stomatal density

Studying the data of distance wise, it was observed that the mean stomatal density of most of the tree species were found to be increased while moving from (750-1000 m) towards nearer to the factory (0-250 m) (Figure 4.11). *Derris elliptica* showed significant increase in stomatal density in east and north direction, *Casearia graveolens* showed significant increase in stomatal density of east, west and south direction except north direction (Annex 11). Likewise, *Cassia fistula* showed significant increase in all direction and *Shorea robusta* showed increase in all three direction except south direction (Annex 11). While observing direction wise data, it was found that there was significant change in stomatal density of most of the species (Annex 11).

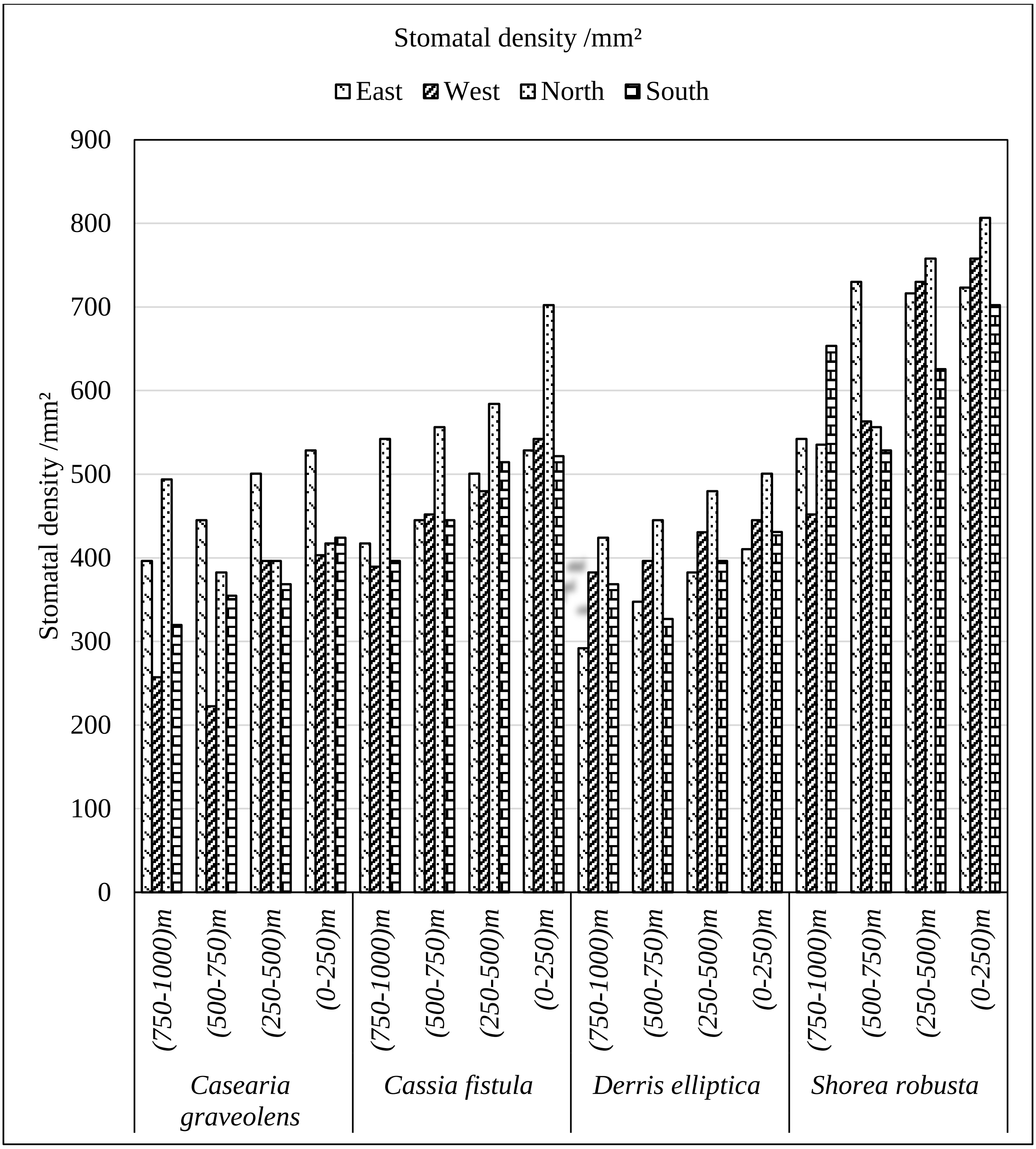


Figure 4. 11. Stomatal density /mm² of different tree species in four different distance in each direction.

Studying distance wise data of shrub species (Figure 4.12), it was observed that, *Melastoma melabathricum* showed significant increase in mean stomatal density of west and north direction and *Clerodendrum viscosum* showed significant increase in east and north direction. While observing direction wise data, it was found that there was insignificant change in stomatal density of *Clerodendrum viscosum* but *Melastoma melabathricum* showed significant change in most of the direction except at the range of (0-250 m) (Annex 12).

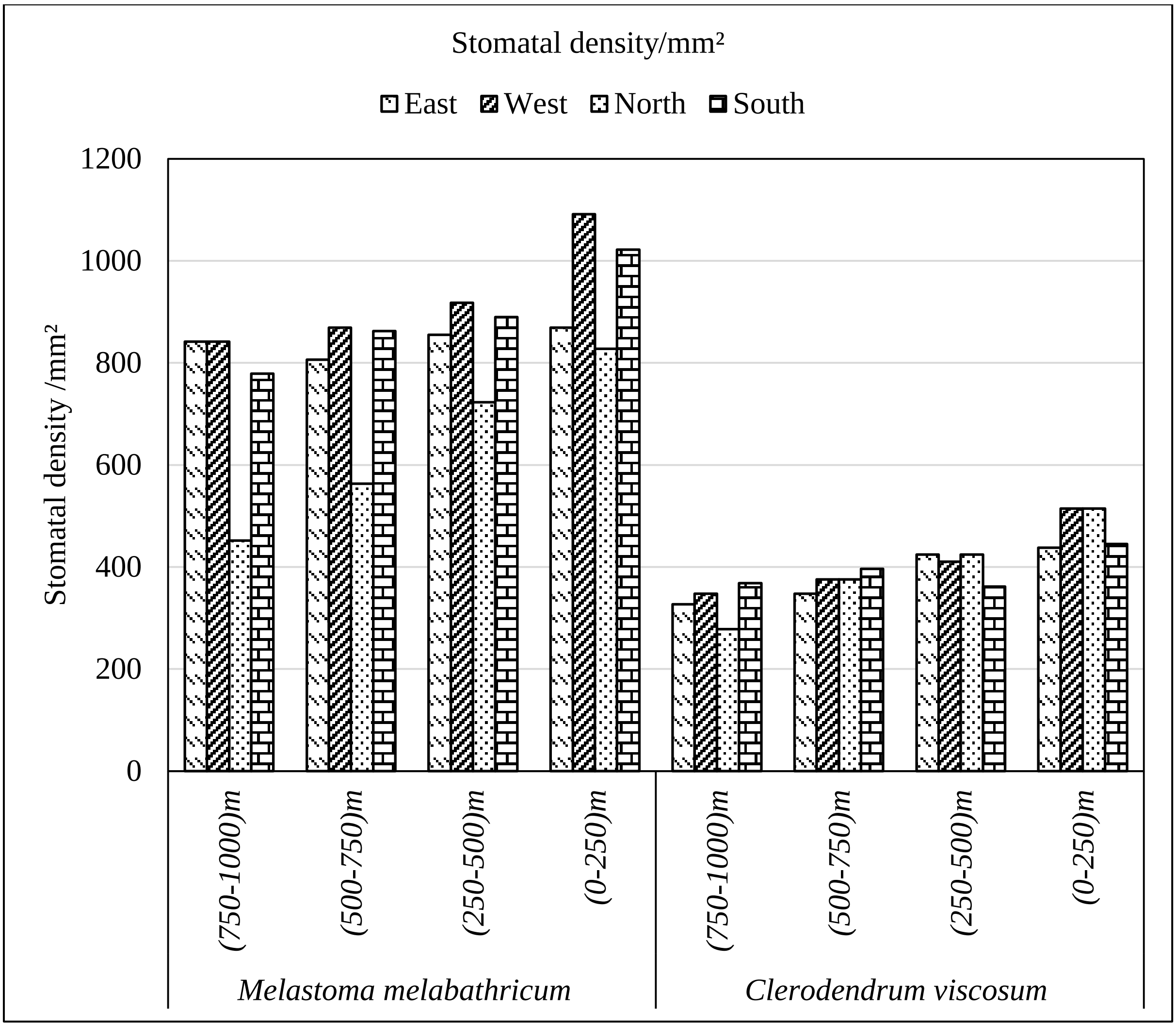
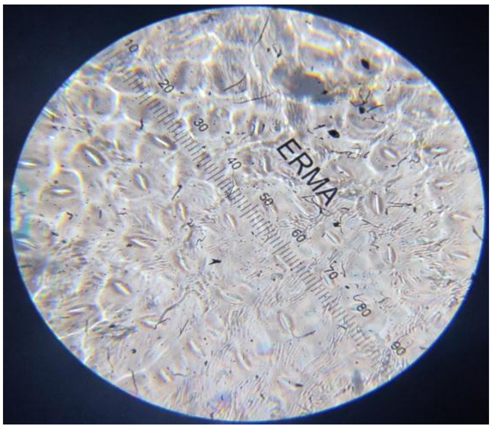
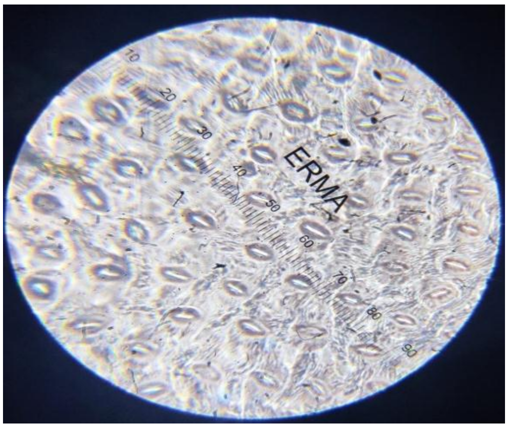


Figure 4. 12. Stomatal density /mm² of different shrub species in four different distance in each direction.



(a)



(b)

Figure 4. 13. Stomata of *Melastoma melabathricum* at (0-250 m) (a) and (750-1000 m) (b) from the cement factory in eastern direction.

4.1.8. Stomatal size (length and breadth)

Data obtained from the analysis showed that the size of stomata decreased while moving nearer towards factory (Figure 4.13). Distance wise study of data showed that there was significant decrease in mean stomatal size of *Derris elliptica* in all three direction (east, west and north) except south direction but *Casearia graveolens* and *Cassia fistula* showed significant decrease in stomatal size of east and north direction (Annex 13). Likewise, *Shorea robusta* showed significant decrease of stomatal size only in east direction while moving nearer distance to the factory (Annex 13).

