ASSESSMENT OF ROAD SIDE TREES IN KATHMANDU VALLEY FOR THEIR AIR POLLUTION TOLERANCE INDEX AND HEAVY METAL BIOMONITORING ABILITY

A THESIS SUBMITTED TO THE

CENTRAL DEPARTMENT OF BOTANY
INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY
NEPAL

FOR THE AWARD OF
DOCTOR OF PHILOSOPHY
IN BOTANY

BY

JAYA PRAKASH HAMAL

JULY 2023

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Institute of Science and Technology
DEAN'S OFFICE
Kirtipur, Kathmandu, Nepal

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EXTERNAL EXAMINERS

The Title of Ph.D. Thesis: "Assessment of Road Side Trees in Kathmandu Valley for their Air Pollution Tolerance Index and Heavy Metal Biomonitoring Ability "

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December 10, 2023

(Dr. Surendra Kumar Gautam)
Asst. Dean

DECLARATION

Thesis entitled **"Assessment of Road-side Trees in Kathmandu Valley for Their Air Pollution Tolerance Index and Heavy Metal Biomonitoring Ability"** which is being submitted to the Central Department of Botany, Institute of Science and Technology (IoST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Prof. Dr. Mukesh Kumar Chettri of Department of Botany, Amrit Campus, Tribhuvan University.

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

....

Jaya Prakash Hamal

RECOMMENDATION

This is to recommend that **Jaya Prakash Hamal** has carried out research entitled **"Assessment of Road-side Trees in Kathmandu Valley for Their Air Pollution Tolerance Index and Heavy Metal Biomonitoring Ability"** for the award of Doctor of Philosophy (Ph.D.) in **Botany** under my supervision. To my knowledge, this work has not been submitted for any other degree.

He has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph.D. degree.

....

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July 2023

TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE AND TECHNOLOGY
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LETTER OF APPROVAL

On the recommendation of Prof. Dr.Mukesh Kumar Chettri this, Ph.D.thesis submitted by Jaya Prakash Hamal entitled **"Assessment of Road-side Trees in Kathmandu Valley for Their Air Pollution Tolerance Index and Heavy Metal Biomonitoring Ability"** is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, T.U.

....
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Jaya Prakash Hamal
July, 2023

ABSTRACT

Rapid urbanization, industrialization, poor maintenance of road and vehicles decline the air quality in Kathmandu valley. Topography of Kathmandu valley is bowl-shaped, hence restricts air movement and promotes thermal inversion, which in turn accelerates air pollution. Air pollution has been identified as a major cause of silent killer for different chronic and infectious ailments. In this context, to minimize the problem of air pollution, tolerant tree species, having good ability to accumulate heavy metals, possibly can play a major role to reduce pollution. The main objectives of this study was to screen some suitable road side trees that can tolerate air pollution stresses as well as accumulate comparatively more heavy metals. For this the work was focused to identify such suitable roadside trees with high air pollution index and good accumulator of heavy metal as well. For this leaves of common roadside trees like *Thuja orientiales, Cedrus deodara, Pinus roxburghii* and *Araucaria arucana* from gymnosperm and *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa, Grevillea robusta, Jacaranda mimosifolia* and *Populus euramericana* from angiosperm were collected from about 3m height above the ground surface during the winter season from polluted sites (Airport, Balaju, Banasthali, Basundhara, Dhumbarahi, Gongbu, Jawalakhel, Ratnapark, Shankpark) and less polluted site, Narayanthan (Budhanilkantha) of Kathmandu valley. Macro and micro morphological parameters (like leaf area, specific leaf area, thickness of cuticle and epidermis from adaxial surface, density of stomata, length and breadth of stomata from abaxial surface) were measured by lasting impression technique. Photographs of anatomical slides were taken and the measurements were taken using Image J computer program. Air pollution tolerances Index (APTI) of different studied plants were calculated and their tolerance levels were categorized. Heavy metals (Zn, Cu and Pb) from the representative leaf samples were extracted using wet digestion method and analyzed using Atomic absorption spectrophotometer and ultimately Metal Accumulation Index (MAI) was calculated. The maximum leaf area was observed in *Ficus reliogiosa* (202.42± 84.61 cm^2) at polluted site while specific leaf area (SLA) in *Purnus cersoides* (212.00±8.31 cm^2/g) at less polluted site. Stomata density was found to be increased at polluted sites than less polluted site and maximum stomata density was observed in *Eucalyptus globules* (231.98±1.84 cm^2). Length and width of stomata were found to be reduced at polluted sites. Maximum length of stomata was observed in *Ficus reliogiosa* (26.82±0.22 µm) and width in *Eucalyptus globules* (15.08±0.39 µm) at less polluted site. Thickness of epidermis and cuticle were found to be reduced at polluted sites. The maximum thickness of cuticle and epidermis were observed in *Callistemon lanceolatus* (11.22±1.66 µm) and *Ficus reliogiosa* (28.98±0.69 µm), respectively, at polluted sites.

Among the studied angiosperm plants, the highest Air Pollution Tolerance Index (APTI) value was observed in *Populus euramericana* (15.67) in Dhumbarahi, while among gymnosperm maximum Air Pollution Tolerance Index (APTI) value was observed in *Pinus roxburghi* (8.94) at Dhumbarahi, indicating their tolerance to air pollution. Among the investigated angiosperms, maximum Zn accumulation was found in *Populus euramericana* (132.38±3.71 mg/kg) at Banasthali, Cu accumulation was in *Jacaranda mimosifoli* (20.27±0.64 mg/kg) at Balaju and Pb in *Callistemon lanceolatus* (40.33±1.84 mg/kg) at Airport. The maximum MAI was recorded in *Populus euramericana* (40.69) at Airport. Similarly, among investigated gymnosperms, maximum Zn accumulation was recorded in *Cedrus deodara* (54.52±1.20 mg/kg) at Dhumbarahi, Cu in *Thuja orientalis* (20.16±0.48 mg/kg) at Airport and Pb in *Pinus roxburgii* (8.85±2.65 mg/kg) at Ratnapark. The highest (MAI) was recorded in *Thuja orientalis* (75.78). Based on maximum metal accumulation index (MAI) and APTI (Air Pollution Tolerance Index) value, the plants like *Populus euramericana, Jacaranda mimoisifolia,* and *Callistemon lanceolatus* with high APTI (Air Pollution Tolerance Index) and MAI value are recommended for plantation along the roadside among angiosperms. Similarly, among studied gymnosperms *Cedrus deodara* and *Thuja orientalis* with high MAI and also tolerant to moderately tolerant value are suggested for plantation along the roadside. Evergreen trees such as *Callistemon lanceolatus, Cinnamomum camphora, Pinus roxburghii, Cedrus deodar* and *Thuja orientalis* are recommended for plantation on roadside along with deciduous trees especially to mitigate pollution during winter season. So these plants were considered as better tolerant plants than others against air pollution. Hence, to mitigate air pollution problem, these tolerant plants were recommended for plantation in Kathmandu valley.

Keywords: Ascorbic acid, Metal Accumulation Index, Micro morphology, Relativewater content, Specific leaf area, Total chlorophyll, Urbanization

शोधसार

काठमाडौं उपत्यकामा भएका सडक छेउका रूखहरूको वायु प्रदूषण सहिष्णुता सूचकांक (APTI) र तिनीहरुमा भएको भारी धातुहरू (Heavy Metal) को जैविक अनुगमन (Bio monitoring) क्षमताको मूल्याङ्कन

तिब्र सहरीकरण, औद्योगीकरण, सडक र सवारीसाधनको कमजोर मर्मतसंभारले काठमाडौं उपत्यकाको हावाको गुणस्तरमा ह्रास आएको छ । काठमाडौं उपत्यकाको स्थलाकृति कचौराको आकारको छ, त्यसैले हावाको आवागमनमा प्रतिबन्ध लगाउँछ र थर्मल इन्भर्सनलाई बढावा दिन्छ, जसले गर्दा वायु प्रदूषण बढ्छ। वायु प्रदूषण विभिन्न दीर्घकालीन र संक्रामक रोगहरूको लागि मौन हत्याराको प्रमुख कारणको रूपमा पहिचान गरिएको छ। यस सन्दर्भमा, वायु प्रदूषणको समस्यालाई न्यूनीकरण गर्न, सहनशील रूख प्रजातिहरू, भारी धातुहरू जम्मा गर्ने राम्रो क्षमता भएकाले प्रदूषण कम गर्न सम्भवतः महत्वपूर्ण भूमिका खेल्न सक्छ। यस अध्ययनको मुख्य उद्देश्य केही उपयुक्त सडक छेउका रूखहरू स्क्रिन गर्नु थियो जसले वायु प्रदूषणको तनावलाई सहन सक्छ र तुलनात्मक रूपमा धेरै भारी धातुहरू जम्मा गर्दछ। यसका लागि उच्च वायु प्रदूषण सूचकांक र भारी धातुको राम्रो सञ्चयकर्ता भएका सडक छेउका उपयुक्त रूखहरू पहिचान गर्ने काममा केन्द्रित थियो। यसका लागि *Thuja orientalis,Cedrus deodara Pinus roxburghii, Aruacaria araucana* जिम्नोस्पर्मबाट र एंजियोस्पर्मबाट *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa, Grevillea robusta, Jacaranda mimosifolia ,Populus euramericana* का पातहरू प्रदूषित साइटहरू (विमानस्थल, बालाजु, बनस्थली, बसुन्धरा, धुम्बाराही, ,गोंगबु, जावलाखेल, रत्नपार्क, शङ्कपार्क) बाट हिउँदको मौसममा जमिनको सतहबाट करिब ३ मिटर उचाइबाट संकलन गरिएको थियो र कम प्रदूषित साइट, नारायणथान (बुढानिलकण्ठ) बाट पनि संकलन गरियो । म्याक्रो र माइक्रो मोर्फोला' जिकल मापदण्डहरू जस्तै पातको क्षेत्र (Leaf Area) विशिष्ट पात क्षेत्र (SLA) ,क्युटिकल र एपिडर्मिसको मोटाई एडाक्सियल(माथिल्लो)सतहबाट मापन लिइयो । यसैगरी एबाक्सियल(तल्लो) सतहबाट स्टोमाटाको घनत्व, लम्बाइ र चौडाइलीइ स्थायी छाप प्रविधिद्वारा (Lasting Impression Technique) द्वारा मापन गरियो। एनाटोमिकल स्लाइडहरूको फोटोहरू लिइयो र छविहरू Image J कम्प्युटर प्रोग्राम प्रयोग गरेर मापन लिइयो। विभिन्न अध्ययन गरिएका वनस्पतिहरूको वायु प्रदूषण सहिष्णुता सूचकांक (APTI) गणना गरियो र तिनीहरूको सहनशीलता स्तरहरू वर्गीकृत गरियो। प्रतिनिधि पात नमूनाहरू (Representative Leaf Samples) बाट भारी धातुहरू (Heavy Metals) Zn, Cu र Pb Wet digestion method प्रयोग गरेर निकालिएको थियो र परमाणु अवशोषण स्पेक्ट्रोफोटोमिटर (Atomic Absorption Spectrophotometer) प्रयोग गरेर विश्लेषण गरियो र अन्ततः Metal Accumulation Index (MAI) निर्धारण गरियो। प्रदूषित साइटमा *Ficus religiosa* मा अधिकतम पात क्षेत्र (Leaf Area) (202.42± 84.61 cm^2) देखियो भने कम प्रदूषित साइटमा *Prunus cersoides* मा विशिष्ट पात क्षेत्र (SLA) (212.00±8.31 cm^2 /g) देखियो। कम प्रदूषित साइटहरू भन्दा प्रदूषित साइटहरूमा स्टोमाटा घनत्व बढेको पाइयो र अधिकतम स्टोमाटा घनत्व (231.98± 1.84 /cm^2) *Eucalyptus globules* मा देखियो। प्रदूषित ठाउँहरूमा स्टोमाटाको लम्बाइ र चौडाइ कम भएको पाइयो। स्टोमाटाको अधिकतम लम्बाइ (26.82± 0.22 μm) *Ficus reliogiosa* मा र चौडाइ (15.08±0.39 μm) कम प्रदूषित साइटको *Eucalyptus globules* मा देखियो। प्रदूषित स्थानहरूमा एपिडर्मिस र क्युटिकलको बाक्लोपन

घटेको पाइयो। क्युटिकल (11.22±1.66 µm) र एपिडर्मिसको अधिकतम मोटाई (28.98±0.69 µm) *Callistemon lanceolatus* र *Ficus reliogiosa* क्रमशः प्रदूषित साइटहरूमा देखियो। अध्ययन गरिएका एन्जियोस्पर्म बिरुवाहरूमध्ये धुम्बाराहीको *Populus euramericana* मा सबैभन्दा बढी APTI (15.67) देखियो भने जिम्नोस्पर्मबाट धुम्बाराहीकै *Pinus roxburghii* मा APTI (8.94) सबैभन्दा बढी देखियो जसले वायु प्रदूषणप्रति उनीहरूको सहिष्णुता (Tolerance) देखाउँछ। अनुसन्धान गरिएका एन्जियोस्पर्महरू मध्ये, बनस्थलीमा *Populous euramericana* मा अधिकतम Zn (132.38±3.71 mg / kg) सङ्कलन भएको भेटियो, Cu (20.27±0.64mg /kg) बालाजुको *Jacaranda mimosifoli* मा र अधिकतम Pb(40.33±1.84 mg/kg) चाहि एयरपोर्टको *Callistemon lanceolatuts* मा भेटियो। अधिकतम MAI (40.96) एयरपोर्टमा भएको *Populus euramericana* मा भेटिएको थियो। त्यसैगरी, अनुसन्धान गरिएका जिम्नोस्पर्महरूमध्ये, अधिकतम Zn (54.52±1.20 mg/kg) धुम्बाराहीको *Cedrus deodara* मा भेटियो यसैगरी अधिकतम Cu (20.16±0.48 mg/kg) एयरपोर्टको *Thuja orientalis* मा र अधिकतम Pb (8.85±2.65 mg/kg) चाहि रत्नपार्कको *Pinus roxburghii* मा पाइयो । उच्चतम MAI (75.78) *Thuja orientalis* मा रेकर्ड गरिएको थियो। अधिकतम MAI र APTI को आधारमा, एंजियोस्पर्म बाट *Populus euramericana, Jacaranda mimoisifolia,* र *Callistemon lanceolatus* र त्यसैगरी, अध्ययन गरिएका जिम्नोस्पर्महरू मध्ये *Cedrus deodara* र *Thuja orientalis* बिरुवाहरू सडकको छेउमा रोप्न सुझाव दिइन्छ। सदाबहार रूखहरू जस्तै *Callistemon lanceolatus, Cinnamomum camphora, Pinus roxburghii, Cedrus deodara* र *Thuja orientalis* लाई विशेष गरी जाडो मौसममा प्रदूषण कम गर्न पतझड बिरुवाहरूसंगै सडकको छेउमा रोप्न उपयुक्त देखिन्छ तसर्थ, वायु प्रदूषणको समस्यालाई कम गर्न यी सहनशील बिरुवाहरू काठमाडौं उपत्यकामा वृक्षारोपण गर्न उपयुक्त देखिन्छ।

मुख्य शब्दहरू: एस्कार्बिक एसिड, धातु संचय सूचकांक (APTI),माइक्रोमोर्फोलजी, सापेक्ष पानी (Relative Water), विशिष्ट पात क्षेत्र (SLA), कुल क्लोरोफिल (Total Chlorophyll), शहरीकरण