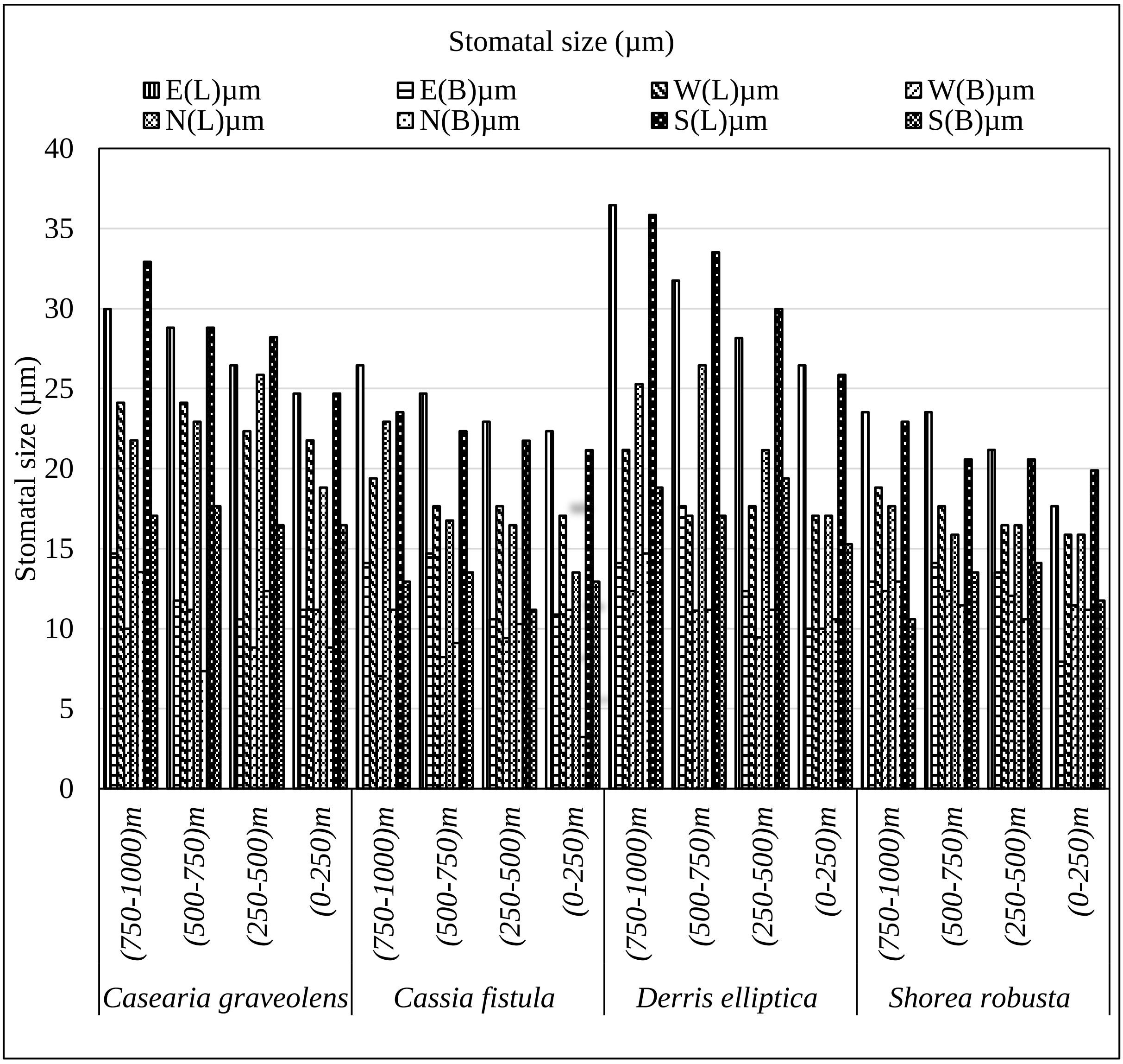


Figure 4. 14. Stomatal size (µm) of different tree species in four different distance in each direction.

Studying the data of mean stomatal size of shrub species it was observed that, *Melastoma melabathricum* showed significant decrease only in length of northern direction and southern direction while moving towards nearer to the factory (Figure 4.14). While *Clerodendrum viscosum* showed significant decrease in east and north direction (Annex 14).

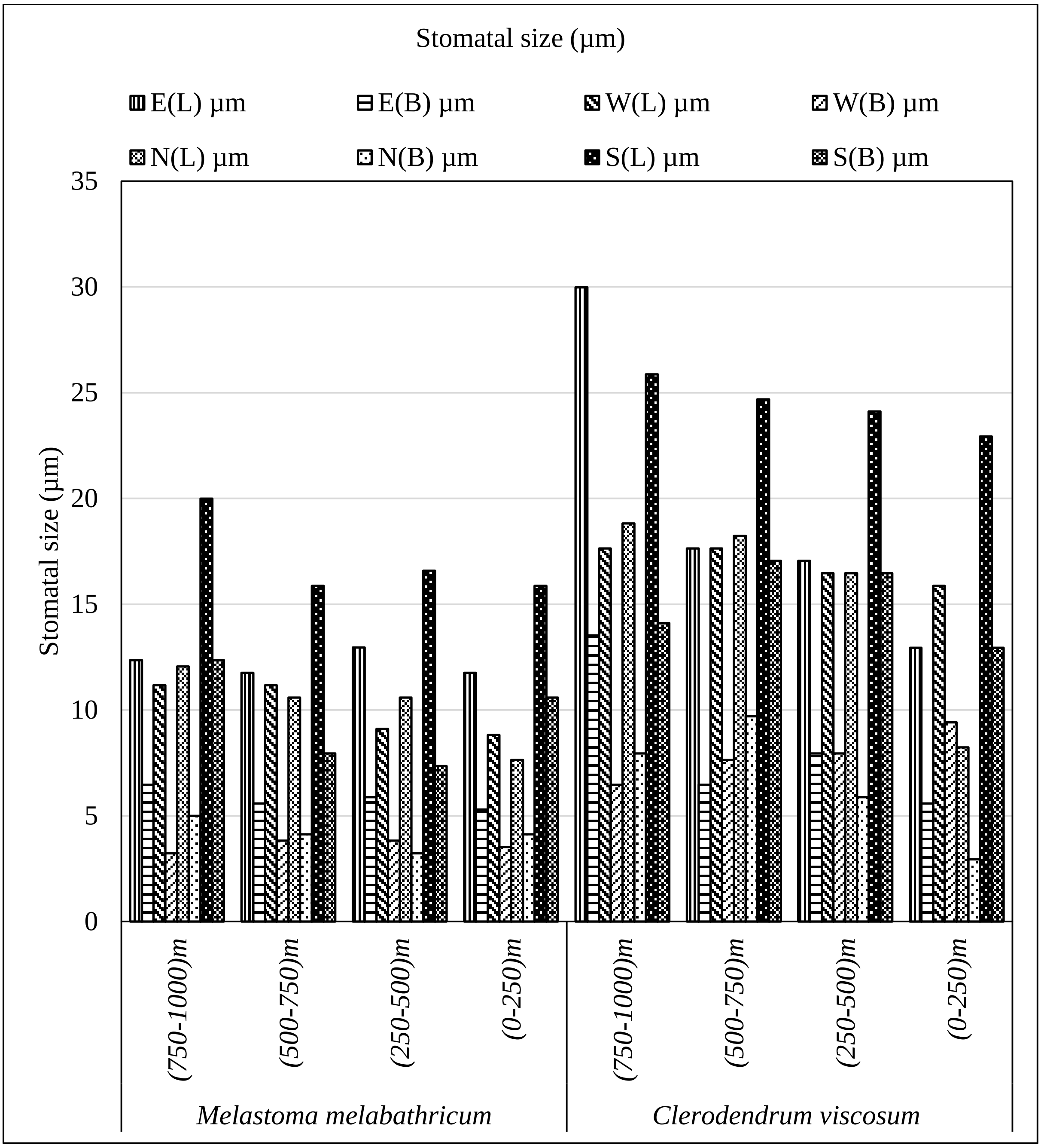


Figure 4. 15. Stomatal size (µm) of different shrub species in four different distance in each direction.

4.1.9. Specific leaf area (SLA)

The result obtained from the analysis showed that, while moving towards nearer to the factory, *Derris elliptica* showed significant decrease in mean specific leaf area (SLA) of all direction except southern direction; *Casearia graveolens* showed significant decrease in all direction except eastern direction; *Cassia fistula* showed significant decrease in all direction except western direction and *Shorea robusta* showed significant decrease only in east and north direction (Figure 4.15) (Annex 15).

Studying the direction wise data, *Derris elliptica* showed no significant change in SLA of all direction. *Casearia graveolens* and *Shorea robusta* showed significant decrease only in (250-500 m). *Cassia fistula* showed significant decrease in almost all ranges (Annex 15).

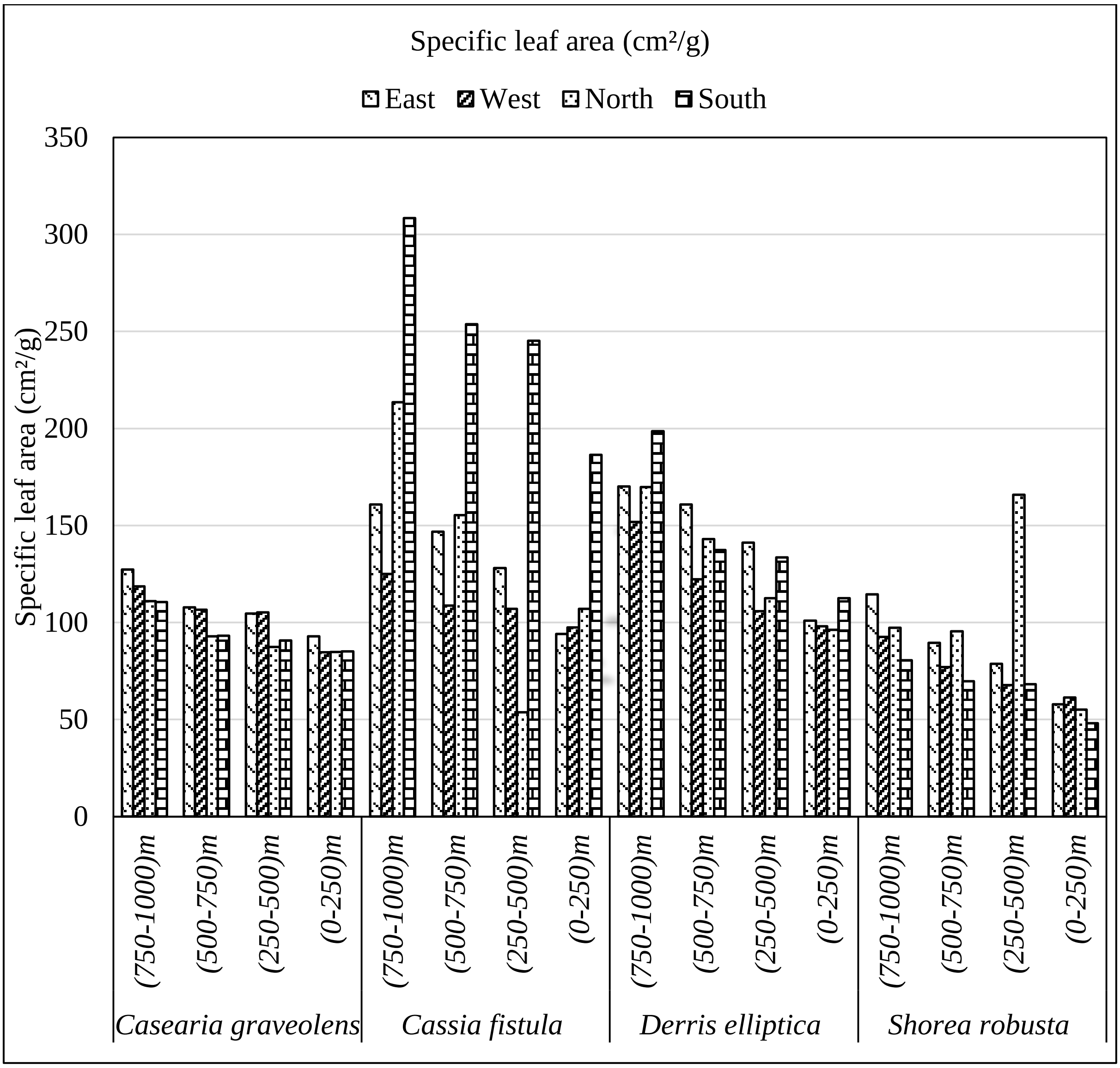


Figure 4. 16. Specific leaf area (cm²/g) of different tree species in four different distance in each direction.

From the analysis of SLA of shrub species it was observed that most of the species except *Colebrookea oppositifolia* have insignificant change in SLA of southern direction (Figure 4.16) (Annex 16). Here, *Colebrookea oppositifolia* showed significant decrease in all direction except western direction. Direction wise data of almost all species shows significant changes in almost all direction (Annex 16).

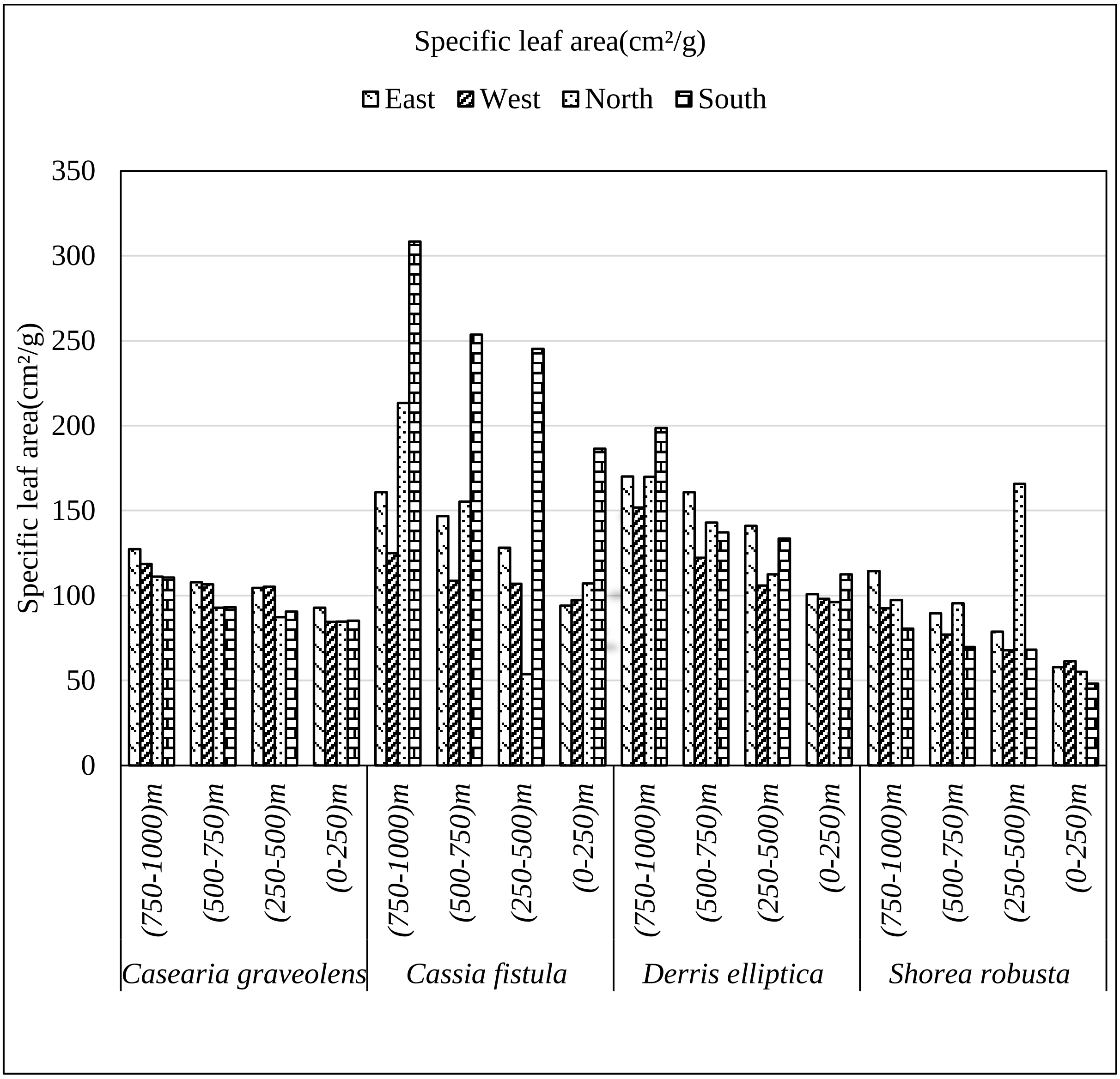


Figure 4. 17. Specific leaf area (cm²/g) of different tree species in four different distance in each direction.

CHAPTER 5: DISCUSSION

5.1. APTI study

The method of air pollution tolerance index (APTI) helps in screening of plants for their tolerance level to air pollutants which is important because the sensitive plants can serve as bio-indicator and tolerant plants as a sink for controlling air pollution (Manjunath and Reddy, 2017). Trees and shrubs are regarded as very efficient for filtering dust pollutants (Farmer, 1993). APTI have four different parameters namely relative water content, total chlorophyll content, leaf extract pH and ascorbic acid. The results of these parameters are discussed below.

5.1.1. Relative Water Content

Relative water content (RWC) of a leaf is a measurement of its hydrated status relative to its maximal water holding capacity at full turgidity. Plant water is necessary to maintain the temperature, nutrient conduction and to help in metabolic processes (Otuu *et al.,* 2014). RWC helps to measure the plant water status in term of physiological consequences of cellular water deficit (Nayak *et al.,* 2018). In the present study, RWC of most of the plant species increased significantly while moving nearer to the factory i.e. 0-250 meters distance (Annex 1 and 2). The higher RWC nearer to the industry might be responsible for normal functioning of biological processes in plants (Meerabai *et al.*, 2012). Plants with higher RWC have greater tolerance capacity towards drought condition (Dedio, 1975). *Casearia graveolens* among the tree species showed highest RWC of 90.47% at 0-250 meters distance in eastern direction of factory and also showed lowest RWC of 40.09% at 750-1000 meters distance from the factory in western direction (Annex 1). Likewise, among the shrub species *Melastoma melabathricum* showed highest RWC at 0-250 meters distance in eastern direction and *Colebrookea oppositifolia* showed lowest RWC of 24.58% at 750-1000 meters distance in southern direction (Annex 2). It has been observed that RWC of plant species increases with pollution in compare to that of less polluted or control site (Radhapriya *et al.,* 2011; Amulya *et al.,* 2015; Sharma, 2019; Yadav and Pandey, 2020). Due to the air pollution the transpiration rate of plants get reduced which damages the leaf engine that pulls water up from the roots (1-2% of the total). Consequently, the plants neither bring minerals nor cool the leaf (Lohe *et al.,* 2015). All the plant species in this study near the factory had higher RWC, which might have helped to maintain its physiological balance under stress condition.

5.1.2. Total chlorophyll content

Chlorophyll content of plant signifies its photosynthesis activity as well as growth and development of biomass (Rai *et al.,* 2013). The variation in chlorophyll content of plants is based upon the age of leaf, plant species, pollution level and atmospheric condition of the particular area such as temperature and light intensity (Katiyar and Dubey, 2001; Hbida and Harikrishna, 2010; Achakzai *et al*., 2017). In the present study, significant decrease in the chlorophyll content of all the species (both tree and shrub) except *Casearia graveolens* was observed while moving nearer to the factory (Annex 3 and 4). In *Casearia graveolens* also the chlorophyll content decreased significantly near the factory except in the eastern direction. Decrease in chlorophyll content might be due to the accumulation of dust particles from the factory. Accumulation of dust causes severe damage in the photosynthetic apparatus (Prajapati and Tripathi, 2008). As the leaf samples was collected during winter season, the reduction in chlorophyll content may be due to the maximum dust accumulation on the leaf surface and its interference with incident light intensity, leading to a reduction in net photosynthesis. Deposition of dust also interferes with gas diffusion between the leaf and air by blocking the stomata (Prajapati and Tripathi, 2008). *Cassia fistula* among the trees and *Phoenix acaulis* among the shrub showed highest chlorophyll content of 0.95mg/g and 0.56mg/g respectively. Likewise, *Casearia graveolens* of tree species and *Phoenix acaulis* of shrub species showed lowest chlorophyll content of 0.068mg/g and 0.05mg/g respectively. Here, highest value of chlorophyll content was observed from 750-1000 meters distance from the factory in southern direction whereas lowest chlorophyll content was obtained from nearer distance to the factory i.e. 0-250meters distance in western direction. Depletion in chlorophyll content might be due to the increased level of pollutants while moving nearer to the factory. According to Kumar (2015), significant loss in total chlorophyll content in the leaves of plants when exposed to air pollution supports an argument that the chloroplast is the primary site of attack by air pollutants such as SPM, SO_x, and NO_X. These pollutants make their entrance into the tissues through the stomata and cause partial denaturation of the chloroplast and decreases pigment content in the cells of polluted leaves (Tripathi and Gautam, 2007). The lower content of SO_2 can fulfil the essential nutrient sulphur requirement of the plant but excess amount of SO_2 may become toxic to the plant, which will injure the chloroplast membranes and also damage the plasmalemma, other important membranes and disrupt enzyme activity (Hopkins, 1999). The study done by Rao and Leblanc (1966) also revealed that reduction in chlorophyll content brought by acidic pollutants like SO_2 causes phaeophytin formation by acidification of chlorophyll. Chlorophyll content also varies with the tolerance as well as sensitivity of the plant species (Jyothi and Jaya, 2010). Higher the sensitivity nature of the plant species, lower is the chlorophyll content (Agrawal and Tiwari, 1997). Among all the studied plants, the degradation of chlorophyll content in *Cassia fistula* was lower than in other tree species near the factory. Hence *Cassia fistula* can be considered as tolerant plant species due to its nature of maintaining chlorophyll content even under stress condition. Chlorophyll content are essential for the photosynthetic activity and decrease in the level of chlorophyll content has been used as an indicator of air pollution (Pawar and Dubey 1985).