LIST OF ACRONYMS AND ABBREVIATIONS

AA : Ascorbic Acid

AAS : Atomic Absorption Spectrometer

ADP : Adenosine Diphosphate

ALRTI : Acute Lower Respiratory Tract Infection

APTI : Air Pollution Tolerance Index

ATP : Adenosine Triphosphate

COPD : Chronic Obstructive Pulmonary Disease

CPCB : Central Pollution Control Board

DMSO : Dimethyl Sulfoxide

DOH : Department of Health

DOTM : Department of Transport Management

EDTA : Ethylenediamine Tetraacetic Acid

HEI : Health Effects Institute

IHD : Ischemic Heart Disease

JICA : Japan International Cooperation Agency

MAI : Metal Accumulation Index

MoPIT : Ministry of Physical Infrastructure and Transport

NCD : Non-communicable Diseases

OECD : The Organisation for Economic Co-operation and Development

PFT : Pulmonary function test

PM : Particulate Matter

ROS : Reactive Oxygen Species

RWC : Relative Water Content

SLA : Specific Leaf Area

VOCs : Volatile Organic Compounds

LIST OF SYMBOLS

& : And

% : Percentage

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CHAPTER 1

1. INTRODUCTION

1.1 Background

When substances are present in excess in the environment at a concentration above than its normal ambient levels is called air pollution. It produces measurable and undesirable effect on living beings and materials (Seinfeld and Pandias, 2006). The quality of air is determined with the concentration of pollutants in the atmosphere. About 6.5 million deaths through out the world are due to air pollution in every year. While premature deaths from household air pollution are projected to decline from 3.5 million today to 3 million by 2040 and it is expected to increase premature deaths from outdoor pollution from 3 million to 4.5 million during the same time period (The Lancet, 2016). Lelieveld *et al.* (2015) assumed that the premature mortality by 2050 could reach up to double due to contribution of outdoor air pollution. One of the leading environmental causes of early death might be air pollution (OECD, 2012). Air pollution impact on human health could range from typical to mild depending upon the exposure as a result increased morbidity and mortality (Gurjar *et al.,* 2010). Cardiovascular sicknesses, lung and respiratory cancer are associated with the acute exposure to air pollutants like aerosols and gases (Shah *et al.,* 2013; Beelen *et al.,* 2014). Many recent European researchers have found notable positive associations between SO_2 and NO_2 concentrations that lead to cardiovascular mortality, and respiratory health in metropolitan areas (Cesaroni *et al.,* 2013; Bentayeb *et al.,* 2015). Longterm exposure to tropospheric ozone (O_3) and particulate matter increases the risk of mortality from cardiopulmonary disorders (Jerrett *et al.,* 2009). Ozone could be alone responsible for about 17,400 premature deaths in European Union (Malkin *et al.,* 2016). Vedrenne *et al.* (2015) stated that degradation of air quality invite many diseases and also reduce life expectancy as a result it increases expenditure in health sector of a citizen. Environment is being threatened by air pollution which affects a wide range of ecosystem through various processes such as eutrophication and acidification. This may results in the loss of environmental services and biodiversity (Lovett *et al.,* 2009; De Vries *et al.*, 2014). Felzer *et al.* (2007) stated that trees like Poplar and Pine exposed to acute high O_3 concentration could lead to reduction in photosynthesis, damage to reproductive process, lower transportation of carbon in roots as well as physiological effects on leaf. High ambient gaseous concentrations of SO_2, NO_2, and O_3 affects agricultural production in numerous crops like beetroot, mung beans, and wheat, especially in suburban areas (Agrawal *et al.*, 2003). Van Dingenen *et al.* (2009) reported that high concentrations of ozone in Europe particularly in summer cause great damage to crops and pastures that showed great economic impact. By 2050, it is predicted that high O_3 concentrations would have damaged crops up to 20% globally, which will have an impact on the world's food output (Chuwah *et al.*, 2015). Acidifying air pollutants exposed in the atmosphere cause damage to materials like glass, bricks, metals, limestone etc. by the effect of weathering, and corrosion (Kucera and Fitz, 1995; Chen *et al.*, 2005). Nord *et al.* (2001) noticed significant damages of building structures and cultural assets (stonework facades, bronze monuments etc.) in Spain as a result of air pollution Furthermore, rubber cracking is produced by oxidative degradation of synthetic and natural rubbers as a result of ozonolysis of rubber chains, which is accelerated by air pollution, resulting in a loss of the physical and mechanical properties of rubbers, like rubber seals and tires (Li and Koenig, 2005). High concentration of air pollutants affect the solar energy by reducing the sunlight radiation reaching on solar cells by which decrease efficiency in electric generation (Chaturvedi and Shashank, 2015). Particulate debris such as sand, dust, and ashes accumulated on the solar panel needs cleaning, which raises the maintenance expenses (Mani and Pillai, 2010). Atmospheric pollution during smog haze affects visibility of light which ultimately affects the movements of vehicles, marine vessels and aircrafts (Watson, 2002; Hyslop and White, 2008).

1.2 Air pollution in Kathmandu valley

Kathmandu valley, Nepal's capital is located in Bagmati Province at an altitude of 1200 to 1400 meters above sea level, with subtropical vegetation. Air pollutants, such as particulate matter (PM), ozone (O_3), carbon monoxide (CO), and volatile organic compounds (VOCs), are expected to have adverse effects on 3.5 million people’s health residing in the valley (Bhardwaj *et al.*, 2018) and cause serious health consequences (Gurung *et al.*, 2017). Due to an increase in the number of automobiles, a high level of population density, and its unique bowl-shaped geography, that stops particulate matter from freely escaping into the atmosphere, human health has been adversely affected in Kathmandu valley (Kanwar *et al.*, 2016). In 2020, on the basis of Environmental Performance Index, the air quality of Nepal was ranked at 145th out of 180 countries (Wendling *et al.,* 2020; Shrestha *et al.,* 2021). Kathmandu valley has been designated as one of the world's worst cities for air quality (Subedi, 2021). In the past 15 years, the number of vehicles in Kathmandu valley has increased significantly. According to data, the number of registered vehicles was 24,003 in 2000/01 and increased to 7, 79,822 in 2015/16 (DOTM, 2017). Among registered automobiles in Bagmati zone, 92% were private vehicles. Out of which 80% were motorbike. For the past ten years, the yearly average growth rate of all registered vehicles in the Bagmati zone was 12%, while the growth rate of motorcycles was approximately 14% alone. Vehicle registrations have increased by almost three times in the past 10 years, causing massive congestion on Kathmandu's streets (MoPIT/JICA, 2012). Emissions from vehicles in metropolitan areas are commonly thought to be harmful to plants (Honour *et al.,* 2009).

1.3 Impacts on human health

Research on the effects of air pollution on human were well documented in Europe and North America (Cohen *et al.,* 2005) while in Nepal on this regard is still very poor (HEI, 2010). Gurung *et al*. (2017) reported that Kathmandu valley is facing a potentially significant human health burden due to exposure of high level of air pollution. Air pollution is going to be major cause of silent killer for different chronic and infectious ailments. An unhealthy environment is solely responsible for 7 million deaths annually and 12.6 million deaths worldwide (WHO, 2017). The improvement in air quality in high-income nations between 1990 and 2015 resulted decrease in the global rates of death from exposure of PM 2.5 (Cohen *et al.,* 2017) but it was predicted that total global mortality due to PM 2.5 might reach up to 76%. The impact on human health in Nepal is still serious. Nearly 24,000 premature deaths have been expected due to outdoor pollution in Nepal each year by 2030 (Shindell *et al.,* 2012). In Nepal, common diseases (like allergies, eye infection, and respiratory illness,) as well as chronic diseases such as ischemic heart disease (IHD), chronic obstructive pulmonary disease (COPD), lung cancer, and stroke are on the rise. Non-communicable diseases (NCDs), which account for 60% of mortality, have also been identified as the main cause of human demise and are the leading cause of death worldwide. The top four leading non-communicable diseases of death are due to cardiovascular and cerebrovascular diseases (44%), cancer (22%), chronic respiratory diseases (9%), and diabetes mellitus (4%) (WHO, 2016). In Nepal, death from ambient air pollution was alarmingly high in 2012, with 33.4% of deaths from IHD, followed by stroke (32%), COPD (17.8%), acute lower respiratory tract infection (ALRTI, 7.4%) and lung cancer (9.3%) (WHO, 2017). Besides this in each ailment, the number of female mortality rate in Nepal was found to be greater than the number of male death in each disease (WHO, 2017). Reports from the Department of Health (DOH) services in Nepal between 2013 and 2014 indicated that COPD was the leading cause of death among patients, with respiratory tract disorders being one of the leading reasons for outpatients (DOH Services, 2014). Study based on the hospitalized patients from different hospitals of Kathmandu valley reveals a significant high frequency of respiratory disorders where COPD was shown to be prominent among the other disorders. According to the gender wise 48.7% of all in patients were female and 51.3% were male. A district wise study in valley reported that the Kathmandu showed the highest number, i.e., 44.4% of patients followed by 10.3% in Lalitpur and 10.2% in Bhaktpur. The average morbidity rate was 44.4%, with COPD, becoming the leading cause of mortality (Karki *et al.*, 2015). Besides Kathmandu valley, 48.4% of patients had COPD for more than five years in Chitwan. Nepal had 14% preterm births, which is quite high percentage as suggested by KC *et al.* (2015). Even though there are no obvious causes for this high preterm birth rate, Subba and Subba (2015) suggest it might be due to influence of air pollution. Due to increase in air pollution hazards in Kathmandu and other cities, traffic police are constantly exposed to dusty roads (Aryal *et al.*, 2015), and as a result, pulmonary functions among traffic police particularly those working in Kathmandu valley have been significantly worsened (Shrestha *et al.*, 2015). Brick kiln employees and sand grocery workers in Kathmandu are both impeded by airborne hazards and their health has been seriously affected (Sanjel *et al.*, 2016). Air pollution has been regarded as one of the leading cause of mortality throughout world leaving behind metabolic hazards, dietary risks, and tobacco smoking. It is also estimated that air pollution will be responsible for one out of every ten deaths (The World Bank, 2017).

1.4 Air pollution and plants

Neat and clean air is essential for entire growth and development of living beings. Now-a-days due to industrialization, urbanization and vehicular emission, air has become highly polluted. Living beings and environment are adversely affected by the introduction of particulate, chemical and biological matter in to the atmosphere. The overuse of fossil fuels in the industrial and transportation sectors increases the amount of gaseous pollutants in the atmosphere (Kulkarni and Ingawale, 2014). Likewise SO_2, NO_2, CO, soot particles, small amount of toxic metals, organic molecules and radioactive isotopes are released by combustion (Agbaire and Esiefarienrhe, 2009; Bhattacharya *et al.*, 2013). The ever-increasing numbers of vehicles, over population pressure, and over-exploitation of open areas have worsened the pollution problem (Sharma and Roy, 1999). So, air pollution is the main cause for decrease or increase in physiological and biochemical responses of various crops and, plants especially growing at the contaminated areas (Chauhan and Joshi, 2008). Plants could play a vital role in all ecosystems as they are stationary in nature and have abundant leaves. They serve as primary receptors for large number of air pollutants (Lohe *et al.*, 2015) and also considered as bio filters as they absorb a huge amount of particles from their surroundings (CPCB, 2007). Plants absorb gaseous pollutants from the air through their leaf stomata and adsorb particles through leaf interception (Kapoor, 2014). Because plants are the primary acceptors of air pollution and serve as scavengers (Mahecha *et al.*, 2013) and plant species with higher performance tolerance can serve as air pollution sinks (Miria and Khan, 2013) as they absorb or adsorb and metabolize pollutants. Leaves play a vital role to sequester carbondioxide from the atmosphere through the process of photosynthesis. Plant species, pollutants type and its concentrations, as well as a number of environmental factors, have an effect on plant's growth (Wuytack *et al.*, 2011). Roadside plant leaves act as sink, as they are constantly exposed to air contaminants and hence have also been considered for bio monitoring potential (Sawidis *et al.*, 2012). High traffic areas are one of the most significant sources for emission of heavy metals and thought to be toxic to living organisms, whereas some heavy metals in traces are considered to be essential for life. Excessive level of toxic metals can damage to biochemical process. In urban environment, heavy metals such as Zn, Pb and Cd can originate from different sources like rubber tire wear, motor oil, auto workshops, electroplating industries and gasoline combustion etc. Though, leaded gasoline has been prohibited, yet much residual lead remains in the soil and heavy metals are transferred to the biosphere constituents as airborne particles. In comparasion to other heavy metals, Cu and Zn are important micronutrients for plants as they participate in a variety of metabolic activities, including protein and enzyme production. These heavy metals, however, can cause a number of toxic symptoms in plants at high concentrations, like inhibited growth, reduction in enzyme activity, reduction in photosynthesis, water imbalance, upset mineral absorption, and alteration in membrane (Sharma and Dubey, 2005; Pandey *et al.,* 2009). Heavy metal resistance in a plant is accomplished through either an avoidance or tolerance mechanism.

1.5 Air pollution and macro morphology of leaf

1.5.1 Leaf surface area

Potential of photosynthesis in leaves is mainly regulated by leaf surface area. The rapid urbanization and industrialization has triggered a lot of researches to combat against urban environmental pollution (Zhang *et al.,* 2017). The particulate matter (PM) is mainly emitted directly by power plants, vehicle exhaust, cement plants, and industrial processors and these particles undergo reactions to produce secondary pollutants in the atmosphere by forming sulfur dioxide (SO_2), nitrogen oxides (NOx), ammonia, and volatile organic compounds (VOCs) (Bosco *et al.*, 2005). Green plants can absorb pollutants from the atmosphere and improve air quality (Gawronski *et al.,* 2017). One study in the city centre of Beijing, China, estimated that trees removed 7,72, 000 kg of PM 10 from air during one year (Yang *et al.*, 2005). PM accumulates on the surfaces of leaves and bark. PM deposition on leaves is increased by air turbulence in tree crowns. Trees accumulate more pollutants like coarse and fine particulate matter (PM), than smaller plants (Fowler *et al.,* 1989). Plants with trichomes and/or rough leaf surfaces are thought to be more effective PM accumulators (Burkhardt, 2010). PM accumulates more effectively in broad-leaved species with rough leaf surfaces than in those with smooth surfaces (Beckett *et al.,* 2000). Coniferous tree with needles capture PM more effectively than broadleaf species due to their unique microstructure and thicker epicuticular wax layer (Beckett *et al.*, 1998). The variation in leaf surface texture and leaf area are important factors to determine air pollutant adsorption ability. Lu *et al.* (2018) studied six species of landscape plants (*Pinus tabuliformis, Pinus bungeana, Salix babylonica*, *Acer elegantulum, Populus* and *Ginkgo biloba*) that grew in diverse levels of pollution in Beijing Xishan National Forest Park (with light pollution, the annual mean PM 2.5 mass concentrations was 80.64±7.13 µg m^{-3}) and Daxing Nanhaizi Park (heavy pollution, the annual mean PM 2.5 mass concentrations was 97.95±12.57 µg m^{-3}) and measured their adsorption and then compared with the the water-soluble ion content and leaf morphology. The findings revealed a positive relationship between pollution level and plant leaf surface PM 2.5 adsorption capacity.

1.5.2 Specific leaf area (SLA)

Specific leaf area (SLA) is the amount of leaf area per unit leaf dry mass (Pierce *et al.,* 1994). It shows the strong relationship with light intensity and declines when intensity of light increases (Kellomaki and Oker-Blom, 1981; Evans and Poorter, 2001). The position of the leaf in the crown determines the rate of SLA, and it is also regarded as one of the key leaf features which is used to measure the growth of plants (Lambers and Poorter, 1992; Wright *et al.*, 2004; Falster *et al.*, 2018). Specific leaf area is an important indicator for plant's adaptation strategies (Grime, 2006). SLA of the most of the terrestrial species ranges between 30 and 330 cm^2 g^{-1} (Poorter *et al.,* 2009), which shows a significant degree of diversity with high variability in SLA. Fast-growing species produce greater leaf area per unit leaf biomass, leading to a higher growth rate (Poorter and Vander Werf, 1998). Low SLA is present in desert, shrub land, and forest species because development is hampered by either dehydration, nutrient limitation, or both (Poorter *et al.,* 2009). Air pollution can also lead to a high variability in SLA for, e.g., *Ligustrum lucidumand* and *Leontodon helveticus* increased SLA due to sulphur dioxide (SO_2) and O_3 pollution, respectively, for compensating the inhibition of photosynthesis (Bassin *et al.,* 2009). However Tiwari *et al.* (2006) reported that SLA of carrot plants decreased due to a decreased leaf production rate, caused by air pollution. In general, elevated carbon dioxide (CO_2) concentrations resulted in decreased SLA (Poorter *et al.*, 2009).

1.6 Air pollution and micro morphology of leaf

Air pollution directly affects the plants either through leaves or indirectly via soil acidification. Many plants experience physiological changes when they are exposed to air borne pollutants before showing visible damage in their leaves (Liu and Ding, 2008). SO_2 in the atmosphere has a negative impact on a variety of physiological and morphological traits of plants. The impacts of vehicular exhaust pollution on the micro morphology of ornamental plants was studied by Saadabi (2011) taking into account the micro morphological features like number of stomata per unit area, the number of epidermal cells per unit area, and the length and width of stomata. In contaminated areas, leaves shortened with lower length, breadth, and low stomatal index per leaf area (Saadabi, 2011). SO_2 is aggravated by high relative humidity and high soil moisture that causes injury in plants (Tankha and Gupta, 1992). In sensitive plant species, pollutants can cause premature senescence, leaf injury, stomata damage, decrease in photosynthetic activity, disturbance in membrane permeability and reduce yielding capacity (Tiwari *et al.,* 2006). Biochemicals (like ascorbic acid, enzymes, pigments, proteins) and physiology (such as relative water content and pH) in plants have been reported to be altered by air pollutants (Karmakar *et al.,* 2016; Kaur and Nagpal, 2017). Air pollution stress leads to stomatal closure, which reduces CO_2 availability in leaves and inhibits carbon fixation (Robinson *et al.,* 1998). Plants can only absorb the pollutants only when pollutants are discharged in to atmosphere (Rai and Panda, 2014). Plants play a crucial role in maintaining an ecological balance by actively involving in the cycling of nutrients and gases (photosynthesis and respiration). Plants with large leaf surface collect and absorb various contaminants from environment (Khanoranga, 2019).