5.1.3. Leaf extract pH

Leaf pH helps plant physiological responses towards stress (Miria and Khan, 2013). In the present study the pH of the leaf extract decreased significantly near the factory than at 750-1000 meters away. Plant species with more acidic pH is more susceptible, whereas neutral (pH 7) and more basic pH are considered as more tolerant (Singh and Verma, 2007). Tree species *Derris elliptica* and shrub *Colebrookea oppositifolia* measured highest pH value of 6.79 and 6.96 respectively whereas tree *Shorea robusta* and shrub *Melastoma melabathricum* showed lowest pH value of 5.09 and 3.81 respectively (Annex 5 and 6). Plants with higher pH have improved tolerance towards air pollution, while lower pH show good correlation with sensitivity to air pollution (Kuddus *et al*., 2011; Yan and Hui, 2008). Higher leaf pH also provide better resistance against pollutants by converting sugars into ascorbic acid and enhancing the reducing power of ascorbic acid (Pravin and Madhunita, 2013). Plant species collected from the industrial site have more acidic pollutants such as SO_2 and NO_2 or in response to different metabolic changes due to the presence of specific pollutants (Ahmad, 2019). When SO_2 diffuses through stomata, gaseous SO2 dissolves in water to form sulfites, bisulfites and their ionic species with the generation of protons, thus affecting the cellular pH (Malhotra and Khan, 1984). Photosynthesis is strongly associated with leaf pH and low leaf pH reduce the photosynthetic process in plants (Yan- Yu and Hui, 2008; Thakar and Mishra, 2010; Rai, 2019).

5.1.4. Ascorbic Acid

Ascorbic acid is an antioxidant that act as reducing agent and influence resistance to harsh environmental stresses including atmospheric pollution (Keller and Schwager, 1977; Lima *et al*., 2000; Rai *et al*., 2013). Being very important reducing agents, ascorbic acid also plays a vital role in cell wall synthesis, defense and cell division (Conklin, 2001). The reducing power of ascorbic acid is directly proportional to its concentration (Lewis, 1976). Among the four tree species studied, three species (*Casearia graveolens*, *Derris elliptica* and *Shorea robusta*), except *Cassia fistula*, showed significant increase in ascorbic acid in all direction while moving nearer to the factory i.e. 0-250 meters distance from 750-1000 meters distance (Annex 7). *Cassia fistula* also showed significant increase in ascorbic acid in all direction except east direction. In case of shrub species, all four species showed significant increase in ascorbic acid content in all four directions (Annex 8) while moving nearer to the factory. Pollution load dependent increase in ascorbic acid content of all species may be due to the increased rate of production of reactive oxygen species (ROS) during photo oxidation process (He and Hader, 2002; Tripathi and Gautam, 2007). The ascorbic acid imparts tolerance to air pollution in plants (Lima *et al*., 2000; Zhang *et al*., 2016) and may prevent the damaging effect in plant tissues (Singh *et al*., 1991; Kapoor and Bansal, 2013). Ascorbic acid also protects chloroplast against SO_2 induced H_2O_2, O_2 and OH^- accumulation and thus protects the enzymes of CO_2 fixation cycle and chlorophyll for inactivation (Tiwari *et al*., 2006). Plants with high ascorbic acid content are resistant plants whereas sensitive plants possess a low level ascorbic acid. *Cassia fistula* of tree species and *Phoenix acaulis* of shrub species possess high amount of ascorbic acid i.e. 47.37mg/g and 44.5mg/g respectively in western direction at the distance nearer to the factory i.e. 0-250meters distance. Thus, these two plant species maintaining high ascorbic acid level under polluted condition are considered to be tolerant to air pollutants (Agbare and Esiefarienrhe, 2009).

5.1.5. Air pollution tolerance index

Air pollution tolerance index (APTI) is an inherent quality of plants in order to determine air pollution stress (Rai *et al*., 2013). Screening of plants on the basis of their susceptibility level is very important. Combining four different physiological parameters (RWC, TChl content, pH and ascorbic acid) of APTI gives a more reliable result than depending on single parameter as they are inter linked to each other (Lui and Ding, 2008; Agbaire, 2009). In the present study, the APTI values of selected tree species ranged from 5.11±2.91 to 42.07±2.96 whereas that of shrub species ranged from 3.54±0.19 to 30.74±0.96 (Annex 9 and 10). The APTI values of most of the species increased significantly while moving nearer to the factory, such results was also observed by Rai *et al*., 2013 in the industrial site. Babu *et al*., 2013 also observed that all the plant species showed higher values of APTI in polluted site as compared to the control site. Cement factory is one of the highly polluting industries, the major impact being confined to air environment (Kumar *et al*., 2008). All the tree species showed highest APTI values in eastern direction and among the shrub species studied

Colebrookea oppositifolia and *Clerodendrum viscosum* have highest APTI values at northern direction, *Melastoma melabathricum* showed highest APTI values in eastern direction and *Phoenix acaulis* have highest APTI values at western direction. Among all four direction, highest APTI values were observed in east direction at 0-250 meters distance from the factory. Different plant species respond differently to air pollution (Rai *et al*., 2013) and plant species known to be sensitive or tolerant in one geographical area may behave differently in another area (Raza *et al*., 1985; Karmak and Padhy, 2019). According to Assadi *et al*., 2011 plants may differ significantly in their responses to air pollution because of various factors like concentration of air pollutants and their temporal distribution, genetic origins, physiological activity, metrological parameters, nutritional status of plants and other environmental factors. In the present study all tree species showed high APTI values in eastern direction which is possibly due to the presence of road between the forests to transport cement from the factory as well as the dust emission from the factory (Radhapriya *et al*., 2012) and these dust are blown to the east direction due to westerly wind in winter (Nayava, 1980), which flow from west to east. APTI values was also observed high among shrubs in north direction which might be due to unpaved road and vehicular movement for transportation of raw material to the factory.

On the basis of APTI values plant species were grouped into three categories (Padmavathi *et al*., 2013). Out of eight species studied, three tree species (*Casearia graveolens*, *Cassia fistula* and *Shorea robusta*) and two shrub species (*Colebrookea oppositifolia and Phoenix acaulis*) were tolerant species with APTI values ranging above seventeen. *Derris elliptica* was considered as intermediate species with APTI value ranging between twelve and sixteen. Shrub species namely *Melastoma melabathricum* and *Clerodendrum viscosum* were kept under sensitive category because their APTI value were less than eleven. Plant species with higher APTI values can help in alleviating air pollution problem whereas plants with lower APTI values can be considered as an indicator of air pollution level (Singh and Rao, 1983). The long term exposure of air pollution in low concentration produces harmful impacts on plant leaves without visible injury (Joshi *et al*., 2009). Previous studies have also clearly shown the impact of air pollution on biochemical parameters (Total chlorophyll content and ascorbic acid) of plants (Pandey *et al*., 2016; Kwak *et al*., 2020). Plants remove pollutants by three mechanism, namely absorption by leaves, deposition of particulates and aerosol over leaf surfaces (Tewari, 1994; Rawat and Banerjee, 1996). The plant species which have been identified as tolerant and intermediate are suggested for urban plantation. Such plantation is the best way for economic growth and sustainable development of cities with industrial structure under air pollution (Chaudhary and Rathore, 2018; Zhu *et al*., 2019; Tahmasbi *et al*., 2019; Chaudhary and Rathore, 2019). Similarly, plant species which are considered as sensitive can act as bio-indicator for air pollution (Singh and Rao, 1983; Seyyednjad *et al*., 2011). Several authors have investigated the significance of APTI in determining the tolerance along with the sensitivity of plant species (Tripathi and Gautam, 2007; Rai *et al*., 2013; Lohe *et al*., 2015, Nayak *et al*., 2018; Dhyani *et al*., 2019; Yadav and Pandey, 2020). Urban forest and development of green belts provides natural ways of cleaning the atmosphere by absorption of gaseous pollutants, particulates and noise through their leaves (Pathak *et al*., 2011).

5.2. Leaf structure (Stomatal Density, Stomatal size, Specific Leaf Area)

Change in morphological traits because of air pollutants could reduce growth in plants (Seyyednezhad *et al*., 2013). Morphological changes occurring in plants are often accompanied by decreasing leaf area and increasing stomatal density, which can lead to disruption in photosynthetic activity and thereby a decrease in growth (Ghorbanli *et al*., 2009). Several researcher have reported the effect of cement dust pollution on micro-morphological characteristics of leaves in different plant species (Shukla *et al*., 2008; Raajasubramanian *et al*., 2011; Kumar and Thambavani 2012; Kumar *et al*., 2015). Present study showed decrease in specific leaf area (SLA) of most of the plant species in all direction near the factory than those present away from the factory. Similar observation was also reported by Kayode and Otoide (2007), and Seyyednejad *et al*., 2009. Continuous exposure of leaf to dust leads to the formation of dense dust layer over the leaf surface which reduces light capturing capacity of plants and hamper plant photosynthetic activities (Pourkhabbaz *et al*., 2010). Reduction in leaf size is an adaptational feature of plant species in order to minimize the entry of poisonous gases into the leaves when exposed to pollution (Zarinkamar *et al*., 2013). SLA also shows strong association with stomatal conductance, photosynthetic rate and plant growth rate (Meziane and Shipley 1999).

Stomatal density of most of the tree species increased significantly while moving from (750-1000 m) towards nearer to the factory (0-250 m). Among the tree species studied *Cassia fistula* showed significant increase in stomatal density in all directions. Shrub species also showed increase in stomatal density near the factory. Several researchers have observed increase in stomatal density of plant leaves in response to pollution (Alves *et al*., 2008; Evans *et al*., 1996, Gostin 2009, Kardel *et al*., 2010; Rasid 2011). Stomatal size (length and breadth) of studied plant species was also found to be decreased while moving nearer to the factory. Smaller stomatal dimensions (length and breadth) are more adaptable to environmental conditions. Gostin (2009) also reported decrease in size and increase in stomatal density in the leaves of polluted site. This kind of modification in stomatal frequency is an adaptive nature of plant to counter the blockage and proper functioning of gaseous exchange and transpiration rate (EI-Khatib *et al*., 2012; Amulya *et al*., 2015; Siqueira-Silva *et al*., 2016a, 2016b). Plant species exposed to cement dust polluted environment leads to a mild morph anatomic alteration in leaves making it more resistance to the pollution (Siqueria-Silva *et al*., 2016). Hamal *et al*., 2018 also observed marked reduction in SLA and stomatal size in polluted area.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1. Conclusion

From the present APTI study, it can be concluded that out of eight species studied, three tree species (*Casearia graveolens*, *Cassia fistula* and *Shorea robusta*) and two shrub species (*Colebrookea oppositifolia and Phoenix acaulis*) were air pollution tolerant species. The tree *Derris elliptica* was of intermediate category and other two shrubs namely *Melastoma melabathricum* and *Clerodendrum viscosum* were sensitive species on the basis of their APTI values. The tolerant and intermediate category species can act as sinks to absorb air pollutants and sensitive species can act as bio-indicator. The study also revealed more air pollution in the eastern direction of the factory as the APTI values of most of the plant species were high in this direction. The stomatal density increased and the stomatal size decreased near the cement factory. SLA of all plant species decreased near the cement factory. On the basis of both biochemical and micro-morphological investigations it can be concluded that the effect of air pollutants is more near the factory.

6.2. Recommendations

1. The tolerant plant species such as *Casearia graveolens*, *Cassia fistula* and *Shorea robusta, Colebrookea oppositifolia and Phoenix acaulis* can be recommended for plantation in degraded areas and roadside around Udayapur Cement Factory and other polluted areas of tropical region of Nepal.
2. As the eastern side is more polluted extensive plantation of tolerant plant species mentioned above are recommended.
3. Protection of forest in its peripheral areas are highly recommended for mitigation of air pollutants from the factory.

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ANNEXES

Annex 1. Relative water content (%) of selected tree species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Distance	East	West	North	South
Casearia graveolens	(750-1000)m	70.29±21.72 Aa	40.09±28.02 Aa	68.97±2.66 Aa	60.02±20.44 Aa
	(500-750)m	72.74±1.63 Aa	60.76±2.87 Aab	70.4±3.42 Aab	62.57±20.31 Aa
	(250-500)m	82.14±3.47 Ba	68.7±2.82 Ab	71.26±4.07 ABab	62.96±12.26 Aa
	(0-250)m	90.47±3.55 Ba	70.01±5.29 Ab	76.22±0.23 Ab	65.05±9.73 Aa
Cassia fistula	(750-1000)m	68.55±6.88 Ba	53.7±2.50 Aa	54.4±0.43 Aa	58.59±10.43 ABa
	(500-750)m	72.02±1.24 Ba	54.42±3.92 Aab	60.72±3.72 Aab	75.15±4.89 Bab
	(250-500)m	75.76±5.32 Ba	59.41±1.78 Abc	63.49±4.29 Ab	78.08±10.79 Bbc
	(0-250)m	76.14±14.05 BCa	60.39±2.48 Ac	64.22±5.21 ABb	80.29±11.94 Cc
Derris elliptica	(750-1000)m	60.84±11.15 Aa	50.1±6.05 Aa	62.4±2.15 Aa	54.23±2.11 Aa
	(500-750)m	68.28±0.81 Bab	54.98±1.74 Aa	64.64±5.19 Ba	59.32±18.23 Aa
	(250-500)m	71.09±3.98 Aab	66.15±3.25 Ab	67.07±3.26 Aa	64.9±13.84 Aa
	(0-250)m	80.28±7.54 Bb	66.83±2.30 Ab	67.88±6.34 Aa	74.17±3.58 ABa
Shorea robusta	(750-1000)m	73.81±10.84 Aa	62.59±9.25 Aa	68.07±4.69 Aa	69.68±5.07 Aa
	(500-750)m	75.74±4.50 Ba	63.46±1.03 Aa	68.25±5.66 ABab	73.66±6.45 Bab
	(250-500)m	78.83±15.89Aa	67.65±0.77 Aab	69.19±5.17 Aa	79.32±0.75Ab
	(0-250)m	84.63±1.68Ba	75.41±4.95Ab	75.59±5.05Aa	81.57±0.93ABb

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P< 0.05$, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 2. Relative water content (RWC %) of selected shrub species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Distance	East	West	North	South
Clerodendrum viscosum	(750-1000)m	45.38±5.46 Aa	44.14±5.05 Aa	44.72±4.98 Aa	39.44±15.64 Aa
	(500-750)m	50.46±9.08 Bab	36.91±3.10 Aa	47.28±4.32 ABab	41.37±4.03 ABab
	(250-500)m	63.91±10.46 Bb	47.41±6.06 Aab	56.15±4.42 ABb	52.52±2.46 ABab
	(0-250)m	64.35±3.15 Ab	54.39±7.92 Ab	56.16±7.69 Ab	56.86±5.78 Ab
Colebrookea oppositifolia	(750-1000)m	32.89±6.49 Ba	28.53±3.04 ABa	26.19±0.68 Aa	24.58±2.35 Aa
	(500-750)m	36.08±6.03 Bab	26.35±1.82 Aa	30.19±3.94 ABa	34.16±4.45 ABb
	(250-500)m	45.98±4.93 Bbc	27.84±3.15 Aa	30.55±6.30 Aa	28.48±0.79 Aab
	(0-250)m	49.19±8.43 Cc	33.7±0.65 ABb	31.36±0.35 Aa	41.85±3.55 BCc

Melastoma melabathricum	(750-1000)m	68.96±4.03 ABa	60.59±3.12 Aa	69.75±6.64 ABa	73.82±8.54 Ba
	(500-750)m	78.46±0.97 Bab	64.28±1.39 Aa	76.63±3.55 Bab	73.96±8.66 Ba
	(250-500)m	85.97±4.30 Bbc	70.24±2.95 Ab	77.65±4.46 ABab	76.05±7.41 Aa
	(0-250)m	89.09±8.51 Ac	73.48±3.66 Ab	80.11±3.76 Ab	82.68±28.22 Aa
Phoenix acaulis	(750-1000)m	59.77±5.75 ABa	63.28±6.44 Ba	52.35±6.29 Aa	65.46±2.83 Ba
	(500-750)m	63.92±17.36 Aa	63.76±2.23 Aa	54.27±2.08 Aa	69.12±10.50 Aa
	(250-500)m	65.16±8.23 Aa	64.24±0.88 Aa	56.82±2.19 Aa	69.67±16.45 Aa
	(0-250)m	66.08±5.74 Aa	64.39±1.94 Aa	59.24±5.10 Aa	70.38±30.02 Aa

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P< 0.05$, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 3. Total Chlorophyll content (mg/g) of selected tree species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Range	East	West	North	South
Casearia graveolens	(750-1000)m	0.23±0.025 Bb	0.14±0.009 Ac	0.14±0.006 Ad	0.3±0.007 Cd
	(500-750)m	0.21±0.005 Cb	0.11±0.003 Ab	0.14±0.002 Bc	0.23±0.007 Dc
	(250-500)m	0.16±0.002 Ca	0.1±0.007 Ab	0.12±0.002 Bb	0.2±0.013 Db
	(0-250)m	0.21±0.005 Db	0.068±0.003 Aa	0.09±0.002 Ba	0.15±0.011 Ca
Cassia fistula	(750-1000)m	0.95±0.019 Bd	0.31±0.005 Ad	0.31±0.016 Ad	1.02±0.008 Cd
	(500-750)m	0.69±0.007 Bc	0.26±0.005 Ac	0.26±0.004 Ac	0.71±0.009 Cc
	(250-500)m	0.56±0.006 Bb	0.22±0.018 Ab	0.23±0.001 Ab	0.59±0.004 Cb
	(0-250)m	0.29±0.003 Ca	0.19±0.009 Ba	0.17±0.001 Aa	0.32±0.006 Da
Derris elliptica	(750-1000)m	0.34±0.002 Cd	0.14±0.036 Ab	0.14±0.002 Ad	0.23±0.007 Bc
	(500-750)m	0.29±0.006 Cc	0.12±0.001 Ab	0.12±0.001 Ac	0.23±0.006 Bc
	(250-500)m	0.25±0.004 Db	0.07±0.001 Aa	0.11±0.001 Bb	0.22±0.006 Cb
	(0-250)m	0.19±0.003 Ca	0.05±0.001 Aa	0.1±0.001 Ba	0.20±0.005 Da
Shorea robusta	(750-1000)m	0.34±0.005 Dd	0.12±0.001 Ac	0.14±0.001 Bd	0.31±0.009 Cc
	(500-750)m	0.33±0.004 Dc	0.1±0.001 Ab	0.13±0.008 Bc	0.29±0.005 Cc
	(250-500)m	0.26±0.004 Cb	0.09±0.015 Ab	0.12±0.001 Bb	0.25±0.008 Cb
	(0-250)m	0.19±0.010 Da	0.07±0.004 Aa	0.11±0.005 Ba	0.13±0.003 Ca

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P< 0.05$, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 4. Total Chlorophyll content (mg/g) of selected shrub species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Range	East	West	North	South
Clerodendrum viscosum	(750-1000)m	0.29±0.014 Cc	0.09±0.002 Ac	0.13±0.001 Bd	0.39±0.005 Dc
	(500-750)m	0.25±0.007 Cb	0.07±0.001 Ab	0.12±0.001 Bc	0.24±0.007 Cb
	(250-500)m	0.21±0.005 Ca	0.076±0.001 Ab	0.09±0.001 Bb	0.24±0.006 Db
	(0-250)m	0.2±0.006 Ba	0.06±0.004 Aa	0.06±0.001 Aa	0.2±0.005 Ba
Colebrookea oppositifolia	(750-1000)m	0.35±0.001 Bd	0.14±0.002 Ac	0.14±0.004 Ac	0.38±0.005 Cc
	(500-750)m	0.28±0.005 Cc	0.12±0.006 Ab	0.14±0.006 Bc	0.38±0.006 Dc
	(250-500)m	0.27±0.003 Cb	0.1±0.004 Aa	0.13±0.003 Bb	0.35±0.007 Db
	(0-250)m	0.25±0.008 Ca	0.09±0.009 Ba	0.08±0.001 Aa	0.25±0.007 Ca
Melastoma melabathricum	(750-1000)m	0.27±0.003 Cd	0.11±0.017 Ab	0.18±0.011 Bd	0.43±0.011 Dd
	(500-750)m	0.24±0.005 Cc	0.11±0.005 Ab	0.14±0.004 Bc	0.33±0.025 Dc
	(250-500)m	0.21±0.003 Cb	0.1±0.002 Aab	0.11±0.001 Bb	0.27±0.009 Db
	(0-250)m	0.15±0.005 Ca	0.08±0.001 Aa	0.09±0.001 Aa	0.13±0.006 Ba
Phoenix acaulis	(750-1000)m	0.48±0.004 Cc	0.09±0.003 Ad	0.12±0.005 Bd	0.56±0.004 Dd
	(500-750)m	0.22±0.008 Cb	0.09±0.001 Ac	0.11±0.002 Bc	0.48±0.005 Dc
	(250-500)m	0.19±0.028 Ca	0.05±0.001 Ab	0.1±0.001 Bb	0.32±0.003 Db
	(0-250)m	0.17±0.008 Ca	0.05±0.001 Aa	0.09±0.001 Ba	0.09±0.009 Ba