1.7 Air pollution and leaf anatomy

Using tree leaves in bio monitoring of air pollution is of incredible environmental significance (Bargagli, 1998; Willekens *et al.,* 1994). The leaves act as a receptor of air pollutants, which also biologically absorbs and filters the pollutants (Free-Smith *et al.,* 2004 and Wagh *et al.,* 2006). Morphological and anatomical characteristics of plants operate as indicators of how the quality of the urban environment is altering, despite their low priority (Balasooriya *et al.,* 2009). Morpho- anatomical adjustments are important measures to gauge the air quality of the urban habitat (EL-Khatib *et al.,* 2011). Studies have revealed that physiological changes in plants take place as a result of a wide variety of environmental causes Kabata-Pendias (2010). Trace elements in soils and plants. Air pollution causes auxiliary changes in the stomata and epidermis, thickening of cell walls, modification of the epicuticular wax layer, and chlorosis in leaves (Rao and Dubey, 1991; Setia, *et al.,* 1994). Foliar epidermis is also considered by few authors as a bio indicator of environmental quality (Masuch *et al.,* 1992; Agrawal and Tiwari, 1997; Balasooriya *et al.,* 2009). As a result of air pollution, the histo-anatomical structure of the leaves of different Fabaceae species showed reduced stomatal size, increased leaf thickness, dark phenolic deposits in palisade, and light parenchyma (Govindaraju *et al.,* 2012). Saadabi (2011) stated that length, breadth and calculated area of stomata showed different percentages of inhibition whereas number of stomata, epidermal cells, and stomatal index exhibited stimulation in *Aristolochia elegans* and *Nerium oleander* growing on the auto-exhaust-polluted roadside at Khartoum city. Due to stomatal closure and clogging in *Boerhaavia, Amaranthus, Cephlandra,* and *Nerium,* help in preventing the entry of toxic gases (Mandal, 2006). Rangkuti (2003) reported that plants that are exposed to air pollution may have different morphological characteristics, such as larger epidermal cells and more trichomes to cope with air pollution. Industrial emissions changed the morphological and anatomical features of normal birch *Betula pendula* leaves, and the noticeably negative changes were reduction in cuticle thickness and epidermal cell width (Neverova *et al.,* 2013). Densities of stomata, trichomes, epidermal cells and length of the trichomes increased while the size of the epidermal cells reduced at polluted areas as comparison to control sites (Aggarwal, 2000).

1.8 Air pollution and biochemical parameters

Relative water content, pH of leaf extract, total chlorophyll, and ascorbic acid content are the four important biochemical parameters by which air pollution tolerance index (APTI) is measured (Singh and Rao, 1983; Thambavani and Kamala, 2010; Thawale *et al.,* 2011; Lohe *et al.,* 2015; Gholami *et al.,* 2016).

1.8.1 Ascorbic acid

The amount of ascorbic acid (AA) content in a plant's leaf plays an important role in pollution tolerance (Seyyednejad *and* Koochak, 2011). Ascorbic acid content increases in response to pollution load in all plant species (Liu and Ding, 2008; Chouhan *et al.,* 2012). This increase in AA might be due to the faster rate of reactive oxygen species (ROS) generation in the course of the photo- oxidation of SO_2 to SO_3, where sulfites are formed from absorbed SO_2. Plants when exposed to the pollutants with high NO_2 and SO_2 are very sensitivity and shut their stomata fast (Srivastava, 1999). The reducing activity of ascorbic acid is dependent on the level of pH, increases at higher pH and decreases at lower pH (Chandawat *et al.,* 2011; Randhi and Reddy, 2012). The conversion of hexose sugar to ascorbic acid may be a result of a high pH. In plants, there is a significant relationship between pollution tolerance and ascorbic acid concentration. Tolerant plants contain a higher concentration of ascorbic acid than sensitive plants (Randhi and Reddy, 2012; Swami and Chauhan, 2015). Such plants that retain high ascorbic acid levels despite growing in polluted environments are thought to be air pollution resistant (Kuddus *et al.*, 2011).

1.8.2 Relative water content

Relative water content (RWC) is a ratio of the amount of water present in the leaf tissue at the time of sampling to that present when fully turgid (Bora and Joshi, 2014), i.e., the state of full saturation. By knowing the amount of water content in plant, the stressed or unstressed condition of a plant could be determined. The high water content of a plant body allows it to maintain physiological equilibrium even during pollution stressed conditions (Tsega and Deviprasad, 2014; Ogunkunle *et al.*, 2015). Plants with a higher RWC are more resistant to drought (Chouhan *et al.,* 2012). Due to air pollution, transpiration rate of leaf decreases, and the plant can not live well in such environment because it loses its motor to pull water and minerals up from the roots to leaves for photosynthesis or other biosynthesis processes as well as hampers cooling effect in leaves (Liu and Ding, 2008).

1.8.3 Chlorophyll

Chlorophyll is the primary photoreceptor in photosynthesis, the light-driven process that converts carbon dioxide into carbohydrates and oxygen. Leaf chlorophyll and carotenoids can provide vital information about a plant's physiological status. A reduction in chlorophyll instantly reduces plant productivity. Plants that retain chlorophyll even in polluted environments are said to be tolerant species (Singh and Verma, 2007). Chlorophyll pigments exist in a highly structured form, and when stressed, they may undergo photochemical processes such reduction oxidation and reversible bleaching; as a result, any change in chlorophyll might impact the plant's physiological, morphological, and biochemical behavior (Giri *et al.,* 2013). Kapoor (2014) observed decreas in Chlorophyll a, chlorophyll b, total chlorophyll content, carotenoid, total sugar, protein, dust catching limit, and leaf size in such plants species growing near to the automobile exhaust and industrial pollutants sites. The chlorophyll content of plant varies from species to species with the age of leaf, pollution level and other biotic and abiotic conditions (Krishnaveni *et al.,* 2012). The chlorophyll concentration decreases with increasing pollution level and also varies with the tolerance and sensitivity of the plant species, i.e., the more sensitive the plant species, the lower the chlorophyll content (Jyothi and Jaya, 2010).

1.8.4 pH

pH being an important biochemical parameter which acts as an indicator of sensitivity of air pollution, and any alteration in leaf extract pH due to air pollution may affect stomatal sensitivity (Chouhan *et al.,* 2012). Plants with low pH are more vulnerable to air pollution, than those of plants with pH about 7 (Srivastava, 1999). Plant having a higher leaf extract pH makes plants more tolerant to pollution (Swami and Chauhan, 2015). It has been observed that when acidic pollutants such as SO_2 and NOx are present in the air, the pH of the leaves drops greatly in the sensitive plants than those of tolerant plants (Scholz and Reck, 1977; Singh and Verma, 2007). Hence, increased pH can enhance plant resistance to contaminants (Agarwal, 1988; Joshi *et al.,* 2016). Higher pH increases reducing activities of ascorbic acid while low pH reduces the efficiency of conversion of hexose sugar to ascorbic acid. So in the presence of acidic pollutants, leaf extract pH decreases and the decrease rate is more in sensitive plants as compared to tolerant plants (Tiwari and Tiwari, 2006; Gholami *et al.,* 2016).

1.9 Air pollution tolerance index (APTI)

Plants show an inherent ability to encounter stress arising from pollution. By assessing the physiological and biochemical response of plants to contaminants, the sensitivity and tolerance of plants can be ascertained (Lakshmi *et al.,* 2009). The susceptibility and sensitivity of plants have been measured by using four biochemical parameters such as relative water content, total chlorophyll content, leaf extract pH and ascorbic acid for Air pollution tolerance index APTI (Singh and Rao, 1983; Rawal *et al.,* 2001; Thawale *et al.,* 2011; Lohe *et al.,* 2015; Kanwar *et al.,* 2016;

Chaudhry and Panwar, 2016; Gholami *et al.,* 2016). The APTI determination provides the plants reaction to air pollution and their ability to fight against air pollution. Hence APTI is regarded as a well-established approach for screening a large number of plants for their susceptibility to pollutants in the air (Tripathi *et al.,* 2009). Singh *et al.* (1991) investigated 69 plant species (herbs, shrubs and trees) for their ability to withstand air pollution and based on their air pollution tolerance index value they categorized them into tolerant, moderately tolerant, intermediate, and sensitive plants. Plants with higher index values are more resistant to air pollutants and can be used as sinks to reduce pollutants, whereas plants with lower index values exhibit less resistance (Singh and Rao, 1983; Shannigrahi *et al.,* 2004; Chandawat *et al.,* 2011). Based on identification of plants into sensitive and tolerant groups, tolerant plants act as sinks for reducing air pollution in urban and industrial regions, while sensitive plants function as markers or indicator (Singh *et al.,* 1991).

1.10 Heavy metals and plants

Generally plants possess physiological mechanisms which make them able to resist with elevated heavy metal concentration in their substrate (Antonovics *et al.,* 1971; Baker, 1981, Woolhouse, 1983). Plants do not always show visible symptoms externally when exposed to pollutants but may have internal injury or changes in metabolic pathways (Barman *et al.,* 1999). Plants when grown on contaminated soils with toxic metals, show different types of responses in anatomical structure (Henry, 2000; Prasad, 2008). High concentrations of Zn and Cu in soil were phytotoxic and had adverse effects on crops, livestock and man (Kiekens, 1995). The phytotoxic effects with high concentrations of Co, Cu and Cr were the reduction in biomass, and that of Fe concentrations was on chlorophyll-a and chlorophyll-b content, and also on activity of catalase in leaves of cauliflower (Chatterjee and Chatterjee, 2000). Degradation of chlorophyll-a with high concentrations of Cu has also been reported in lichens (Chettri *et al.,* 1998) and mosses (Shakya *et al.,* 2008). Excess heavy metals cause internal water deficit in soil as well as in the plant body via reduced conductivity of stems and poor root system development (Lamoreaux and Chaney, 1977) which ultimately reduces nutritional quality and biomass production (Cottenie *et al.*, 1976).

1.11 Rationale of the study

In Nepal, there is an increasing trend of urbanization. According to the National Population and Housing Census (NPHC, 2021) the total population of Nepal, is 29,164,578, comparasion to other districts, Kathmandu has highest population density (5,169 per square Kilometer) with population of 2,041,587. The main cause of air quality degradation in Kathmandu is uncontrolled infrastructure development, vehicular emission, improper solid waste management, and industrialization. According to the Kathmandu Valley Traffic Police offie, the number of vehicles that ply on the valley road at present is 1.75 million. Just a year ago Traffic police data showed 1.4 million (Ojha, 2022). With the rapid increase in vehicle registration, population density, as well as road conditions, the air pollution in Kathmandu valley is higher than other cities of Nepal. Furthermore, waste burning in hospitals and other sectors, as well as coal burning and open burning of products such as tires and tubes, plastics, and papers, are additional sources of air pollution. Kathmandu, the bowl-shaped valley that encompasses three districts (Kathmandu, the capital city, Lalitpur, and Bhaktapur) has extremely high levels of particulate ambient air pollution, with levels five times higher than acceptable guidelines (Shrestha, 2018). The worsening of air quality of Kathmandu has been blamed for an increasing number of vehicles and construction activities (like buildings, roads, excavations).The main sources of elemental carbon (black carbon) in Kathmandu have also been identified from brick klins (40%), motor vehicles (37%) and biomass burning (22%) (Kim *et al.,* 2015). In Kathmandu the pulmonary function test (PFT) among the traffic police was found to be decreasing with the number of years involved in vehicular traffic duty (Shrestha *et al.*, 2015) and this clearly indicated that the continuous exposure in the urban areas of Nepal would pose serious respiratory problems. More efforts and investments are required to combat with this air pollution problem in Kathmandu. But this air pollution issue can be minimized by identifying the ‘natural sinks’, i.e., the suitable plants that can absorb and/or adsorb and detoxify contaminants from the air as well as could play a significant role in lessening air pollution and improving air quality by absorbing gases, and bio-accumulating heavy metals. In Kathmandu valley, from previous study of APTI, plants like *Cinnamum camphora*, *Jacaranda mimosifolia,* *Grevillea robusta, Pinus roxburgii, Thuja orientalis* and other different plants were described as moderately tolerant in one study (Rawal *et al.,* 2001; Rajbansi and Pradhanagar, 2017; Ter *et al.,* 2020) but sensitive in another (Kanwar *et al.,* 2016). In this context, some plants may be sensitive or tolerant to air pollution (Horaginamani and Ravichandran, 2010), but the contradictory results from different studies made it difficult to ascertain if a plant is tolerant or sensitive. Only understanding tolerant and/or sensitive plant species is not enough, as plants have different survival strategy especially in heavy metal contaminated environment. Some plants accumulate more heavy metals in root portion and allow small amount to reach the shoot portion (Hall, 2002) and thus reduces the toxic effect in their physiology but in some others metals are equally available in all parts (Rascio, 1977). Hence it is very important to understand the underlying mechanism in them. For this it is very important to assess if the plants are good accumulator or avoiders of toxic heavy metals. Some tree species may survive without accumulating heavy metals even when it is present in their environment (avoiders) and some can accumulate and withstand its toxicity to some extent (accumulator). Therefore, it is very important to know the relationship between APTI parameters and metal accumulation in different plant species specially to understand if a plant is an accumulator or an excluder/avoider. Heavy metal accumulator will act as a sink and will help in reducing concentration of heavy metals in ambient air. So the outcome of current study will assist to select the tolerant road side trees, which can accumulate heavy metals and grow in contaminated environment, and will act as natural sink to reduce the air pollutants.

1.12 Hypothesis

Based on above facts it is hypothesized that trees with large leaf surface area, thin cuticle and large stomatal pore, will absorb and accumulate heavy metals in leaves. It is also hypothesized that due to differences in metal accumulation in the leaf, same plant species exposed at different pollution level will have different APTI value.

1.13 Objectives

The general objective of this study is to identify the most suitable tree species which can absorb large quantity of air pollutants and helps in mitigating heavy metal pollution problem. To achieve this main objective, the following specific objectives have been set for the study.

1. To study the macro and micro morphology of road side trees leaf surface.
2. To evaluate biochemical parameters and Air Pollution Tolerance Index (APTI) of different road side trees.
3. To measure the absorption of heavy metal concentrations (Zn, Cu and Pb) in tree leaves growing along the road side.
4. To evaluate correlation among morphological characters (macro and micro) biochemical parameters and metal content.
5. To investigate the relation between metals and biochemical parameters.

1.14 Limitations

1. All the plants were not found in all the study sites hence could not include.
2. Only road side plants were included.
3. Samples were only collected in winter season.

CHAPTER 2

2 LITERATURE REVIEW

In developing countries, air pollution is a major threat to urban areas. The chief factors for air pollution are industrialization and urbanization (Bilal *et al.*, 2019; Lelieveld, 2020). Fast urbanization, uncontrolled industrialization, lack of strict environmental laws and poor emission control lead to the serious problems in the environment which severely affect both human and environmental health (Sanchez-Chardi, 2016). Metals circulate in the environment via both natural and human activities. Mobilization of pollutants from volcanic eruptions, rock, ocean evaporation, forest fires, and soil formation are natural sources (Ugulu *et al.,* 2012; Masindi and Muedi, 2018) while rubber tire wear, auto workshops, motor oil, gasoline combustion, electroplating industries etc. are urban sources of metal pollutants (Ugolini *et al.,* 2013).

2.1 Plants and air pollution

Plants have long been used in the detoxification of the air. Although trees help to reduce air pollution, pollutants have an adverse effect on them (Ghorbanli *et al.,* 2009). This injury appears in the form of morphological, anatomical, deformations and physiological changes in plants (Tiwari *et al.,* 2006; Liu and Ding, 2008). Plant leaves covered with dust receive less light, reducing photosynthetic efficiency and productivity (Šuškalo *et al.*, 2018). Wang *et al.* (2019) stated that dust and particulate matter are the commenst types of environmental air pollutants which are responsible for severe problems in plants. Trees are the most efficient in trapping and absorbing for various particles which play an important role and act as pollutant absorbers. Age, density, size, plant height, canopy and leaf area all influence dust capturing capacity. Particulate matters deposited on the epidermis of leaf disturb gaseous exchange and repiration as well as reduce photosynthesis too (Préndez *et al.,* 2019). The leaf area was greatly covered by dust particles in an urban area due to which the leaf size and stomatal density were found to be lower, and also observed thin cuticle and stomata closed than in a rural area (Pourkhabbaz *et al.,* 2010). Significant increase in trichome density on the leaf lower surface, and also increase in trichome length on both the upper and lower surfaces in *Robinia pseudoacacia* and *Ailanthus altissima* trees growing near the Iranian Aluminium Company (northeast of Arak province) have been speculated due to air pollution (Amini *et al.,* 2016). Decrease in leaf surface area, leaf lengths, and maximum width under air pollution conditions was obserd in *Acer negundo*, an ornamental tree in an urban forest (Babapour *et al.,* 2014). Molashahi and Feizi (2014) conducted a study on the capacity to absorb pollutants in *Pinus eldarica, Fraxinus rotundifilia* and *Morus alba,* and observed that *Morus alba* was the most capable of trapping pollutants due to its larger leaf surface area than the other two.The investigation of epidermal characters in the leaves of *Fraxinus pennsylvanica* and *Ficus benjamina* showed significantly variation with their stomata length and width between urban and rural areas, and width being higher in rural areas (Arriaga *et al.,* 2014). Squires (2016) observed that air pollution decreased stomatal conductance in light conditions while increasesed in dark conditions. Pollutant particles also inhibited photosynthesis by masking the leaves. Plant morphology plays a significant role in determining plant tolerance to air pollution. As a result, certain parameters like dimensions of stomatal aperture, thickness of cuticle, density of epidermal cells, and their cell wall properties, play important role for reducing air pollution and preventing pollutants from entering the plant. Those plants which are sensitive to air pollutants, they change in their anatomy, morphology, physiology, and biochemistry and it was supported by many researchers (Rai, 2016). Celik *et al.* (2005) reported that plants like *Robinia pseudoacacia* accurately reflected environmental changes and served as an effective biomonitoring tool for environmental quality. Manning and Feder (1985) suggested that the bio monitoring approach is a reliable as well as cost-effective alternative monitoring techniques for detecting the presence of pollutants in the environment. Plants act as natural bioindicators and help in monitoring of air quality through lessening the adverse impacts of toxicity of metals (Ugulu *et al.,* 2015). Plants are well known for accumulating and absorbing of potentially toxic substances (Piczak *et al.,* 2003) and they transfer trace elements from abiotic to biotic environment (Hu *et al.,* 2014; Martnez-López *et al.,* 2014). Heavy metal concentrations in air are strongly correlated with heavy metal concentrations in plants (Ugulu *et al.,* 2012). Root uptake and soil acidification govern heavy metal concentration in plants that is related to the bioavailability of trace element either in soil, or dry and wet deposition on the outer surfaces of leaf (Socha *et al.,* 2017). Plant leaves have a large surface area for soaking up, impingement, and accumulating air pollutants, so they serve as an environmental sink (Balasubramanian *et al.,* 2018). Many researchers have suggested that while developing green belts, tolerant tree species should be planted to improve air quality in town areas (Hu *et al.,* 2014). Different types of plants have been used in bio monitoring studies since long time (Baslar *et al.,* 2009; Sánchez-Chardi, 2016). Plants act as bio monitors and thought to be the most helpful and eco friendly method for monitoring as well as reducing of urban air pollution (Rossini *et al.,* 2007). Green plants particularly in industrial sectors or along the streets can provide an eco friendly and cost-effective way to reduce air pollution by which they help in beautifying to the environment (Bharti *et al.,* 2018). Air Pollution Tolerance Index (APTI) is thought to be a useful tool to evaluate whether a plant species is tolerant or susceptible to air pollution (Shrestha *et al.,* 2021).

2.2 Macro morphology of leaf

Leaf area is a trait that can be used to study environmental factors (like light interception, irrigation response, and evapotranspiration) as well as ecological factors (such as plant growth and photosynthetic efficiency) in urban green spaces (Tian *et al.,* 2019). Leaf area in tree species is directly related to particulate matter concentrations (Rodríguez-Santamaría *et al.,* 2022) because tree species retain more particulate matter at higher leaf area (Zha *et al.*, 2018). Earlier study has revealed that these leaf characteristics (stomatal density and trichomes) can be used to assess air quality in the context of urban tree species. No doubt, leaf traits have become a tool that can assist for the study in the management of urban tree air quality and ecosystem benefit (Rodrguez *et al.*, 2022). In comparison to all other plant parts, the leaf is the most sensitive to air pollutants. Numerous studies have found that when exposed to pollution, leaf area and petiole length reduce (Dineva, 2004; Tiwari *et al.,* 2006). According to Abdulmoniem (2011), polluted sites resulted in smaller leaves with reduced length, width, and stomatal index per leaf area. According to Saadullah and Mudassir (2013), all plant species studied showed a significant reduction in leaf area at polluted sites when compared to the same plant species at non-polluted sites. Meerabai *et al.* (2012) found a slight decrease in leaf area, size of stomata, density of stomata, stomata frequency, and stomata index in pigeon pea as a result of pollution.Specific leaf area (SLA), that measures leaf surface area per unit mass, is a key factor in plant's development. SLA indicates the plant’s photosynthetic capacities and adaptation to the environment. SLA is a major element in several ecological researches on photosynthesis, biomass, resource use, respiration, and growth strategies of different species of plants (Reich *et al.,* 1991; Wilson *et al.,* 1999; Yulin *et al.,* 2005; Liu *et al.,* 2018). Environmental factors such as light regime, water supply, nutrient intake and plant's status (like leaf and plant age) may all have an impact on the SLA of an individual plant. Several studies have been conducted to determine the impact of plant and leaf age in regulating leaf characteristics particularly SLA. These studies were mostly focused on agricultural plants and evergreens plants particularly in *Eucalyptus* (Biemond *et al.,* 1995; Bertin and Gary, 1998; Li *et al.,* 1999; Day *et al.,* 2001; England and Attiwill, 2006; Jullien *et al.,* 2009). Previous studies have shown that SLA decreased with increasing leaf age (Reich *et al.,* 1991; Luo *et al.,* 2005; White and Scott, 2006; Milla *et al.,* 2008), however, no change in SLA was observed in red spruce with plant age (Day *et al.,* 2001). SLA generally decreases from the bottom to the top of the canopy in evergreen species (Hollinger, 1989), but little information is available for seasonal deciduous trees or semi deciduous forest (Fotis and Curtis, 2017). Abbasi *et al.* (2018) conducted a study in *Ulmus* sps. at polluted sites of Azadi and Gisha Bridge and observed a reduction in specific leaf area, which has been suggested to be a strategy to improve stress tolerance (Xu *et al.,* 2009).

2.3 Micro morphology of leaf

The leaves of plants are the most exposed part and affected by air pollution (Treshow, 1984). As a result leaf area and petiole length were reduced due to stress of air pollution (Dineva, 2004; Tiwari *et al.,* 2006). Different plant species growing in polluted regions have shown negative impacts of air pollutants on stomatal density and opening (Verma *et al.,* 2006). Air pollution stress causes stomatal closure (Woo *et al.,* 2007). With the stomatal closure, CO_2 availability in leaves is limited, which hinders carbon fixation. In severely polluted areas, Gostin (2009) observed that pollution stress affected the structure of *Plantago lanceolata* leaves, the stomata of plants found to be decreased in size but increased in number. Nevertheless, this species is much resistant to the effects of air pollutants, and despite the changes observed, it continues to grow and mature (flowering stage). Pollutants absorbed by plants from the air have negative impact on photosynthesis, respiration, leaf conductance, and leaf life span (Tripathi *et al.,* 2009). All of these parameters in trees have a negative impact on canopy carbon fixation and net chlorophyll content. Ogunkunle *et al.* (2013) studied the impact of cement industry dust pollution on the anatomical features of the leaves of two plants, *Pennisetum purpureum* and *Sida acuta,* and observed significant reduction in stomatal size and increased stomatal index, which might be the strategy for anatomical adaptations in a polluted environment. Similarly, *Lantana camara* and *Calotropis procera* exposed in cement dust pollution has also been found to change the anatomical features and noticed modification in epidermal and stomatal characteristics in both species (Tiwari and Pandey, 2017). Amulya *et al.* (2015) reported a higher stomatal index, reduction in stomatal width,pores and length in *Tabernaemontana divaricata* and *Hamelia patens* growing at a very high traffic density (at K.R. Circle and Chikkahalli, Mysore city) than that of plants growing in the control areas. Leaf size was also reduced in polluted areas as compared to the control site. The cement dust pollutants also showed hidden damage in apricot tree such as gradual loss in photosynthetic activity, closing of stomata, and ultimately affected growth and production (Rafiq and Kumawat, 2016). Air pollutants also showed impacts on stomatal opening and number in the leaves of roadside plants (Zeb *et al.,* 2017).

2.4 Biochemical parameters for APTI

2.4.1 Ascorbic acid content (AA)

Ascorbic acid (AA) is a powerful reducing agent that activates several physiological processes like photosynthetic carbon fixation and defensive mechanisms and its reduction power is dependent on its concentration (Raza and Murthy, 1988; Thakar and Mishra, 2010; Yannawar and Bhosle, 2013). Pollution causes a rise in the ascorbic acid content of plant species, which may be related to an increase in the rate of formation of reactive oxygen species during the photo oxidation process (Chandawat *et al.,* 2011). Increased ascorbic acid concentration may be an effective method for preventing thylakoid membranes from oxidative damage during water stress conditions (Tambussi *et al.,* 2000). Ascorbic acid (AA) plays an important role in cell division, defense mechanism and synthesis of the cell wall in plants (Conklin, 2001). Ascorbic acid is a stress-reducing chemical found in higher amounts in stress-tolerant plant species (Rai *et al.,* 2013). The defense mechanism of plant responds by increasing the concentration of ascorbic acid, which enhances its ability to tolerate pollutants (Keller and Schwager, 1997).It is believed that plant species with high ascorbic acid content in polluted areas may withstand the stress of air pollution (Swami and Chauhan, 2015). Ascorbic acid levels in the leaves are an indicator of plants tolerance towards pollution (Subramani and Devanandan, 2015). Mishra and Pandey (2011) reported low ascorbic acid in plants like *Ficus religiosa, Ficus glomerata, Phyllanthus embilca* and *Eucalyptus globus* from polluted Tiruchirappalli city. Krishnaveni *et al*. (2012) evaluated the biochemical changes in the leaf of plants collected in and around Salem city and found high ascorbic acid content for *Albizia lebbeck,* and *Albiziz amara;* moderate amount of ascorbate with *Tridax procumbens, Syzygium cumini, Morinda pubescens, Delonix elata, Annona squamosa, Strychnos nux-vomica, Alangium salviifolium, Holarrhena antidysenterica;* and very low levels of ascorbate in *Aegle marmelos*. Tak and Kakdea (2017) observed that ascorbic acid decreased significantly in the plant leaves of *Alstonia scholaris, Azadirachta indica, Azadirachta indica, Tamarindus indica, Tectona grandis, Annona squamosa* and *Acacia nilotica* growing on the roadside with exposed to vehicular air pollution. Ascorbic acid acts as an antioxidant and develops the mechanism in a plant to resist against the adverse atmospheric conditions (Keller and Schwager, 1997).

2.4.2 Total chlorophyll content (Tchl)

Chlorophyll act as a bioindicator for air pollution levels (Darrall and Jager, 1984). Impact of air pollution on plants can be known by measuring the chlorophyll concentration of leaves of that plant. Plants with more chlorophyll may be more resistant to pollutants (Joshi and Swami, 2009). Photosynthetic pigments are degraded due to air pollution (Bansal, 1988; Singh *et al.,* 1990; Sandelius *et al.,* 1995). Air pollutants, particularly particulate matter, make their entrance into the tissues through the stomata and cause partial denaturation of the chloroplast and decrease pigment contents in the cells of polluted leaves. Swami *et al.* (2004) found a significant decrease in chlorophyll content, carotenoid content, ascorbic acid content, pH, and moisture content in the leaves of two tree species, *Shorea robusta* and *Mallotus philippinensis,* growing along the automobile polluted road side. Decrease in the concentration of chlorophyll in polluted area is caused by the shading effects with deposition of suspended particulate matter on the leaf surface which might clog the stomata and interfere with the gaseous exchange which ultimately hinder chlorophyll synthesis (Prajapati and Tripathi, 2008). Seyyednejad and Koochak (2011) observed highest chlorophyll content in the leaves of *Eucalptus camaldulensis* from polluted site as compared to the control. Plants growing in polluted environments showed lower level of chlorophyll'a', chlorophyll'b', and total chlorophyll (Raina and Sharma, 2006) but Tripathi and Gautam (2007) reported chlorophyll content in the leaves of *Mangifera indica* found to be increased in response to air pollution.

2.4.3 Leaf extract pH

In the presence of an acidic pollutant, the leaf pH is reduced and the reducing rate is more in sensitive plants compare to that in tolerant plant species (Scholz and Reck, 1977). pH of plant acts as a biochemical parameter and indicates sensitivity of plants in context with air pollution. Plants with pH about 7 are more tolerant while with low pH are more susceptible and less tolerant (Singh and Verma, 2007; Kumar and Nandini, 2013). Photosynthetic efficiency decreases with the decrease in pH (Escobedo *et al.,* 2008). Variations in the pH of leaf extract due to air pollution may have an impact on stomatal sensitivity (Chouhan *et al.,* 2012). Declining rate of pH in the presence of an acidic pollutant (such as SO_2 and NOx) in the ambient air is more in sensitive plants compare to tolerant plants (Singh and Verma, 2007; Paulsamy *et al.,* 2000; Scholz and Reck, 1977). Photosynthetic efficiency depends on pH of leaf and soil in that location which indicates the condition of the plant (Subramani and Devaanandan, 2015). Higher pH can provide resistance to the plant against the pollutants (Joshi and Bist, 2016). Kumar and Nandini (2013) found leaf extract pH moderately acidic in the species like *Ficus religiosa, Ficus benghalensis, Delonix regia, Albizia saman, Spathodea campanulata* and *Michelia champaca* collected from urban Bangalore, whereas of other species were slightly acidic collected from the same area. Nwadinigwe (2014) observed variable results of leaf extract pH in plants growing at polluted and control area and found more acidic at polluted site as compared to control site in plants (like *Delonix regia, Bougainvillea spectabilis,* and *Durantaerecta Ixora*) and low pH leaf extract (in *Ixora coccinea* and *Anacardium ocidentale*). Plants with high pH may increase the efficiency of conversion from hexose sugar to ascorbic acid (Escobedo *et al.,* 2008), whereas plants with low leaf extract pH show the good correlation of sensitivity towards air pollution (Yan-Ju and Hui, 2008). Rai *et al.* (2013) reported that plants growing at the industrial area showed acidic range while the plants growing at the non-industrial site showed neutral to slightly alkaline range.

2.4.4 Relative water content (RWC)

Water is vital precondition for plant‘s life (Swami *et al.,* 2004). RWC of a leaf is the amount water present in it relative to its full turgidity condition (Bora and Joshi, 2014). Relative water content is associated with protoplasmic permeability in cells, resulting in the loss of water and dissolved nutrition and early leaf senescence (Agrawal and Tiwari, 1997; Tsega and Deviprasad, 2014; Ogunkunle *et al.,* 2015). Several studies reported that high temperature and drought stress greatly infulence physiology and plant growth (Ro *et al.,* 2021; Chen *et al.,* 2022).High water content within a plant body helps to maintain its physiological balance under stressful conditions, such as exposure to air pollution (Agarwal and Tiwari, 1997; Innes and Haron, 2000).Under air polluted conditions, transpiration rates are frequently high, which may lead to desiccation. Therefore, the maintenance of RWC by the plant may determine its relative tolerance to pollution (Verma, 2003). Increased RWC in a particular species improves its drought tolerance. High RWCs in most of the plants growing in an industrial sites and polluted areas might be responsible for maintaining the normal physiological function (Rai *et al.,* 2013) Tree species may be more resistant to pollutants due to greater relative water content at polluted areas (Kumar *et al.,* 2019).