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P < 0.05$, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 5. Leaf extract pH of selected tree species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Range	East	West	North	South
Casearia graveolens	(750-1000)m	6.68±0.035 Dd	6.24±0.017 Cc	5.94±0.017 Ac	6.12±0.015 Bb
	(500-750)m	6.15±0.021 Cc	6.14±0.055 Cb	5.92±0.025 Abc	6.01±0.020 Bb
	(250-500)m	6.05±0.020 Cb	6.15±0.042 Db	5.89±0.011 Bb	5.62±0.015 Aa
	(0-250)m	5.72±0.050 Ba	6.02±0.026 Ca	5.3±0.006 Aa	5.71±0.146 Ba
Cassia fistula	(750-1000)m	6.48±0.015 Cd	6.38±0.010 Bb	6.11±0.010 Ac	6.42±0.066 Bb
	(500-750)m	6.39±0.035 Bc	6.36±0.025 Bab	5.65±0.005 Ab	6.39±0.095 Bb
	(250-500)m	5.64±0.045 Aa	6.35±0.012 Cab	5.61±0.015 Aa	6.19±0.136 Ba
	(0-250)m	5.86±0.046 Bb	6.29±0.057 Da	5.62±0.020 Aa	6.03±0.010 Ca
Derris elliptica	(750-1000)m	6.79±0.091 Dc	6.45±0.010 Cb	6.07±0.015 Ac	6.19±0.010 Bab
	(500-750)m	6.33±0.015 Ba	6.41±0.017 Ba	5.91±0.015 Ab	6.33±0.130 Bb
	(250-500)m	6.3±0.006 Ba	6.4±0.031 Ca	5.93±0.040 Ab	6.27±0.025 Bbc
	(0-250)m	6.45±0.015 Db	6.39±0.021 Ca	5.72±0.026 Aa	6.13±0.010 Ba

Shorea robusta	(750-1000)m	6.24±0.060 Db	5.83±0.045 Bb	5.46±0.011 Ab	6.1±0.020 Cc
	(500-750)m	6.21±0.017 Db	5.81±0.020 Bb	5.46±0.074 Ab	6.04±0.032 Cb
	(250-500)m	6.21±0.015 Cb	5.76±0.044 Bab	5.09±0.229 Aa	6.04±0.021 Cb
	(0-250)m	5.87±0.035 Da	5.74±0.025 Ca	5.23±0.015 Bab	4.51±0.021 Aa

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at P< 0.05, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 6. Leaf extract pH of selected shrub species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Range	East	West	North	South
Clerodendrum viscosum	(750-1000)m	6.72±0.025 Cd	5.95±0.025 Ab	6.24±0.312 ABb	6.29±0.036 Bc
	(500-750)m	6.66±0.015 Dc	5.81±0.026 Ba	6±0.045 Cab	5.59±0.035 Aa
	(250-500)m	6.44±0.006 Db	5.77±0.084 Ba	5.93±0.030 Cab	5.57±0.060 Aa
	(0-250)m	5.58±0.055 Aa	5.74±0.006 Ba	5.91±0.006 Ca	6.05±0.006 Db
Colebrookea oppositifolia	(750-1000)m	6.96±0.015 Bd	6.67±0.032 Bb	6.67±0.032Ba	5.96±0.46Aa
	(500-750)m	6.87±0.026 Cc	6.69±0.070 BCb	6.42±0.386 ABa	6.17±0.010 Aa
	(250-500)m	6.45±0.015 Ab	6.39±0.075 Aa	6.33±0.467 Aa	6.05±0.020 Aa
	(0-250)m	6.23±0.025 Ba	6.44±0.040 Ca	6.28±0.070 Ba	6.11±0.040 Aa
Melastoma melabathricum	(750-1000)m	5.51±0.100 Bd	5.28±0.225 ABc	6.2±0.020 Cc	5.07±0.162 Ab
	(500-750)m	4.81±0.010 Ac	4.78±0.071 Ab	4.83±0.038 Ab	5.07±0.175 Bb
	(250-500)m	4.52±0.126 Bb	4.45±0.010 Ba	4.75±0.045 Cb	3.81±0.010 Aa
	(0-250)m	4.3±0.040 BCa	4.41±0.010 Ca	4.27±0.125 Ba	3.86±0.015 Aa
Phoenix acaulis	(750-1000)m	6.64±0.035 Cd	5.97±0.055 Ab	6±0.066 Ac	6.5±0.036 Bc
	(500-750)m	6.58±0.029 Bc	5.84±0.010 Ab	5.51±0.015 Ab	5.81±0.501 Aa
	(250-500)m	6.45±0.015 Bb	5.42±0.096 Aa	5.42±0.083 Aab	6.34±0.015 Bbc
	(0-250)m	5.77±0.010 Ba	5.41±0.175 Aa	5.32±0.020 Aa	6±0.031 Cab

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at P< 0.05, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 7. Ascorbic acid content (mg/g) of selected tree species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Range	East	West	North	South
Casearia graveolens	(750-1000)m	2.8±0.088 Ca	1.73±0.208 Aa	2.4±0.167 Ba	2.3±0.067 Ba
	(500-750)m	5.63±0.300 Cb	1.97±0.333 Aa	3.03±0.120 Bb	2.6±0.067 Bb
	(250-500)m	6.05±0.020 Dc	5.47±0.202 Cb	3.2±0.115 Bb	2.63±0.088 Ab
	(0-250)m	5.72±0.050 Bb	6.33±0.252 Cc	6.43±0.153 Cc	2.63±0.088 Ab
Cassia fistula	(750-1000)m	6.13±0.371 Aa	13.77±3.877 Ba	9.6±0.218 Aa	5.93±0.176 Aa
	(500-750)m	7.33±1.766 Aa	33.2±1.126 Cb	12.43±1.244 Bb	11.33±1.097 Bb
	(250-500)m	5.64±0.045 Aa	44.6±0.145 Dc	21±0.353 Cc	15.33±0.088 Bc
	(0-250)m	5.86±0.046 Aa	47.37±1.617 Dc	36.03±0.561 Cd	21.3±0.584 Bd
Derris elliptica	(750-1000)m	1.38±0.006 Aa	2.13±0.218 Ba	1.5±0.100 Aa	1.33±0.088 Aa
	(500-750)m	1.87±0.033 Bb	2.43±0.167 Ca	2±0.088 Bb	1.4±0.033 Aa
	(250-500)m	6.3±0.006 Cc	2.61±0.279 Ba	2.93±0.202 Bc	1.5±0.033 Ab
	(0-250)m	6.45±0.015 Cd	6.9±0.945 Cb	3.2±0.120 Bd	1.93±0.033 Ac
Shorea robusta	(750-1000)m	2.63±0.305 Ca	1.87±0.153 ABa	2.17±0.145 Ba	1.73±0.033 Aa
	(500-750)m	3.3±0.348 BCb	3.53±0.260 Cb	2.83±0.333 Ba	1.87±0.088 Aa
	(250-500)m	6.21±0.015 Dc	3.8±0.208 Cb	2.97±0.251 Ba	2.23±0.218 Ab
	(0-250)m	5.87±0.035 Bc	9.9±1.550 Cc	7.3±0.901 Bb	2.83±0.100 Ac

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P < 0.05$, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 8. Ascorbic acid content of selected shrub species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Range	East	West	North	South
Clerodendrum viscosum	(750-1000)m	1.53±0.133 Ba	1.53±0.202 Ba	1.73±0.202 Ba	1.17±0.033 Aa
	(500-750)m	4.13±1.467 Bb	2.13±0.067 Ab	2.33±0.145 Ab	1.4±0.100 Aa
	(250-500)m	6.37±1.801 Bbc	2.43±0.067 Ac	2.7±0.153 Ac	1.9±0.203 Ab
	(0-250)m	8.83±1.377 Cc	2.4±0.167 Ac	2.8±0.153 Ac	4.47±0.361 Bc
Colebrookea oppositifolia	(750-1000)m	1.6±0.176 Aa	1.87±0.185 ABa	1.87±0.115 Ba	1.7±0.057 ABa
	(500-750)m	1.6±0.067 Aa	2.5±0.133 Bc	2.53±0.133 Ba	1.77±0.057 Aab
	(250-500)m	4.33±0.318 Cb	2.2±0.067 Ab	2.93±0.491 Ba	1.83±0.033 Ab
	(0-250)m	18.03±1.133 Bc	3.5±0.089 Ad	14.65±18.687 Ba	2.37±0.033 Ac
Melastoma melabathricum	(750-1000)m	2±0.233 Ba	2±0.088 Ba	1.53±0.057 Aa	1.47±0.057 Aa
	(500-750)m	3±0.033 Cb	2.46±0.208 Bab	2.43±0.133 Bb	1.83±0.067 Ab
	(250-500)m	3.93±0.451 Cc	3.2±0.088 Bb	1.8±0.404 Aa	2.23±0.088 Ac

	(0-250)m	4.96±0.096 Bd	8.03±1.050 Cc	7.37±0.133 Cc	2.9±0.067 Ad
Phoenix acaulis	(750-1000)m	3.1±0.982 Ba	1.9±0.202 Aa	3.25±0.135 Ba	1.4±0.033 Aa
	(500-750)m	8.53±1.850 Cb	1.73±0.167 Aa	3.83±0.120 Bb	1.9±0.153 Aab
	(250-500)m	8.26±0.176 Cb	8.83±0.821 Cb	5.4±0.185 Bc	2.3±0.033 Ab
	(0-250)m	16.2±2.450 Bc	44.5±0.833 Cc	8.23±0.333 Ad	9±0.617 Ac

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P < 0.05$, using one way ANOVA followed by Duncan Multiple Range Test ($N = 80$).