2.4.5 Air pollution tolerance index (APTI)

Air pollution tolerance index (APTI) has been widley used to calculate the response of plants towards air pollution (Singh and Rao, 1983; Lakshmi *et al.,* 2009). Plants with higher APTI value are designated as more tolerant towards air pollution and assist to reduce air pollution, whereas plants with a lower APTI value are less tolerant and indicate sensitivity towars air pollutants. Agbaire and Esiefarienrhe (2009) studied the plants that were continuously exposed to pollution, which absorbed, stored, and accumulated pollutants into their systems. These plants which were exposed to pollutants exhibited visible modifications along with changes in biochemical processes and accumulation of specific metabolites. Change in biochemical parameters in leaves is utilized as an early stress indicator or as a marker for physiological damage before visible injury symptoms appear. Joshi and Bora (2011) evaluated the APTI of plants growing alongside a highway in northern India, including*, Psidium guajava, Eucalyptus spp., Ficus benghalensis, Saraca indica, Cassia fistula, Azadirachta indica, Bougainvillea glabra* and *Ficus religiosa* and found that *Ficus religiosa* had a high APTI value. Some road side plants of Ahmadabad city, India, were evaluated for APTI by Chandawat *et al.* (2011) and found that the sequence of APTI value as *Ficus religiosa>Ficus glomerata>Azadiracta indica>Polyalthia longifolia.* Chouhan *et al.* (2012) investigated APTI in *Dalbergia sissoo, Euginia jambolana, Azadirachta indica, Mangifera indica Nerium indicum,* and *Calotropis gigantean,* growing in Pithampur industrial area, Madhya Pradesh, India and found that *Calotropis gigantea* had the highest APTI and *Azadirachta indica* had the lowest. They recommended that *Calotropis gigantea, Dalbergia sissoo* as efficient tree species in developing green belts and help in reducing air pollution. Similarly some tree species were evaluated for APTI (Kumar and Nandini, 2013) growing in Bangalore, India, and reported that *Pongamia pinnata, Michelia champaca, Millingtonia hortensis, Polyalthia longifolia, Albizia saman*, *Azadiracta indica* and *Tamarindus indica* showed tolerance to air pollutants and were suggested for use in bio monitoring of air pollution in comparable places. In another study, APTI of trees such as *Ficus palmata, Populus deltoides*, and *Pistacia integerrima* growing at several locations in Himachal Pradesh, India, was investigated by (Sharma, 2014) and found an APTI trend in the order *of F. palmata>P. deltoides>Pistacia integerrima*. Furthermore, study reported that at the rainy season had the greatest APTI value, followed by winter, and the summer season had the lowest. APTI of six different trees was studied by Bora and Joshi (2014), and reported that *Saraca indica>Azadirachta indica>Shorea robusta>Eucalyptus* sp.*>Ficus religiosa>Tectona grandis* had the highest APTI. Similarly APTI of three prominent tree species (*Dalbergia sissoo, Delonix regiai,* and *Cassia siamea,*) growing along road sides of Harda (Madhya Pradesh, India), was evaluated by Jain and Kutty (2014) and observed that APTI of *Cassia siamea* was greater in polluted sites (i.e., road side) than at residential area considered as controlled site. Rai and Panda (2014) studied the APTI of five plants growing along a road in Aizawl India and found that *Artocarpus heterophyllus* had the highest APTI and *Lagerstroemia speciosa* had the lowest. Lohe *et al.* (2015) calculated APTI in several plant species growing in different cities in northern India and observed that *Eucalyptus globus* had the highest APTI, then followed by *Ficus religiosa>Mangifera indica>Polyalthia longifolia>Phyllanthus emblica>Citrus limon>Lantana camara.* The APTI of tree species such as *Polyalthia longifolia, Psidium guajava, Ficus religiosa, Azadirachta indica, Mangifera indica,* and *Syzygium cumini* growing in various cities of northern India was evaluated by Madan and Chauhan (2015) and found that *Mangifera indica* showed the highest APTI and *Polyalthia longifolia* had the lowest. Similarly Swami and Chauhan (2015) investigated APTI in diverse tree species in *Eucalyptus citridora, Shorea robusta, Tectona grandis,* and *Mangifera indica,* and found that *Shorea robusta* had the highest APTI and *Eucalyptus citridora* had the lowest. Similar APTI study of four plant species (*Pongamia pinnata, Azadirachta indica, Tamarindus indicus,* and *Neerium oleander*), growing at different sources of air pollutants such as vehicles and industry in southern India, showed highest APTI in *Neerium oleander*and also indicated a pollution status in different sites (Akilan and Nandhakumar, 2016). APTI of three trees from India (*Tectona grandis, Polyalthia longifolia,* and *Ficus religiosa*) was measured by Pradhan *et al.* (2016) and reported highest APTI of *Tectona grandis.* Similarly in another industrial area of Tarapur, Maharashtra, Joshi *et al.* (2016) evaluated the APTI of 30 plants and found order of tolerant species as *Putranjiva roxburghii> Mangifera indica> Ficus racemosa> Ficus hispida>Morinda citrifolia* and the order of sensitive species as *Nyctanthes arbor-tristis> Bauhinia purpurea> Peltophorum pterocarpum> Psidiu guajava> Morinda pubescens.* APTI of plants growing near a gas flare station in Nigeria was studied by Tanee and Albert (2013) and observed APTI in the order of *Psidium guajava> Puerenia phaseoloides> Mallotus oppositifolia> Musa paradisiaca> Telfairia occidentalis> Cymbopogon citratus> Talinum triangulare> Vernonia amygdalina> Manihot esculenta.* Nwadinigwe (2014) investigated the APTI in plants like *Bougainvillea spectabilis, Ixora coccinea, Anacardium occidentale, Mangifera indica, Delonix regia* and *Duranta erecta* that grew near industrial locations across Nigeria. *Delonix regia* had the greatest APTI, followed by *Bougainvillea spectabilis and Duranta erecta*, while *Anacardium occidentale* had the lowest. Irerhievwie *et al.* (2014) evaluated APTI of seven plant species growing in a Nigerian metropolitan city and revealed APTI in the order of *Ceiba pentandra>Dendro calamus* *calostachyus>Irvingia gabonesis>Hevea brasiliensis>Vernonia amygdalina>Citrus sinensis>Chrysophyllum albidum.*

2.5 Air pollution tolerance index (APTI) studies in Nepal

Rawal *et al.* (2001) studied APTI of different tree species namely *Cinnamomum camphora, Grevillea robusta, Callistemon citrinus* and *Jacaranda mimosifolia* from Kathmandu valley and found high APTI value in *Cinnamomum camphora* followed by *Jacaranda mimosifolia, Callistemon citrinus* and *Grevillea robusta*. The study suggested *Cinnamomum camphora* as appropriate plant for plantation on roadside areas. Kanwar *et al.* (2016) studied tree species such as *Prunus persica, Populus deltoids* and *Grevillea robusta* from Kathmandu and found high APTI in these plant species whereas plants like *Pyrus pyrifolia, Celtis australis* and *Punica granatum* were reported as sensitive. Rajbanshi and Pradhananga (2017) studied on APTI of tree species from heavy traffic roadside of Kathmandu and reported high APTI value in lianas *Bougainvillea glabra* followed by *Albizia saman>Ficus benjamina>Psidium guajava>Grevillea robusta.* Dhyani *et al.* (2019) studied APTI of *Duranta repens, Bougainvillea glabra, Ricinus communis, Lantana camara* and *Sambucus hookeri* along the roadsides in Kathmandu valley and found high APTI in polluted site as compared to less polluted site. *Bougainvillea glabra* and *Duranta repens* were found to be the most tolerant species with high APTI values and recommended that these plants can be used in green belt development. They also recommend *Lantana camera* and *Ricinus communis* as useful plant for bio monitoring because of low APTI. Ter *et al.* (2020) compared the APTI of some common tree species like *Cinnamomum camphora, Ficus elastica, Ficus religiosa, Ficus benghalensis, Grevillea robusta* from Pashupati area of Kathmandu Metropolitan City and compared them with the plants from clean Shivapuri area. Their results showed high APTI in *Cinnamomum camphora, Ficus elastica, Ficus religiosa, Ficus benghalensis* and *Grevillea robusta,* whereas *Phyllanthus emblica* and *Schima wallichii* were found to be sensitive tree species. Sapkota and Devkota (2021) studied APTI of *Ficus religiosa, Tecoma stans, Buddleja asiatica, Lecosceptrum canum* and *Nyctanthes arbortristis* from different places of Lalitpur metropolitan city and found high APTI in *Ficus religiosa* and *Tecoma stans*. *Buddleja asiatica* showed lowest APTI. Shrestha *et al*. (2021) studied air pollution tolerance index of nine different plant species (*Albizia julibrissin, Cinnamomum camphora, Dypsis lutescens, Ficus benjamina,Ficus sp., Nerium* *oleander, Psidium guajava, Schefflera pueckleri* and *Thuja sp.*) in the Kathmandu valley and reported *Cinnamomum camphora* with the highest air pollution tolerance index where as *Schefflera pueckleri, Psidium guajava* and *Ficus benjamina* as sensitive species. Likewise *Ficus sp., Nerium oleander, Thuja sp., Dypsis lutescens* and *Albizia julibrissin* reported as moderate level of tolerance to air pollution. Timilsina *et al.* (2022) studied *Mangifera indica, Thuja occidentalis, Cinnamomum camphora, Ficus elastica, Callistemon lanceolatus, Pinus roxburghii, Grevillea robusta, Prunus persica, Salix babylonica, Sambucus canadensis, Buddleia asiatica, Cryptomeria japonica,* and *Celtis australis* from different areas of Kathmandu city and found high APTI value in *Mangifera indica* and the lowest for *Buddleia asiatica.*

2.6 Metal content in plants

Heavy metals are the elements with grater than 5 g cm^{-3} densities (Adriano, 2001). In our environment both essential and non-essential heavy metals are present. Among these heavy metals, the metals like, Fe, Mg, Mn Mo, Ni, Se, Co, Cu Zn., and Cr are essential and required in very small amounts for the growth and development of plants (Alloway, 2013). These essential heavy metals play an important biochemical and physiological role in plants, especially in constituent of various cellular enzymes and take active part in numerous oxidation-reduction reactions (Emamverdian *et al.*, 2015). These heavy metals elements are high reactivity and have a direct influence on senescence, plant's development, energy-producing activities, and other physiological processes. Heavy metal concentration in soil over permissible limits are hazardous to plants, causing oxidative stress through free radicals or deteriorating enzyme activities by replacing necessary metals and minerals (Henry, 2000; Prasad, 2008; Sharma and Chettri, 2012). Heavy metals alter cell metabolism and decrease plant development. However, the toxicity of metals varies depending on the stage of their development (Skórzyska and Baszynski, 1997). Cadmium (Cd) is one of the most dangerous pollutants due to its high toxicity and water solubility (Pinto *et al.*, 2004). Cd interacts with the absorption of metals (like Zn, Cu, Mn, and Fe) in some plant species. Cd enhanced production of reactive oxygen species (ROS) in plants besides inducing a process of peroxidation and breakdown of chlorophyll (Hegedüs *et al.*, 2004). Heavy metals have an impact on the biological and physiological processes of plants and due to their metallic features that are extremely harmful to plants. Heavy metal accumulation in plants causes decrease in biomass, chlorophyll-a, carotenoid, seed production, and root deformity (Sharma and Chettri, 2012). Phytoremediation is a natural biological process in which plants detoxify or immobilize heavy metals from the environment.

Heavy metals can be taken up and accumulated by plants through their root and leaf surfaces (Sawidis *et al.,* 2001). Trees may absorb and hold a lot of heavy metals because they have a large canopy area and extensive shoot and root system, that are useful as biological indicators of pollutants when assessing air pollution (Moreno *et al.,* 2003; Kardel *et al.,* 2010). Furthermore, a number of plant species have already been used as bioindicators (Aksoy *et al.,* 2000; Celik *et al.,* 2005; Baycu *et al.,* 2006; Mingorance and Oliva, 2006). For example, *Acerpseudo platanus* has been utilized as a bioindicator for measuring air pollution in urban ecosystems in Europe (André *et al.,* 2006), while *Quercus ilex* has been employed as a bio accumulator for heavy metals in urban environments (Ugolini *et al.,* 2013). However, most of the studies only focused at a single tree species (Aksoy *et al.*, 2000; Mingorance and Oliva, 2006; Al-Alawi and Mandiwana, 2007), and just a few studies have done at various types of plants at the same time (Piczak *et al.,* 2003), particularly evergreen and deciduous species (Hu *et al.,* 2014). The structure and chemical features of leaves have been shown to influence on the accumulation of airborne particles and/or the retention of particles on leaf surfaces, influencing heavy metal accumulation in plants (Gratani *et al.,* 2008). Furthermore, understanding the efficiency of heavy metal accumulation by various plant species is essential for developing planting strategies that improve urban air quality (Dzieranowski *et al.,* 2011). Metal accumulation in foliage are influenced by number of factors (such as atmospheric dust, direct atmospheric wet deposition, soil pH, soil texture, local flora, land use type, local soil contamination, bioavailability of metal in soil, bioaccumulation potential, and metal transfer) within the plant (Tomasevic *et al.,* 2004; Norouzi *et al.,* 2015). measured atmospheric heavy metal pollution in leaves of two tree species (*Tilia* sp. and *Aesculus hippocastanum*) from Belgrade, Serbia, for their bioaccumulation potential and found the accumulation of metals were higher in majority of the leaf samples, with, Cd (4.9 mg/g DW), Pb (20.3 mg/g DW) and Cu (110.2 mg/g DW) than the “reference plant” values. It has been widely used to compare with different plant species (Markert, 1992). Heavy metal accumulation was significantly higher at the mature stage than that of beginning of the vegetative stage (Tomasevic *et al.,* 2004). Even plants exposed to similar sources of heavy metals under similar environmental circumstances do not have same accumulation pattern because an individual plant species characteristic also determines accumulation patterns. Cicek and Koparal (2004) studied the plants such as in *Robinia pseudoacacia, Quercus infectoria, Salix alba, Pinus nigra* and *Populus tremula.,* within a 10 km radius of a thermal power plant in Kütahya Province and observed significant accumulations of Cd, Cr, Cu, and Pb in the leaves showing a progressive reduction in accumulation as distance increased from the power station. Celik *et al.* (2005) investigated heavy metal contamination in the leaves of *Robiniapseudo acacia*in Denizli City, Turkey and found that samples taken nearer to the industrial areas showed higher accumulations of Zn, Mn, Pb, Cu, Fe, and Cd than samples taken from roadways. Similarly Santos-Jallath *et al.* (2012) found notable accumulation of As in *Tecoma stans* (9.22 mg/kg) and *Nicotiana glauca* (91.94 mg/kg) leaves, but levels were lower in *Casuarina* sp. (3.95 mg/kg) and *Prosopis* sp. (6.94 mg/kg) grown adjoining tailings dams in Queretaro, Mexico. Sawidis *et al.* (2011) evaluated the bioaccumulation of Cu, Fe, Cr, and Pb in the leaf samples of *Pinus nigra* and *Platanus orientalis* from three European cities (Salzburg, Belgrade, and Thessaloniki) and reported that bioaccumulation of all metals were higher in plants grown in polluted areas compared to control and showed a significant correlation between soil surface metal and metal accumulation in leaves.

2.6.1 Heavy metals and their physiological role in plants

Essential heavy metals like Zn, Cu, Mn, Fe, Ni, and Mo shows an important role in physiology and biochemistry of plants (Zhuang *et al.,* 2009). Metals like Zn, Cu and Mo are essential metals for plant’s growth and development because they drive as an enzyme reaction cofactor or activator of a catalytic element in metalloproteinase. These essential heavy metals take part in electron transport, redox reactions, and basic activities in metabolisms of the nucleic acid (Nagajyoti *et al.,* 2010).They have direct involvement in various enzymes. Nagajyoti *et al.* (2010) reported that the presence of these essential metals in growth media at a specified concentration is essential; however their excess concentration results in several toxic effects. Industrialization, synthetic chemicals, fungicides and pesticides are expanding sources of harmful metal contaminations especially probably in farming soil and water bodies (Khanna *et al.,* 2018). Besides Zn and Cu other metals like Pb, Ni, Ag and Hg are supposed to be non- essential trace elements, and are extremely hazardous in nature. Plants are stationary in a terrestrial system and their roots serve as the primary contact sites for trace metal ions while in aquatic condition the whole plant body is exposed and metal ions are directly absorbed from the water through leaf surface (Kibria, 2014). Heavy metals have negative impact on the development and activity of soil microorganisms and also have an indirect impact on plant growth (Kumar *et al.,* 2019). Physiological processes like root nutrient absorption, plant water balance, metabolism, photosynthesis, respiration, germination, and reproduction are influenced through the interference with ionic homeostasis and enzyme activity. Chlorosis, senescence, necrosis, and stunted growth wilting, poor biomass output, reduced seed counts, and ultimately mortality are visible indications of heavy metal poisoning. Plants growing under heavy stress of metal must spend more energy for survival, which would otherwise this energy could be accessible for other processes. This insufficient quantity of energy leads in a decline in total plant development in such a difficult metal-stressed environment (Kumar and Aery, 2016).

2.6.2 Response of plants with different heavy metals

Toxic heavy metals remaining for prolonged in the soil ecosystem are become major concern in living beings and plants together. Essential and non-essential metals contain in the growing medium but in excess, it becomes toxic, inhibiting the growth and development of plant and even cause death. Plants develop some effective and precise ways to survive against metal stress. These include immobilization, absorption and transport limitation, exclusion of metals, plasma membranes exclusion, chelation and sequestration (Kumar *et al.,* 2019). Induction of stress proteins and synthesis of specific heavy metal transporters are performed through specific ligands (Clemens, 2001; Adrees *et al.,* 2015). There are two basic strategies for heavy metal tolerance in plants, either avoiding toxic metal from entering through the plasma membrane or by binding them with protein. It is possible to do this by either pumping metal out of the cell using active efflux pumps or enhancing metal ion binding to the cell wall. Tong *et al.* (2004) also revealed that plant detoxifies toxic metal ions either through deactivating them by chelation or converting the toxic metal ion concentration to low toxic concentration. Plants have strong capacity of absorbing and solubilizing of different kinds of soil nutrients by helping in the production of chelating agents and the modification of pH and redox processes. Plants also have incredibly precise systems for translocating and accumulating certain nutrients or metals. Number of factors such as life cycle of plant, plant's structure, pH of soil, vigority of plants, carbohydrate level, depth of root system, temperature, respiration rate, partial oxygen pressure, nutrient interface, and microbial presence impact metal accumulation in plants (Chen *et al.,* 2006). Plants may precipitate heavy metals either by excreting anions such as PO_3 or by modifying the pH of the rhizosphere (Chen *et al.,* 2006). During the adsorption process, the root surface can bind a variety of heavy metals. Heavy metals such as Ni, Pb, Cd, and Sr accumulate fast in plant root tissues (Kumar *et al.,* 2019). Plants have been categorized into three types depending on their method of action for survival under stress conditions: accumulators, excluders, and indicators (Hossain *et al.,* 2012). Plants which accumulate metals between 0.1-1% of their dry weight are called hyper accumulator species (Baker and Brooks, 1989). Hyper accumulator plants can deposit and resist large amount of metal pollutants. Several plant species may grow in heavy metal contaminated soil. They collect large amount of metals through leaf surface containing in soil indicating as an ecological adaptation (Lombi *et al.,* 2002).

2.6.2.1 Zinc (Zn)

Zinc is an essential and basic mineral for plant's growth and normal cell metabolism at an optimal level (Dhankhar *et al.,* 2012). Zn functions as a cofactor in several physiological processes including (multiple biomolecule metabolism, enzyme activation, gene expression and control, protein synthesis, and reproductive development). Zn, however, alters physiological processes and inhibits development in plants at greater concentrations (> 300 g/dry weight) (Cakmak, 2000). Multiple metabolic processes in plants are inhibited by high zinc exposure in the growing media, which results in senescence and reduced development. Zn toxicity restricts the growth of shoot and root (Fontes and Cox, 1998). Marschner and Cakmak (1986) noted that the chlorosis might develop partially by an induced deficiency of iron (Fe) as hydrated of Fe^{2+} and Zn^{2+} ions. Zinc toxicity inhibited the development of root and shoot in plants (Choi *et al.,* 1996) and induced chlorosis on both young and old leaves that were exposed to Zn for an extended period of time (Ebbs and Kochian, 1997). Excess amount of Zn cause deficiency in essential elements like Cu and Mn in shoots and that also limits the transport of essential micronutrients from root to shoot

(Sharma and Chettri, 2012). The possible cause of interference of translocation of this micronutrient is due to the manganese and iron concentrations in a plant being higher in the root than in the shoot growing on zinc-rich medium in a plant (Ebbs and Kochian, 1997). Zn and Cadium also altered enzyme catalytic performance in *Phaseolus vulgaris* (Van Assche *et al.,* 1988). Zn levels were found to be higher in polluted soil than that of requirement which may lead to phytoyoxicity. High concentration of Zn in soil reduced metabolic processes in many plants, showing decrease in development and causing senescence. Plants with Mn and Fe concentrations when grown in Zn-rich medium, Mn and Fe concentrations found to be higher in root than shoot (Ebbs and Kochian, 1997). Zn toxicity also causes typical effect with appearance of purple red color in leaves due to lack of phosphorous (Lee *et al.,* 1996). Studies of Zn binding hyper accumulator (Salt *et al.,* 1999) indicated that histidine plays an important role in the roots, whereas organic acids which are commonly found in plant vacuoles are involved in xylem transport and Zn storage in shoots. Yang *et al.* (2014) studied 12 Willow clones (Salix spp.) to observe the tolerance and accumulation of Zn and Cu and found that the majority of Willow clones (Salix spp.) were good accumulators of Zn and Cu.

2.6.2.2 Lead (Pb)

Lead is naturally present in all soils. It generally occurs in the range of 15 to 40 milligrams lead per kilogram of soil (mg/kg) (James, 1988). Primary and secondary leads are found in terrestrial environment. Primary Pb is integrated into minerals at the time of their formation and is geogenic in origin while secondary Pb is radiogenic and originated from the uranium and thorium (Weis, 2016). Lead are mostly used in the the manufacture of lead acid batteries and in alloys, wires, and chemicals. One of the most common and harmful non-essential elements in soil is lead. Although roots of plants have a greater capacity to absorb Pb, but aerial parts is quite limited (Niazi and Burton, 2016). Amount of lead accumulation in plants are dependent on the soil characteristics such as size of particle, pH, and capacity for cation exchange. Other parameters like root surface area, root exudation, mycorrhization also determine the amount of lead. Rate of transpiration is also important for availability of Pb. Plant roots absorb Pb via apoplastic route or Ca^{2+} permeable channels andaccumulates largely in root cells following take-up due to the Casparian strips' blocking of the endodermis (Pourrut *et al.,* 2011). Cell wall of the roots trap lead with negative charge

(Seregin and Ivanov, 2001) and lead accumulation has various adverse effects on plant physiological, morphological, and biochemical processes, either directly or indirectly. Pb toxicity occurs in cells through changing the cell membrane's permeability and then interacts with active metabolic enzyme groups, substituting essential ions, and forming complexes with the ADP or ATP phosphate group. Water imbalance, enzyme inhibition, disrupted mineral nutrition, hormonal disruption, lipid peroxidation, DNA damage due to over production of reactive oxygen species (ROS), suppression of ATP synthesis, and variations in membrane permeability, are disturbed by lead toxicity Pourrut *et al.* (2011). Germination of seed in *Pinus helipensis* and *Spartiana alterniflora* were inhibited due to lead (Morzeck and Funicclli, 1982). Lead may interfere with important enzymes, which might prevent seed germination. Besides this lead inhibited the growth of roots, stems, and leaves in *Raphanus sativa,* barley, and species of *Allium* (Juwarkar and Shende, 1986). Root elongation is hindered due to varying degrees in the pH medium, lead content, and ionic makeup (Goldbold and Hutterman, 1986). Root growth was found to be increased in *Sesamum indicum* but excessive lead levels in soil accelerated reduction in morphology of many plant species, such as lignification of cortical parenchyma, unevenradial cell walls of the endodermis and thickenings of roots in pea (Kumar *et al.,* 1993). Proliferation was induced by the Pb that effects in the restoration process of vascular plants (Kaji *et al.,* 1995).

2.6.2.3 Copper (Cu)

Copper is an essential metal for normal plant growth and development. Copper is considered as a micronutrient for plants and plays an important role in CO_2 assimilation and ATP synthesis (Pichhode and Nikhil, 2015). Demirevska-Kepova *et al.* (2004) reported that Cu is an essential component of various proteins like plastocyanin of photosynthetic system and cytochrome oxidase of respiratory electron transport chain. Cu pollution in the soil is incorporated from various human sources like smelting and mining of Cu containing ores. Mining activities yield a large amount of waste rocks and tailing, which is deposited on the surface. High concentration of Cu in the soil is cytotoxicity causing stress and harm to plants, as well as leaf chlorosis and plant growth retardation (Katare *et al.,* 2015). Similarly Cu being as a redoxactive metal, promotes the formation of reactive oxygen species (ROS) through the Fenton-Haber-Weiss reactions (Stohs and Bagchi, 1995). Overproduction of reactive oxygen species (ROS) can cause severe damage to membrane lipids, protein synthesis, and DNA (Lequeux *et al.,* 2010). Excessive copper levels can also cause a number of physiological and morphological problems. Furthermore, excess copper can reduce germination rate, shoot enlargement, plant biomass, and water content (Ahsan *et al.,* 2007). Cu toxicity can also cause reduction in mitotic index, cell division inhibition as well as induce chromosomal aberrations, and structural changes of the cell wall (Liu *et al.*, 2009; Bouazizi *et al.*, 2010).

2.7 Metal accumulation index (MAI)

Plants accumulate different types of metals from contaminated environment. Metal Acuumulation Index (MAI) is a useful approach to assess the metal accumulation ability of plants growing in polluted regions. Khanoranga (2019) studied wild plant and cultivated species from three districts of Balochistan, Pakistan (Mastung, Pishin, and Quetta). Nine plants (*Convolvulus arvensis, Elaeagnus angustifolia, Vitis vinifera, Chenopodium album, Triticum aestivum, Medicago sativa, Lepidium sativum, Prunus armeniaca* and *Punica granatum*) were collected in Quetta while five plants (*Lepidium sativum, Morus alba,Malcolmia Africana, Triticum aestivum* and *Peganum harmala*) were collected from Pishin and Mastung.

MAI of various plants arround brick kilns sites of Balochistan, Pakistan showed that *Lepidum sativum* with maxmimum value of MAI and *Malcolmia africana* with lowest. Plants showing with higher MAI value can be used as a barrier between polluted and unpolluted environment. Plants with higher MAI value are thought to be greater accumulation capacity of metals and considered as more tolerant (Hu *et al.,* 2014; Nadgorska Socha *et al.,* 2017). Thus plants with higher MAI value are recommended for plantation in heavy metalloid polluted sites and better to consider indigenous plants with high MAI values as a natural pollution control in contaminated areas. Plants with higher MAI are thought to be resistant and can serve as a sink for heavy metalloid contamination (Nadgorska-Socha *et al.,* 2017). Plants with higher MAI values have more accumulation capacity of metals so considered to be tolerant species (Hu *et al.*, 2014) and also have been suggested to be influenced by a number of factors such as local atmospheric condition, weather, sample height of trees, time of sampling, and features of plants (Hu *et al.*, 2014).

2.8 Research gap

In our country, few studies have been carried out about physiological response of plants to air pollution. From previous studies high APTI was recorded among *Prunus persia, Populus deltoids, Thuja* sp. and *Grevillea robusta* (Kanwar *et al.*, 2016) but the other study reported *Cinnamomum camphora* with high air pollution tolerance index and *Grevellia robusta* with the lowest APTI (Rajbanshi and Pradhananga, 2017; Rawal *et al.*, 2001). So in the case of *Grevillea robusta,* it was regarded as the most tolerant in one study (Kanwar *et al.*, 2016) but sensitive in another (Rawal *et al.*, 2001; Rajbanshi and Pradhananga, 2017). The results of APTI obtained are contradictory and confusing. It is important to know if a plant species is tolerant (i.e., with high APTI value) or sensitive (with low APTI value) especially to plant them near the road side. Planting tolerant plant species with a high APTI and metal accumulation index value can help to solve the problem of poor air quality in general. The tolerant tree species, serve a vital role near polluted areas, as they not only act as an efficient sink but also as a filter for pollutants. Understanding only tolerant and/or sensitive plant species is not sufficient since plants have different survival strategies under pollution stress and identifying the underlying mechanism in them is important. It is essential to determine if the plants are good accumulators or avoiders of toxic heavy metals. Some tree species can thrive in their surroundings without accumulating heavy metals (avoiders), while others may accumulate and withstand its toxicity to certain extend (accumulator). So it is essential to understand the relationship between APTI parameters, and metal accumulation in different plants, particularly to identify if a plant is an accumulator or an excluder/avoider. In Kathmandu valley, most of the studies have been conducted with physiological response of plants towards air pollution (Kanwar *et al.*, 2016, Rajbanshi and Pradhananga, 2017). But the studies with relation among biochemical parameters, macro and micro morphological study and heavy metals accumulation in them are missing. It is very important to understand the underlying mechanism for their survival in adverse environment. It is not known if the plants growing along the road side of Kathmandu valley are either good accumulator or avoiders of toxic heavy metals. Do all the plants along the road side of Kathmandu valley show the same tolerance to air pollution or not? What could be relation among biochemical parameters, macro and micro morphological characters and heavy metals accumulation? Can these sensitive or tolerant tree species be used as bioindicator of air quality in Kathmandu valley?

CHAPTER 3

3 MATERIALS AND METHODS

3.1 Study area

Kathmandu valley lies in between latitude 27°27′ - 27°49′ E and longitude 85°10′ N - 85°32′ N and its altitude varies from 1200 to 1400 meters above sea level in subtropical zone. The hills around the valley are Phulchowki (2782 meters) in the south west, Shivapuri (2713 meters), in the north, Nagarjun (2100 meters) in the north-west and Champa Devi (2400 meters) in the west, and are the prominent boundary of the valley. Bowl-shaped geography makes Kathmandu valley more prone to air pollution (Shrestha, 2001; Pradhan *et al.*, 2012). In summer an average temperature lies between 19°C to 35°C and in winter it lies between 2°C to 12°C. Valley is characterized by typical monsoon climate with dry winter and wet summer. An average annual rainfall in the valley is 1343 mm with 97 rainy days per year having more than 0.1 mm rainfall.

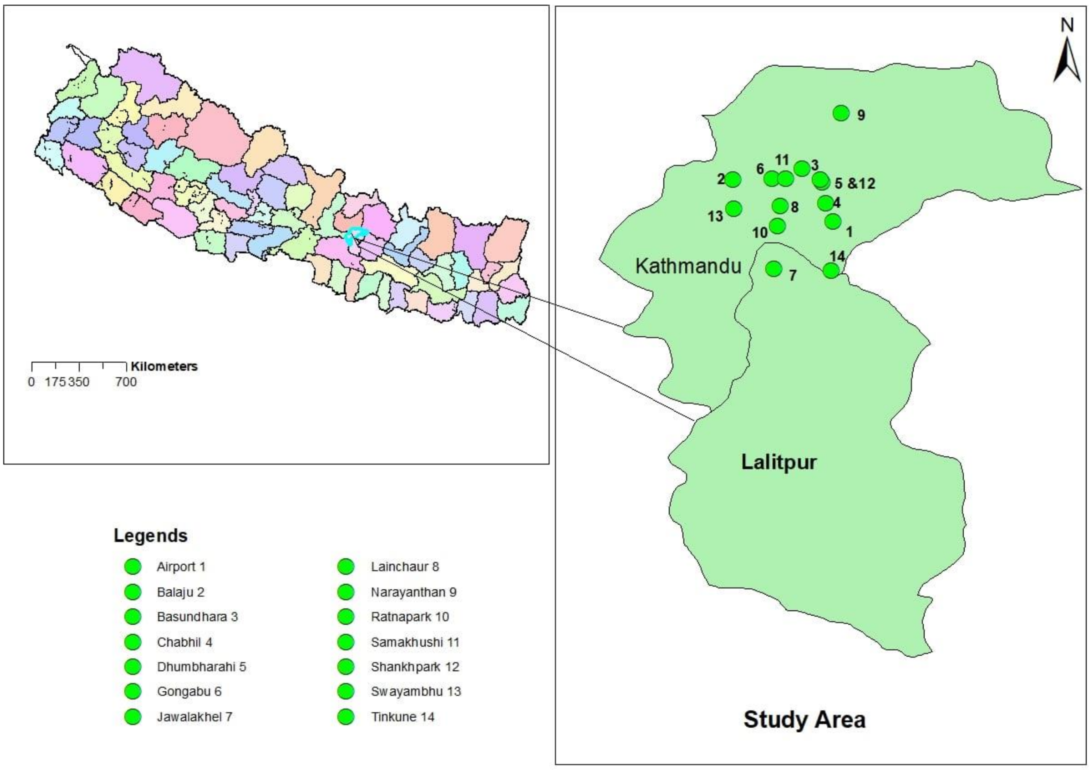

Figure 1: Map of Kathmandu valley showing study site: 1-Airport, 2-Balaju 3-Basundhara, 4-Chabhil, 5-Dhumbarahi, 6-Gongbu, 7-Jawalakhel, 8-Lainchaur, 9-Narayanthan, 10-Ratnapark, 11-Samakhusi, 12-Shankpark, 13- Swayambhu, and 14-Tinkune

The study was mainly focused along the ring roads of Kathmandu valley which were dusty with heavy traffic areas like Airport, Balaju, Gongbu, Samakhusi Basundhara, Chabhil, Dhumbarahi, Sankhapark, Swayambhu, and Tinkune. Besides this the areas inside the ring road with high traffic (like Jawalakhel, Lainchaur, Ratnapark) and a comparatively less polluted site with less traffic like Narayanthan (also called Budhanilkantha) (Shakya *et al.,* 2012;Chauhan *et al.,* 2021) were also considered for the study (Fig. 1).