Annex 9. Air Pollution Tolerance Index (APTI) of selected tree species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Range	East	West	North	South
Casearia graveolens	(750-1000)m	8.96±2.184 Aa	5.11±2.912 Aa	8.36±0.343 Aa	7.48±2.029 Aa
	(500-750)m	10.86±0.355 Ba	7.31±0.484 Aa	8.88±0.278 Aab	7.88±1.995 Aa
	(250-500)m	14.79±1.837 Cb	10.28±0.158 Bb	9.05±0.353 ABb	7.83±1.233 Aa
	(0-250)m	16.09±1.741 Cb	10.86±0.685 Bb	11.09±0.107 Bc	8.05±1.041 Aa
Cassia fistula	(750-1000)m	11.42±0.953 Aa	14.58±2.859 Ba	11.61±0.176 ABa	10.27±1.060 Aa
	(500-750)m	12.39±1.344 Aa	27.41±0.868 Cb	13.42±0.485 Ab	15.55±0.915 Bb
	(250-500)m	14.98±1.621 Aa	35.24±0.294 Cc	18.63±0.509 Bc	18.21±1.181 Bc
	(0-250)m	42.07±2.957 Db	36.78±0.941 Cc	27.29±0.334 Bd	21.56±1.013 Ad
Derris elliptica	(750-1000)m	7.07±1.117 Aa	6.42±0.669 Aa	7.17±0.251 Aa	6.28±0.154 Aa
	(500-750)m	8.06±0.071 Ba	7.08±0.073 Aa	7.67±0.487 Ba	6.85±1.819 Aab
	(250-500)m	12.94±1.067 Bb	8.3±0.221 Ab	8.48±0.338 Ab	7.46±1.375 Aab
	(0-250)m	14.65±1.326 Cb	11.13±0.714 Bc	8.65±0.566 Ab	8.64±0.361 Ab
Shorea robusta	(750-1000)m	9.11±1.269 Aa	7.37±0.992 Aa	8.02±0.508 Aa	8.08±0.526 Aa
	(500-750)m	9.73±0.517 Ba	8.43±0.154 Aab	8.41±0.701 Aa	8.55±0.666 Aab
	(250-500)m	10.82±1.657 Ba	8.99±0.122 Ab	8.46±0.455 Aa	9.34±0.178 ABbc
	(0-250)m	20.69±1.485 Db	13.29±0.928 Cc	11.45±0.177 Bb	9.47±0.053 Ac

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P < 0.05$, using one way ANOVA followed by Duncan Multiple Range Test ($N = 80$).

Annex 10. Air Pollution Tolerance Index (APTI) of selected shrub species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

(APTI) Scientific name	Range	East	West	North	South
Clerodendrum viscosum	(750-1000)m	5.61±0.515 Aa	5.34±0.540 Aab	5.57±0.584 Aa	4.72±1.566 Aa
	(500-750)m	7.9±0.884 Ca	4.95±0.345 Aa	6.16±0.351 Bab	4.95±0.456 Aa
	(250-500)m	10.62±2.243 Bb	6.16±0.549 Abc	7.24±0.538 Ab	6.35±0.351 Aa
	(0-250)m	11.54±0.975 Cb	6.83±0.753 Ac	7.29±0.821 ABb	8.48±0.748 Bb
Colebrookea oppositifolia	(750-1000)m	4.46±0.574 Ba	4.12±0.178 ABa	3.89±0.150 ABa	3.54±0.194 Aa
	(500-750)m	4.75±0.597 Aa	4.34±0.176 Aa	4.68±0.481 Aa	4.57±0.417 Ab
	(250-500)m	7.51±0.286 Cb	4.21±0.296 Aa	4.94±0.417 Ba	4.02±0.095 Aa
	(0-250)m	16.62±1.600 Bc	5.66±0.077 Ab	12.37±11.747 ABa	5.69±0.323 Ac
Melastoma melabathricum	(750-1000)m	8.05±0.375 Aa	7.14±0.313 Aa	7.95±0.633 Aa	8.18±0.867 Aa
	(500-750)m	9.36±0.095 Cb	7.64±0.223 Aa	8.87±0.328 BCb	8.38±0.867 ABa
	(250-500)m	10.45±0.251 Bc	8.48±0.311 Ab	8.64±0.312 Aab	8.52±0.713 Aa
	(0-250)m	11.11±0.834 Ac	10.96±0.626 Ac	11.22±0.403 Ac	9.43±2.794 Aa
Phoenix acaulis	(750-1000)m	8.18±1.027 Aa	7.48±0.699 Aa	7.23±0.576 Aa	7.54±0.263 Aa
	(500-750)m	12.2±0.650 Bb	7.4±0.318 Aa	7.58±0.191 Aa	8.11±1.025 Aa
	(250-500)m	12±0.964 Bb	11.26±0.493 Bb	8.66±0.249 Ab	8.49±1.671 Aa
	(0-250)m	16.23±1.763 Bc	30.74±0.957 Cc	10.38±0.677 Ac	12.52±3.007 Ab

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P < 0.05$, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 11. Stomata density/mm^2 of selected tree species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Distance	East	West	North	South
Casearia graveolens	(750-1000)m	396.33±20.85BCa	257.27±60.22Aa	493.67±73.25Ca	319.85±43.42ABa
	(500-750)m	445±24.11Bab	222.5±31.86Aa	382.43±94.06Ba	354.61±20.85Ba
	(250-500)m	500.63±75.21Bb	396.33±55.19Ab	396.33±20.85Aa	368.52±12.08Aa
	(0-250)m	528.44±43.42Bb	403.28±24.11Ab	417.19±20.86Aa	424.14±31.86Ab
Cassia fistula	(750-1000)m	417.19±20.85Aa	389.38±31.86Aa	542.35±20.85Ba	396.33±41.71Aa
	(500-750)m	445±24.11Aab	451.96±31.86Aab	556.26±24.11Ba	445±67.05Aab
	(250-500)m	500.63±75.21Aab	479.77±55.18Aab	584.07±41.71Aa	514.54±31.86Ab
	(0-250)m	528.44±43.42Ab	542.35±83.43Ab	702.27±31.86Bb	521.49±20.86Ab
Derri s	(750-1000)m	292.03±20.85Aa	382.43±12.08BCa	424.14±31.86Ca	368.52±31.86Ba
	(500-750)m	347.66±63.72Aab	396.33±36.14Aa	445±24.10Aab	326.79±102.89Aa

	(250-500)m	382.43±12.08Ab	430.76±47.09ABa	479.76±2.085Bab	396.33±20.85Aa
	(0-250)m	410.24±12.08Ab	445±31.86Aa	500.63±36.14Bb	431.09±24.10Aa
Shorea robusta	(750-1000)m	542.35±75.21ABa	451.95±114.88Aa	535.39±12.08ABa	653.59±73.25Bb
	(500-750)m	730.08±20.85Bb	563.21±20.85Aa	556.25±24.10Aa	528.44±52.49Aa
	(250-500)m	716.18±78.97ABb	730.08±20.85Bb	757.89±43.42Bb	625.78±41.71Aab
	(0-250)m	723.13±86.84Ab	757.89±52.49Ab	806.56±43.42Ab	702.27±31.86Ab

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at P< 0.05, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 12. Stomata density/mm^2 of selected shrub species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Range	East	West	North	South
Melastoma melabathricu	(750-1000)m	841.34±114.88Ba	841.34±174.93Ba	451.95±52.49Aa	778.76±179.84Ba
	(500-750)m	806.57±197.15ABa	869.15±31.86Bab	563.21±20.85Aa	862.19±193.81Ba
	(250-500)m	855.24±62.57ABa	917.82±36.14Bab	723.13±94.06Ab	890.01±134.10ABa
	(0-250)m	869.15±177.40Aa	1091.65±156.56Ab	827.43±52.49Ab	1022.12±146.01Aa
Clerodendrum viscosum	(750-1000)m	326.79±52.49Aa	347.66±159.31Aa	278.13±31.86Aa	368.52±12.08Aa
	(500-750)m	347.66±52.49Aab	375.47±55.18Aa	375.47±83.43Aab	396.33±36.14Aa
	(250-500)m	424.14±31.86Abc	410.24±43.42Aa	424.14±67.05Abc	361.56±43.42Aa
	(0-250)m	438.05±41.71Ac	514.54±31.86Aa	514.54±52.49Ac	445±86.84Aa

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at P< 0.05, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 13. Length (L) and breadth (B) of stomata (µm) in the plant leaves of selected tree species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plants	Distance	E(L)µm	E(B)µm	W(L)µm	W(B)µm	N(L)µm	N(B)µm	S(L)µm	S(B)µm
Casearia graveolens	(750-1000) m	29.98±2.45Bc	14.7±2.07Bb	24.108±2.45Aa	9.996±1.89Aa	21.756±2.72Aab	13.524±2.72Bb	32.928±2.54Bc	17.052±2.45Ca
	(500-750)m	28.812±2.45Bbc	11.76±2.94Bab	24.108±3.83Aa	11.172±1.49Ba	22.932±3.83Aab	7.35±2.19Aa	28.812±2.45Bb	17.64±2.07Ca
	(250-500)m	26.46±2.07Bab	10.584±1.45ABa	22.344±1.89Aa	8.82±2.07Aa	25.872±2.45Bb	12.348±2.45Bb	28.224±2.72Bb	16.464±3.35Ca
	(0-250)m	24.696±1.75Ba	11.172±2.45Aa	21.756±1.75Ba	11.172±1.49Aa	18.816±3.35Aa	8.82±2.07Aa	24.696±1.75Ba	16.464±2.72Ba
Cassia fistula	(750-1000) m	26.46±2.07Cb	14.112±2.45Cb	19.404±1.77Aa	7.056±1.76Aa	22.932±2.45Bb	11.172±1.49Bb	23.52±2.07Ba	12.936±1.75BCa
	(500-750)m	24.696±3.35Bab	14.7±2.07Bb	17.64±2.94Aa	8.232±2.45Aab	16.758±1.97Aa	9.114±0.96Ab	22.344±2.72Ba	13.524±1.76Ba
	(250-500)m	22.932±2.45Ba	10.584±1.75Aa	17.64±2.07Aa	9.408±3.29Aab	16.464±2.72Aa	10.29±2.19Ab	21.756±1.76Ba	11.172±2.45Aa
	(0-250)m	22.344±1.75Ca	10.878±1.32Ba	17.052±2.45Ba	11.172±1.49Bb	13.524±2.75Aa	3.234±0.89Aa	21.168±2.45Ca	12.936±2.14Ba
Derris ellipica	(750-1000) m	36.456±1.76Cc	14.112±2.45Ab	21.168±3.83Bb	12.348±1.49Ab	25.284±2.72Ac	14.7±2.07Ab	35.868±2.45Cc	18.816±3.35Ba
	(500-750)m	31.752±2.45Cb	17.64±2.94Bc	17.052±2.45Aa	11.132±1.47Aab	26.46±4.15Bc	11.172±1.49Aa	33.516±2.72Cc	17.052±2.45Ba
	(250-500)m	28.166±1.83Ca	12.348±1.49Aab	17.64±2.07Aab	9.448±1.74Aa	21.168±2.45Bb	11.172±1.49Aa	29.988±1.49Cb	19.404±3.35Ba
	(0-250)m	26.46±2.07Ba	9.996±1.76Aa	17.052±2.45Aa	9.996±1.89Aa	17.052±2.45Aa	10.584±1.75Aa	25.872±3.22Ba	15.288±2.45Ba
Shorea robusta	(750-1000) m	23.52±2.94Bb	12.936±3.35Ab	18.816±1.63Aa	12.348±1.63Aa	17.64±2.08Aa	12.936±1.78Ab	22.932±2.46Ba	10.584±1.25Aa
	(500-750)m	23.52±2.08Cb	14.112±3.39Ab	17.64±2.08ABa	12.348±1.50Aa	15.876±1.82Aa	11.466±0.99Aab	20.58±3.60BCa	13.524±2.63Aab
	(250-500)m	21.168±2.46Bb	13.524±1.72Bb	16.464±2.63Aa	12.054±1.61ABa	16.464±2.63Aa	10.584±1.29Aa	20.58±2.07Ba	14.112±2.46Bb
	(0-250)m	17.64±2.08ABa	7.938±1.32Aa	15.876±1.63Aa	11.466±0.64Ba	15.876±1.78Aa	11.172±1.51Bab	19.992±2.46Ba	11.76±2.07Bab

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at P< 0.05, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 14. Length (L) and Breadth (B) of stomata (µm) in the plant leaves of selected shrub species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Name	Distance	E(L) µm	E(B) µm	W(L) µm	W(B) µm	N(L) µm	N(B) µm	S(L) µm	S(B) µm
Melastoma melabathricum	(750-1000)m	12.348±2.45Aa	6.468±0.87Ba	11.172±2.45Aa	3.234±1.12Aa	12.054±1.84Ab	4.998±0.89ABa	19.992±1.64Bb	12.348±2.04Cb
	(500-750)m	11.76±2.07Aa	5.586±0.96Aa	11.172±2.45Aa	3.822±1.37Aa	10.584±2.68Ab	4.116±2.17Aa	15.876±3.54Ba	7.938±1.49Ba
	(250-500)m	12.936±2.53Ba	5.88±1.03Ba	9.114±2.18Aa	3.822±1.45Aa	10.584±1.76ABb	3.234±1.15Aa	16.584±2.22Ca	7.35±1.63Ba
	(0-250)m	11.76±1.03Ba	5.292±1.07Aa	8.82±2.07Aa	3.528±1.83Aa	7.644±1.76Aa	4.116±2.23Aa	15.876±1.46Ca	10.584±1.76Bb
Clerodendrum viscosum	(750-1000)m	29.988±2.45Cc	13.524±1.76Bc	17.64±2.07Aa	6.468±1.49Aa	18.816±1.77Bb	7.938±1.49Ac	25.872±3.22Aa	14.112±2.45Bab
	(500-750)m	17.64±2.07Ab	6.468±1.49Aab	17.64±2.94Aa	7.644±2.73ABa	18.228±2.45Ab	9.702±1.49Bc	24.696±3.94Ba	17.052±2.45Cb
	(250-500)m	17.052±2.45Ab	7.938±1.49Ab	16.464±1.77Aa	7.938±1.49Aa	16.464±1.89Ab	5.88±2.07Ab	24.108±2.45Ba	16.464±1.62Bb
	(0-250)m	12.936±1.89Ba	5.586±1.22Ba	15.876±2.72Ba	9.408±2.45Ca	8.232±2.56Aa	2.94±0.03Aa	22.932±2.45Ca	12.936±1.75Da

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at P< 0.05, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

Annex 15. Specific leaf area (cm^2/g) of selected tree species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Distance	East	West	North	South
Casearia graveolens	(750-1000)m	127.34±18.24Aa	118.63±12.67Ab	111.05±10.21Ab	110.58±10.24Ab
	(500-750)m	107.78±27.12Aa	106.54±19.84Aab	92.9±10.30Aab	93.18±9.91Aab
	(250-500)m	104.54±11.64Ba	105.24±3.88Bab	87.36±10.31Aa	90.65±5.06ABa
	(0-250)m	92.91±26.83Aa	84.58±6.79Aa	84.76±9.61Aa	85.12±11.01Aa
Cassia fistula	(750-1000)m	160.83±11.70Ac	125.07±9.78Aa	213.43±29.22Bd	308.33±32.19Cb
	(500-750)m	146.75±20.54Abc	108.64±11.19Aa	155.31±24.87Ac	253.66±51.08Bab
	(250-500)m	128.09±2.94Bb	107.01±23.38Ba	53.65±0.84Aa	245.18±9.21Cab
	(0-250)m	94.03±5.45Aa	97.36±11.85Aa	107.09±19.36Ab	186.37±47.41Ba
Derris elliptica	(750-1000)m	170.16±37.55Ab	151.84±22.19Ab	169.84±38.71Ac	198.6±11.25Aa
	(500-750)m	160.84±15.03Ab	122.25±23.86Aab	143.03±11.45Abc	137.26±44.97Aa
	(250-500)m	141.11±6.48Aab	105.87±27.53Aab	112.56±7.84Aab	133.55±47.28Aa
	(0-250)m	100.9±23.89Aa	98.05±20.59Aa	96.24±15.46Aa	112.58±58.75Aa
Shorea robusta	(750-1000)m	114.47±18.66Ac	92.57±36.08Aa	97.33±6.22Ab	80.47±13.68Aa
	(500-750)m	89.55±5.55Ab	77.06±10.99Aa	95.48±20.01Ab	69.71±14.68Aa
	(250-500)m	78.68±7.01Aab	67.74±23.47Aa	165.76±15.74Bc	68.12±36.37Aa
	(0-250)m	57.80±11.84Aa	61.28±10.33Aa	55.15±4.27Aa	48.14±2.18Aa

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P< 0.05$, using one way ANOVA followed by Duncan Multiple Range Test ($N= 80$).

Annex 16. Specific leaf area (cm^2/g) of selected shrub species at different distance and direction (east, west, north and south) around Udayapur Cement Factory.

Plant species	Range	East	West	North	South
Clerodendrum viscosum	(750-1000)m	172.85±59.26Aa	113.7±6.55Ab	165.88±30.96Ac	114.25±52.42Aa
	(500-750)m	159.92±31.60Ba	101.17±22.86ABab	132.21±39.68ABbc	91.96±25.38Aa
	(250-500)m	142.57±49.16Ba	78.04±17.21Aa	75.53±6.02Aa	88.51±17.56Aa
	(0-250)m	117.22±41.16Aa	72±18.45Aa	94.15±21.64Aab	83.63±29.19Aa
Colebrookea oppositifolia	(750-1000)m	178.18±23.96Ab	153.18±36.80Aa	277.48±7.85Bd	393.86±21.71Cb
	(500-750)m	161.06±13.40Aab	144.48±26.92Aa	190.58±17.44Ac	288.58±88.39Bb
	(250-500)m	154.33±13.38Bab	125.48±8.55ABa	91.42±14.27Aa	277.72±55.56Cb
	(0-250)m	119.55±32.82Aa	125.03±23.61Aa	146.91±20.30Ab	148.18±64.01Aa
Melastoma melabathricum	(750-1000)m	229.49±10.93ABb	277.64±42.42Bb	268.81±49.36Bd	188.06±9.29Aa
	(500-750)m	214.28±36.63Ab	220.49±28.86Aab	169.59±17.27Ac	179.95±21.22Aa
	(250-500)m	208.84±14.60Cb	170.3±26.69Ba	26.85±4.51Aa	171.14±9.03Ba
	(0-250)m	158.59±12.48ABa	193.83±47.29Ba	111.63±12.38Ab	146.89±56.75ABa
Phoenix acaulis	(750-1000)m	53.47±4.83Ab	102.69±5.62Cc	84.02±7.27Bc	50.34±5.97Aa
	(500-750)m	41.46±5.27Aa	62.82±4.87Bb	52.35±7.10ABb	49.61±5.51Aa
	(250-500)m	44.13±7.64Aab	54.27±4.62Ab	45.04±7.32Aab	48.37±10.81Aa
	(0-250)m	37.03±2.20ABa	29.66±2.99Aa	33.48±0.45Aa	45.33±8.10Ba

Same capital letters in the row and same small letters in the column after mean ± standard deviation do not differ significantly at $P < 0.05$, using one way ANOVA followed by Duncan Multiple Range Test (N= 80).

PHOTO PLATES

PHOTOPLATE 1. Plant species used for analysis

Photo 1. *Cassia fistula*

Photo 2. *Shorea robusta*

Photo 3. *Casearia graveolens*

Photo 4. *Derris elliptica*

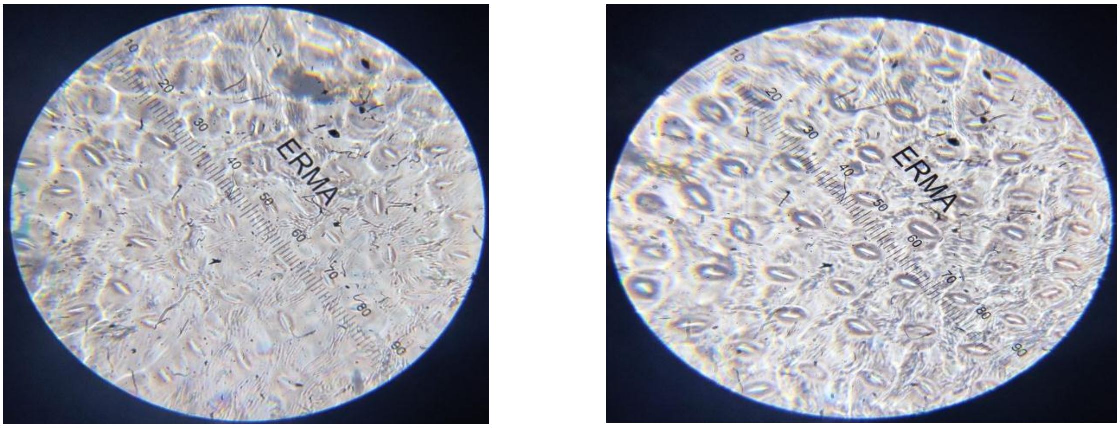
Photo 5. Flower and fruit of *Clerodendrum viscosum*

Photo 6. *Melastoma melabathricum*

Photo 7. *Phoenix acaulis*

Photo 8. *Colebrookea oppositifolia*

PHOTOPLATE 2:



(a) (b)

Photo 9. Stomata of *Melastoma melabathricum* at (0-250 m) (a) and (750-1000 m) (b) from the cement factory in eastern direction.

Photo 10. Dust deposition on surrounding vegetation

PHOTOPLATE 3:


Photo 11. Leaf sample collection



Photo 12. Lab work