3.2 Sample collection

Plants were selected on the basis of common occurrence, easy accessibility and abundance at selected sites of the study (Table 1). At each site three individuals of same species were selected and about 10 to 15 matured leaves were collected from all sides of each tree. Leaves of commonly available gymnosperm trees along the roadside like *Pinus roxburghii, Cedrus deodara, Thuja orientalis* and *Araucaria araucana* were collected from polluted area (Jawalakhel, Airport, Sankhapark, Dhumbarahi, and Ratnapark) and comparatively less polluted site Narayanthan, i.e., also called Budhanilkantha. Similarly commonly available angiosperm trees along the road side like *Populus euramericana, Ficus religiosa, Jacaranda mimosifolia, Grevillea robusta, Callistemon lanceolatus* and *Cinnamomum camphora* were also collected from selected site. Leaves from lower surface but above 3 m height from the ground surface were collected for the study. Leaves were collected from all sites during winter months (December and January) in the morning 9-10 AM and study conducted in 2015-2016. Among collected samples, leaves of gymnosperm trees were used only for biochemical parameters (pH, total chlorophyll, relative water content, and ascorbic acid and heavy metal analysis while macro and micro morphological characters were not considered because of their small leaf area. Among the collected leaf samples of angiosperm, all the biochemical parameters, heavy metal content, as well as micro and macro morphological characters were measured due to their large leaf area. All the biochemical parameters were performed at laboratory of Amrit campus, Tribhuvan University, on the same day of collection, whereas micro and macro morphological characters were measured on the next day keeping the collected samples at low temperature in the refrigerator.

Table 1. Name of plant species collected from different sites

Botanical name (Common name)	Family	Sites of collection
Angiosperm		
Callistemon lanceolatus (Sw.) DC (Bottle brush)	Myrtaceae	Airport, Balaju, Banasthali, Basundhara, Gongbu Dhumbarahi, Narayanthan (Budhanilkantha)
Celtis australis L. (European nettle tree)	Cannabaceae	Airport, Balaju, Basundhara, Dhumbarahi
Cinnamomum camphora (L.) J. Presl. (Camphor tree)	Lauraceae	Airport, Balaju, Basundhara, Dhumbarahi, Narayanthan (Budhanilkantha)
Eucalyptus globulus Labill. (Gum trees)	Myrtaceae	Airport, Balaju, Dhumbarahi, Swayambhu, Narayanthan (Budhanilkantha)
Ficus elastica Roxb. ex Hornem. (Rubber plant)	Moraceae	Airport, Chabhil, Samakhusi, Tinkune, Narayanthan (Budhanilkantha)
Ficus religiosa L. (Peepal tree)	Moraceae	Airport, Balaju, Basundhara, Dhumbarahi, Narayanthan (Budhanilkantha)
Grevillea robusta A. Cunn. ex R. Br. (Silky oak)	Proteaceae	Airport, Balaju, Basundhara, Dhumbarahi, Lainchaur, Narayanthan (Budhanilkantha)
Jacaranda mimosifolia D. Don (Kikuyu)	Bignoniaceae	Airport, Basundhara, Dhumbarahi, Lainchaur, Swayambhu, Narayanthan (Budhanilkantha)
Populus euramericana L. (Poplar tree)	Salicaceae	Airport, Basundhara, Chabhil, Dhumbarahi, Samakhusi, Tinkune, Narayanthan (Budhanilkantha)
Prunus cerasoides D. Don (Sour cherry)	Rosaceae	Airport, Balaju, Basundhara, Dhumbarahi, Narayanthan (Budhanilkantha)
Gymnosperm		
Araucaria araucana (Molina) K. Koch (Monkey puzzle tree)	Araucariaceae	Airport, Dhumbarahi, Jawalakhel, Ratnapark, Shankhapark, Narayanthan (Budhanilkantha)
Cedrus deodara D. Don (Deodar)	Pinaceae	Airport, Dhumbarahi, Jawalakhel, Ratnapark, Shankhapark, Narayanthan (Budhanilkantha)
Pinus roxburghii Sarg. (Chir pine)	Pinaceae	Airport, Dhumbarahi, Jawalakhel, Ratnapark, Shankhapark, Narayanthan (Budhanilkantha)
Thuja orientalis L. (Black Juniper)	Cupressaceae	Airport, Dhumbarahi, Jawalakhel, Ratnapark, Shankhapark, Narayanthan (Budhanilkantha)

3.3 Criteria for sample collection sites

Samples were collected at polluted and less polluted sites (Table 2). Polluted sites were selected on the basis of heavy metal concentrations, number of vehicles plying on the route (Shakya *et al.,* 2012; Chauhan *et al.*, 2021), commercial and undisturbed area where as less polluted site was selected on the basis of less contamination of heavy metals (Shakya *et al.*, 2012) and low traffic zone (Chauhan *et al*. 2021).

Table 2. Description of study sites

Studied sites	Location	Description of sites	Categorized
Along the ring road	Airport	Heavy traffic load, densely populated, commercial area linked with air field	Polluted
	Balaju	Heavy traffic load, densely populated, commercial area and linked with Balaju industrial area	Polluted
	Basundhara	Heavy traffic load, densely populated, commercial area	Polluted
	Chabhil	Heavy traffic load, densely populated with commercial area and linked to Shaku	Polluted
	Dhumbarahi	Heavy traffic load, densely, populated, commercial area and linked to the Narayan Gopal Chowk	Polluted
	Gongbu	heavy traffic load with main bus terminal linking major cities, densely populated with commercial areas	Polluted
	Samakhusi	Heavy traffic load, densely populated, commercial area	Polluted
	Sankhapark	A public park, but with heavy traffic load, densely populated and commercial area	Polluted
	Swayambhu	A holy place included in world heritage site, but with heavy traffic load at ring road, densely populated and commercial area	Polluted
	Tinkune	Heavy traffic load, densely populated, commercial area and links the Bhaktapur with ring road	Polluted
Inside ring road (with in the city)	Jawalakhel	Situated at Lalitpur district, heavy traffic load; densely populated, commercial area with central zoo nearby	Polluted
	Lainchaur	Heavy traffic load, densely populated and commercial area and inner to the ring road	Polluted
	Ratnapark	Heavy traffic, central substation of buses linking most of the places in the valley, densely populated and commercial area	Polluted
Outside the ring road (control)	Narayanthan (Budhanilkantha)	Low traffic, sparse residential and undisturbed area. Out of ring road and adjacent to Shivpuri-Nagarjun National Park	Less Polluted

3.4 Leaf morphological character

The leaf characters under study were divided into macro morphological characters and micro morphological characters. Leaf area and specific leaf area were included under macro morphology whereas stomata density, stomata size (length and breadth), thickness of cuticle and thickness of epidermis were included under micro morphological characters.

3.4.1 Macro morphological characters

Three largest leaves of each species at each site were collected for this study. Dusts on the leaf samples were cleaned and rinsed with soft wet cloth and then photos were taken along with scales. The photographs were managed in a folder on computer and were used for measuring two macro morphological characters (leaf area and specific leaf area). Leaf area was measured using Image J program version 1.31 (Kovacic and Nikolic, 2005) from the photos taken. For Specific leaf area (SLA), the same leaf sample which were used for leaf area were dried in hot air oven at 60°C for 24 hrs and then weight of the dried leaves were measured with the help of three digital electronic balance. Ultimately SLA (cm^2g^{-1}) of each leaf was measured using the leaf area and it's corresponding dry weight (Chaturvedi *et al.*, 2013).

SLA = Leaf area/dry weight (cm^2g^{-1})

3.4.2 Micro morphological characters

For the study of micro morphological study all the samples of tree leaves were collected from study sites. Largest leaf sample was considered for all the micro morphological study. For the measurements of stomatal density, length and breadth of guard cells, the peelings of abaxial leaf surface were carefully taken with extra care and slides were made by the method of lasting impression technique (Eisele *et al.*, 2016; Jayakody *et al.*, 2017). In this method nearly one square centimeter of the lower leaf surface was painted with a transparent nail polish. The nail polish was then allowed to dry and then nail polish was peeled out. The leaf impression was taped on slide and was examined under 15x × 45x magnification using compound microscope (BM-3) and 3 microscopic fields from 3 different leaf samples were studied. Number of stomata per microscopic field was recorded for stomatal density. To measure stomatal size, the length and breadth of stomata were measured ocular division that was fitted in the eye piece. The ocular division was calibrated with the stage micrometer under the same magnification. To measure thickness of cuticle and epidermis of adaxial leaf surface, thin vertical sections of leaves were taken and then photographs were captured along with ocular division. The thickness of cuticle and epidermis were measurements from the photograph using Image J computer software program version 1.31 (Kovacic and Nikolic, 2005). Percantage increase or decrease in SLA, stomata density, length of stomata, breadth of stomata, thickness of epidermis and thickness of cuticle was calculated from the differences in data of respective parameters at polluted sites from that of less polluted sites.

3.5 Biochemical parameters

3.5.1 Total Chlorophyll (Tchl) content

For total chlorophyll measurement, 0.05 g leaves were measured using digital electric balance (NLB, 2004). The measured leaves were then placed in a test tube containing 5 ml DMSO and incubated in a water bath (Memmert water bath) at 60-65°C for an hour until complete decolourisation of leaf tissues was seen. After that test tube containing leaf tissues and DMSO were cooled at room temperature for about 30 minutes. These filtrate solutions were made to centrifuge (R 4C) and then analyzed at a 665 nm and 648 nm using a Spectrophotometer (Model No. LT31). Measurement of absorption of blank DMSO was also measured with the help of a Spectrophotometer to calibrate the absorption (Barnes *et al.,* 1992). Lastly, total chlorophyll (Tchl) concentration (a, b, and total) was calculated by using the equations given by Barnes *et al.* (1992) and were expressed as mg/g fresh weight.

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

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

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

Where A665 = absorption value at 665 nm and A648 = absorption value at 648 nm

3.5.2 Leaf extract pH

5 gram of leaf sample was crushed with a motor and pestle, then 50 ml deionized water was added and filtered. The resulting suspension was then measured using a digital pH meter (LT10) (Apriyantono *et al.,* 1989)

3.5.3 Ascorbic acid (AA)

Ascorbic acid content was determined according to the method of Bajaj and Kaur (1981). In this method 1 g of fresh leaf, was taken and then 4 ml of the oxalic acid EDTA extraction solution was added which was then mixed with 1 ml of orthophosphoric acid, 1 ml of 5% tetraoxosulphate acid, 2 ml of ammonium molybdate, and 3 ml of water. The resultant solution was allowed to remain still for 15 minutes and then absorbance at 760 nm with a spectrophotometer (Model No. LT 31). Ascorbic acid concentrations (mg/g) in the sample were then calculated using the absorbance of a standard ascorbic acid curve.

3.5.4 Estimation of relative water content (RWC)

Relative water content (RWC) is the total water content in a given leaf relative to its fully turgid or hydrated state (Bora and Joshi, 2014). For this the fresh weight of a composite sample of leaf was measured and then the measured sample leaves were floated in water for up to 24 hours and again their weight was measured. Then these leaves was dried in oven to a consistent weight at around 85°C for 24 hours and reweighted. Relative Water Content (RWC) was determined by using the following formulae given by (Barrs and Weatherly, 1962)

Relative water content (%) = $\{(F-D) / (T-D)\} \times 100$

F = Fresh weight of leaves (g)

D = Dry weight of leaves (g)

T = Turgid weight of leaves (g)

3.5.5 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.

3.5.6 Air pollution tolerance index (APTI) categories

APTI value of tree species were grouped into different categories on the basis of their tolerance level according to Thakar and Mishra (2010) and Padmavathi *et al.* (2013). Based on APTI mean value of all species and their standard deviation, Thakar and Mishra (2010) Categorized APTI into the following four types.

a) Tree species APTI higher than mean APTI + SD =Tolerant

b) Tree species APTI value between mean APTI and mean APTI + SD = Moderatel tolerant

c) Tree species APTI value between mean APTI-SD and mean APTI = Intermediate

d) Tree species APTI lower than the mean APTI - SD = Sensitive

Padmavathi *et al.* (2013) approached only APTI values of tree species and categorized as below.

a) APTI value above 17 = Tolerant

b) APTI between 12 and 16 = Intermediate

c) Less than 12 = Sensitive

3.6 Metal analysis

3.6.1 Sample preparation

The collected leaf samples were cleaned in dry condition using fine brush, and then the leaves were washed with running tap water; rinsed with double deionized water and then dried at room temperature (20°C) about 24-48 hours, and then again dried at 60^{o}C for 48 hours. The oven dried leaf samples were then crushed using motor and pestle and ultimately a representative sample of each species for each site was obtained (Chapman and Pratt, 1961).

3.6.2 Metal analysis

For metal analysis, representative sample (0.5 g) was taken and wet digested using 10 ml of diacid (HCl: HNO_3) mixture. The specimen and the 5 ml mixture of diacid was added and heated gentle to 95±5°C without allowing to boil at covered condition initially and then 5 ml dicarboxylic acid was added and further heated at 180°C for 2-3 hours until white effervescence appeared. The digested aliquot was then cooled and filtered through Whatman filter paper no. 41 in a 100 ml volumetric flask and ultimately volume of 100 ml was adjusted by adding distilled water up to the mark. The heavy metal concentration in an aliquot was determined using an atomic absorption spectrophotometer. For quality control, the blank without plant materials was run in parallel following the same procedure (U.S. EPA, 1996). The metal analysis following this procedure was conducted at the laboratory of Nepal Environmental and Scientific Services at Thapathali, Kathmandu.

3.6.3 Metal accumulation index (MAI)

Metal accumulation index (MAI) of the each plant species was calculated by the formula given by Liu *et al.* (2007).

$MAI = (1\ N)\ \sum N\ j = 1Ij$

where N is the total number of metals analyzed.

$Ij = x\ \delta x$is the sub-index for variable j, obtained by dividing the mean value (x) of each metal by its standard deviation.

3.7 Statistical analysis

To evaluate the significant differences in SLA between polluted and control sites one sample t-test was conducted using SPSS program version 25. The data obtained from micro morphological study between polluted and control sites were compared, and increase or decrease percentages of the investigated parameters at polluted sites were analyzed statistically using one sample t-test. To understand the significant differences of various biochemical parameters (for the APTI) and heavy metals (Zn, Cu, and Pb) concentrations among the study sites of the same species, the data obtained were analyzed statistically using one-way ANOVA followed by Duncan’s multiple range test. Data obtained from the biochemical measurements (for APTI) and of different heavy metals concentration (Zn, Cu and Pb) were further subjected to Pearson’s correlations test to access the relationship between biochemical parameters and heavy metal content in leaves; the% decrease in SLA and metal content in leaves; and percentage (%)decrease in SLA and and biochemical parameters of APTI.

CHAPTER 4

4 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Leaf morphology

4.1.1.1 Macro morphological character

4.1.1.1. a Leaf area

Leaf area was found to be reduced in all the studied tree leaves at polluted sites in comparison to less polluted sites (Table 3). Leaf area ranged from 4.60 cm^2 to 202.42 cm^2 at less polluted site and ranged from 3.83 cm^2 to 137.19 cm^2 at polluted sites**.** Maximum decrease in leaf area was observed at polluted sites in *Celtis australis* (18.75%) and minimum decreased was recorded in *Ficus elastica* (2.16%). Based on one sample t-test, significant decrease in leaf area at polluted sites was observed in all studied trees except *Celtis australis.*

Table 3: Leaf area of different trees species at polluted sites and less polluted sites and t-values obtained from % decrease in leaf area characters

Plant species	Polluted site (P) Mean±Sd cm^2	Less polluted site (LP) Mean±Sd cm^2	% Decrease Mean±Sd	t-value
Callistemon lanceolatus	3.83±0.42	4.60±0.34	16.73±1.33	7.79*
Celtis australis	27.96±0.88	32.46±0.57	18.75±10.25	3.16
Cinnamomum camphora	22.34±3.47	29.45±2.08	24.14±2.34	5.71*
Eucalyptus globulus	31.25±1.37	32.65±1.43	4.54±1.25	6.99*
Ficus elastica	90.31±15.46	107.33±9.58	12.16±3.49	5.85*
Ficus reliogisa	137.19±5.47	202.42±84.61	34.87±0.44	19.19**
Grevillea robusta	10.21±0.91	13.10±1.50	21.67±2.46	6.81*
Jacaranda mimosifolia	7.98±3.76	10.14±6.22	21.17±6.97	2.61
Populus euramericana	58.97±8.02	105.33±4.92	44.66±0.94	8.63*
Prunus cerasoides	44.17±3.46	57.28±4.92	26.11±0.89	11.91**

t value marked by *indicates $p<0.05$ and ** indicates $p<0.01$ obtained from one sample t-test (for % decreased leaf area data for each tree species with N value ranging from 15 to 21)

4.1.1.1.b Specific leaf area (SLA)

Specific leaf area was found to be decreased in all the studied tree leaves at polluted areas in comparison to less polluted areas (Table 4). SLA values in the tree leaves in this study varied from 24.48 to 188 cm^2 g^{-1}. SLA decreased from 3.27 (in *Ficus elastica* at Chabhil) to 78.18% at polluted sites (in *Callistemon lanceolatus* at Dhumbarahi). Although all tree species showed a reduction in SLA at polluted sites, but even found significant at some polluted sites. For instance, *Grevillea robusta* at Lainchaur and Basundhara; *Jacaranda mimosifolia* at Basundhara, Lainchaur, and Swayambhu; *Celtis australis* was significantly reduced at Dhumbarahi and Airport*;* *Eucalyptus globulus* at Airport and Balaju; *Ficus religiosa* at Airport, Basundhara, Dhumbarahi, and Balaju, and *Ficus elastica* at Tinkune and Samakhusi. The leaves of *Callistemon lanceolatus* showed significant % decrease in SLA at all polluted sites.

Table 4: SLA (cm^2g-^{1}) and % decrease in SLA of different tree species growing along the road side in various polluted (P) and less polluted sites (LP) in Kathmandu

Plants	**Polluted (P) and less polluted site (LP)**	**SLA (Cm^2 g^{-1}) Mean±Sd**	**% Decrease in SLA mean± Sd**	**t-value**
Callistemon lanceolatus (SW.) DC	Airport (P)	61.79±22.33	40.84±30.55	4.793*
	Balaju (P)	35.60±13.45	31.37±7.47	4.584*
	Dhumbarahi (P)	24.48±0.87	78.18±4.47	48.605**
	Swayambhu (P)	69.31±5.66	21.05±9.11	21.211**
	Narayanthan (LP)	116.1±28.74		
Celtis australis L.	Airport (P)	139.69±22.33	11.94±0.36	56.79***
	Balaju (P)	140.37±10.68	11.54±5.93	3.37
	Basundhara (P)	132.94±12.97	16.18±8.22	3.41
	Dhumbarahi (P)	141.82±2.82	10.57±2.83	6.46*
	Narayanthan	158.63±2.90		
Cinnamomum camphora (L.) J. Presl	Airport (P)	104.54±25.05	32.94±22.68	2.514
	Balaju (P)	99.74±0.08	16.90±7.52	3.891
	Basundhara (P)	88.74±9.58	32.72±22.63	2.504
	Dhumbarahi (P)	91.11±14.18	10.90±10.83	1.743
	Naraynthan (LP)	139.44±37.04		
Eucalyptus globulus Labill.	Airport (P)	130.59±7.10	14.69±3.19	7.97**
	Balaju (P)	131.54±7.68	14.07±3.53	6.90*
	Dhumbarahi (P)	127.76±25.39	16.80±13.83	2.10
	Swyambhu (P)	132.00±6.62	13.76±2.93	8.12

	Narayanthan (LP)	153.06±5.16		
Ficus elastica Roxb. ex Hornem	Airport (P)	40.96±0.35	4.10±1.94	3.65
	Chabhil (P)	41.30±0.38	3.27±3.15	1.80
	Samakhusi (P)	40.75±0.82	4.60±1.71	4.65*
	Tinkune (P)	39.86±11.8	6.71±1.11	10.44**
	Narayanthan	42.73±1.18		
Ficus religiosa L.	Airport (P)	61.12±6.81	53.87±7.59	12.289**
	Balaju (P)	69.31±9.00	47.31±1.79	45.790**
	Basundhara (P)	81.85±6.35	34.02±14.07	4.189*
	Dhumbarahi (P)	99.13±17.38	25.21±2.90	15.069**
	Narayanthan (LP)	132.70±23.55		
Grevillea robusta A cum ex R. Br.	Airport (P)	80.08±25.83	28.98±19.05	2.634
	Balaju (P)	61.55±31.94	48.20±26.83	3.111
	Basundhara (P)	101.28±6.94	13.38±1.70	13.638**
	Dhumbarahi (P)	72.12±6.03	27.48±13.71	3.471
	Lainchaur (P)	84.12±17.64	25.69±10.48	4.245
	Narayanthan (LP)	114.58±29.75		
Jacaranda mimosifolia D. Don	Airport (P)	128.52±4.74	23.77±14.07	2.925
	Basundhara (P)	74.22±17.06	41.83±14.73	4.91*
	Dhumbarahi (P)	112.47±86.85	40.94±44.98	1.576
	Lainchaur (P)	164.08±36.56	13.00±5.66	3.974*
	Swyambhu (P)	41.77±8.67	73.57±1.26	101.335**
	Narayanthan (LP)	188.08±35.12		
Populous euramericana L.	Airport (P)	112.73±20.60	20.69±7.68	4.668*
	Basundhara (P)	114.90±34.87	9.32±4.80	3.363
	Chabhil (P)	93.50±31.32	27.93±16.78	2.883
	Dhumbarahi (P)	61.03±12.33	50.67±10.11	8.685*
	samakhusi (P)	89.92±37.40	34.80±21.99	2.740
	Tinkune (P)	78.48±19.66	38.99±13.47	5.015*
	Narayanthan (LP)	131.07±32.21		
Prunus cerasoides D. Don	Airport (P)	173.25±6.31	18.40±10.48	3.04
	Balaju (P)	175.26±24.62	17.13±13.10	2.27
	Basundhara (P)	178.17±24.75	16.14±9.05	3.09
	Dhumbarahi (P)	187.70±20.62	11.45±9.06	2.19
	Narayanthan (LP)	212.00±8.31		

t value marked by indicates $*p<0.05$, $**p<0.01$,$***p<0.001$ obtained from one sample t-test (for % decreased specific leaf area data for each tree species with N value ranging from 15 to 21)

4.1.1.2 Micro morphological characters

4.1.1.2.a Stomata density

Stomata density was found to increased in all the studied tree leaves in polluted areas when compared to less polluted sites and ranged from 122.19 per cm^2 (in *Grevillea robusta*) to 231.98 per cm^2 (in *Eucalyptus globulus*). Stomata density ranged from 110.74 to 219.01 per cm^2 at the less polluted site in them. Increase in stomata density at polluted sites ranged from 2.5% (in *Grevillea robusta*) to 31.31% (in *Celtis australis*). At polluted sites, all the studied tree species showed an increase in stomata density and was found to be significant ($p<0.05$) in all tree species except *Eucalyptus globulus*. (Table 5).

Table 5: Percentage increase in number of stomata per cm^2 in different trees species at polluted sites and t-values obtained from one sample t-test

Plant species	Polluted site (P) Mean±Sd (µm)	Less polluted site (LP) Mean±Sd (µm)	% Increase Mean±Sd	t-value
Callistemon lanceolatus	137.2±1.8	124.40±2.82	9.56±0.73	6.31*
Celtis australis	126.91±1.45	113.20±4.59	31.31±2.87	16.53*
Cinnamomum camphora	155.04±3.95	124.36±5.99	18.04±1.33	12.6**
Eucalyptus globulus	231.98±1.84	219.01±3.20	8.51±5.42	3.85
Ficus elastica	154.72±4.14	124.31±3.28	25.94±1.07	8.85*
Ficus reliogisa	154.02±2.62	120.36±9.79	14.43±4.26	8.16*
Grevillea robusta	122.19±1.29	110.74±5.80	2.5±1.72	14.72**
Populus euramericana	223.97±8.29	181.87±2.96	24.98±1.85	8.74*
Prunus cerasoides	188.34±6.67	159.84±8.14	16.01±1.53	20.0**

t value marked by indicates *$p<0.05$, **$p<0.01$,***$p<0.001$ obtained from one sample t-test (for % increased stomatal density data for each tree species with N value ranging from 15 to 21)

4.1.1.2. b Size of stomata

Length and width of stomata were reduced at polluted areas than at less polluted sites. Length of stomata ranged from 14.17 (in *Callistemon lanceolatus*) to 26.82 µm (in *Ficus reliogisa*) in less pollute site and it ranged from 11.45 (in *Callistemon lanceolatus*) to 25.44 µm (in *Ficus religiosa*) at polluted sites. The length of stomata reduced from 5.10 (in *Ficus religiosa*) to 23.79% (*Populus euramericana*) at polluted sites (Table 4). Similarly, breadth of stomata ranged from 9.79 (in *Cinnamomum camphora*) to 15.08 µm (in *Eucalyptus globulus*) in less polluted site and it ranged from 7.95 (in *Cinnamomum camphora*) to 13.23 µm (in *Ficus religiosa*). The breadth of stomata reduced from 8.46 (in *Ficus religiosa*) to 29.43% (in *Prunus cerasoides*) at polluted sites (Table 5). Minimum reduction in length (5.10%) and breadth (8.46%) of stomata was observed in *Ficus religiosa.* Maximum reduction in length (26.79%) was observed in *Celtis australis* and maximum reduction in breadth (29.43%) was observed in *Prunus cerasoides*. Both length and breadth of stomata reduced significantly in most of the species studied (Tables 6 and 7).

Table 6: Length of stomata in different tree species at polluted and less polluted sites along with t-values obtained from % decreased data using one sample t-test

Plant species	Polluted site (P) Mean±Sd (µm)	Less polluted site (LP) Mean±Sd (µm)	% Decrease Mean±Sd	t-value
Callistemon lanceolatus	11.45±0.80	14.17±1.03	16.88±5.36	5.45*
Celtis australis	17.16±0.44	23.15±1.35	26.79±0.91	50.83***
Cinnamomum camphora	14.31±3.79	23.31±2.97	10.66±2.27	8.13*
Eucalyptus globulus	20.29±1.36	24.17±0.75	14.71±1.34	18.97**
Ficus elastica	17.33±0.66	19.81±0.44	13.66±2.62	9.03*
Ficus reliogisa	25.44±0.66	26.82±0.22	5.10±0.88	10.04*
Grevillea robusta	22.95±1.44	24.57±1.26	7.65±2.62	5.05*
Populus euramericana	14.03±1.47	18.70±1.76	23.79±1.64	25.00**
Prunus cerasoides	14.96±0.27	18.51±0.58	20.63±1.90	18.73**

t value marked by indicates **p<0.05, **p<0.01,***p<0.001* obtained from one sample t-test (for % decreased length of stomata data for each tree species with N value ranging from 15 to 21)

Table 7: Breadth of stomata in different tree species at polluted and less polluted sites along with t-values obtained from % decreased data using one sample t-test

Plant species	Polluted site (p) Mean±Sd (µm)	Less polluted site (LP) Mean±Sd (µm)	% Decrease Mean±Sd	t-value
Callistemon lanceolatus	8.51±0.77	11.02±0.59	21.30±1.27	29.06**
Celtis australis	8.47±0.47	9.94±0.06	14.68±1.82	13.98*
Cinnamomum camphora	7.95±0.76	9.79±0.25	14.89±5.22	4.94*
Eucalyptus globulus	11.85±0.55	15.08±0.39	20.43±0.92	38.31**
Ficus elastica	8.95.21	11.15±0.34	19.48±1.20	28.07**
Ficus reliogisa	13.23±0.68	14.02±0.72	8.46±5.15	2.85
Grevillea robusta	10.98±0.25	14.10±0.31	23.64±2.82	14.51*
Populus euramericana	8.73±0.17	12.06±0.57	26.92±1.91	24.46*
Prunus cerasoides	8.57±0.51	12.41±0.41	29.43±1.98	25.71*

t value marked by indicates **p<0.05 and **p<0.01,***p<0.001* obtained from one sample t-test (for % decreased in breadth of stomata data for each tree species with N value ranging from 15 to 21

4.1.1.2. c Thickness of cuticle

Among the studied tree leaves, highest cuticle thickness was observed in *Callistemon lanceolatus* (11.22 µm) and lowest was observed in *Prunus cerasoides* (6.23 µm) at less polluted site. The cuticle thickness was found to be highly reduced at polluted sites and ranged from 4.24 µm in *Cinnamomum camphora* to 7.83 µm in *Eucalyptus globulus***.** Maximum decrease in cuticle thickness (36.07%) at polluted sites was observed in *Callistemon lanceolatus* and minimum (7.30%) in *Populus euramericana.* Cuticle thickness reduced significantly ($p<0.05$) in all the studied tree species at polluted sites (Table 8).

Table 8: Percentage reduction in cuticle thickness of different trees at polluted areas and t-values obtained from one-sample t-test

Plant species	**Pollute site (P) Mean±Sd (µm)**	**Less polluted site (LP) Mean±Sd (µm)**	**% Decrease Mean±Sd**	**t-value**
Callistemon lanceolatus	6.94±1.74	11.22±1.66	36.07±1.83	1.84**
Celtis australis	4.79±0.56	6.86±0.34	27.23±3.51	3.51**
Cinnamomum camphora	4.24±0.86	6.39±1.05	30.50±6.90	6.91*
Eucalyptus globulus	7.83±0.39	10.72±0.52	27.51±0.51	0.51***
Ficus elastica	7.05±0.22	9.78±0.38	28.85±1.40	1.40**
Ficus reliogisa	5.72±1.44	6.78±1.53	16.34±2.32	2.32**
Grevillea robusta	5.77±0.34	7.11±0.87	18.98±3.23	3.24*
Populus euramericana	5.45±0.42	7.21±0.38	24.41±2.53	2.54**
Prunus cerasoides	4.82±0.42	6.23±0.58	21.97±1.47	1.47**

t value marked by indicates $*p<0.05$, $**p<0.01$,$***p<0.001$ obtained from one sample t-test (for % decreased in cuticle thickness for each tree species with N value ranging from 15 to 21)

4.1.1.2. d Thickness of epidermis

Thickness of epidermis ranged from 13.06 µm in *Celtis australis* to 28.98 µm in *Ficus religiosa* at less polluted site. Thickness of epidermis was found to be reduced in all the tree species at the polluted sites and ranged from 10.84 µm in *Celtis australis* to 26.96 µm in *Ficus religiosa*. Maximum decrease in epidermis (18.98%) at polluted sites was observed in *Grevillea robusta* and minimum decreased in *Ficus reliogisa* (7.79%). One sample t-test showed significant decrease in epidermis at polluted sites in the tree species (Table 9).

Table 9: Percentage decrease in epidermis thickness (µm) of different trees species at polluted sites and t-values obtained from one sample t-test

Plant species	Polluted site (P) Mean±Sd (µm)	Lesspolluted site (LP) Mean±Sd (µm)	% Decrease Mean±Sd	t-value
Callistemon lanceolatus	18.15±2.78	22.34±1.92	15.41±4.18	6.38*
Celtis australis	10.84±0.44	13.06±0.65	17.47±0.78	38.44**
Cinnamomum camphora	16.54±0.55	19.17±0.89	12.86±1.92	11.55**
Eucalyptus globulus	15.16±0.53	17.48±0.78	14.08±0.78	31.05**
Ficus elastica	16.71±0.41	19.92±0.67	16.49±0.68	41.87**
Ficus reliogisa	26.96±0.55	28.98±0.69	7.79±1.74	7.73*
Grevillea robusta	15.14±1.79	18.82±1.21	18.98±2.76	11.89**
Populus euramericana	19.77±0.42	23.77±0.43	16.18±1.27	22.04**
Prunus cerasoides	19.93±1.18	22.96±0.94	13.85±1.42	16.80**

t value marked by indicates *$*p<0.05$ and $**p<0.01$* obtained from one sample t-test (for % decreased in thickness of epidermis for each tree species with N value ranging from 15 to 21)

4.1.2 Air pollution tolerance index (APTI)

4.1.2.1 Total chlorophyll content (Tchl)

4.1.2.1. a Total chlorophyll in angiosperm

Among the studied angiosperm tree species total chlorophyll (Tchl) ranged from 0.11 to 1.65 mg/g (Table 10). In *Callistemon lanceolatus*, total chlorophyll content ranged from 0.14 to 0.74 mg/g (at Airport and Gongbu, respectively), in *Cinnamomum camphora* from 0.11 to 0.72 mg/g (at Airport and Basundhara, respectively) and in *Ficus religiosa* from 0.2 to 1.17 mg/g (at Airport and Narayanthan, respectively). Similarly, in *Grevillea robusta* it ranged from 0.42 to 1.14 mg/g (at Balaju and Basundhara, respectively), in *Jacaranda mimosifolia* from 0.31 to 1.25 mg/g (at Airport and Basundhara, respectively) and in *Populus euramericana* from 0.58 to 1.4 mg/g (at Airport and Basundhara, respectively). Except for *Ficus religiosa*, total chlorophyll (Tch) content was found to be significantly higher in all studied tree species in some polluted areas compared to less polluted sites. in all tree species studied. Total chlorophyll content (Tch) in all the studied plants, except *Grevillea robusta*, was lowest in Airport. Highest total chlorophyll content (Tchl) content in *Cinnamomum camphora, Grevillea robusta* and *Populus euramericana* was recorded in Basundhara, a polluted site. Similarly, highest total chlorophyll content (Tch) content in *Callistemon lanceolatus* and *Jacaranda mimosifolia* was also recorded in polluted sites, Gongobu and Balaju, respectively.

4.1.2.1.b Total chlorophyll in gymnosperm

Total chlorophyll content (Tchl) content among the studied gymnosperms plant species ranged from 0.59 to 3.73 mg/g (Table 11). In *Pinus roxburghii* total chlorophyll content ranged from 0.62 to 3.73 mg/g (at Ratnapark and Dhumbarahi, respectively), in *Thuja orientalis* from 0.59 to 3.66 mg/g (at Shankhpark and Jawalakhel, respectively) and in *Cedrus deodara* from 0.67 to 3.47 mg/g (at Ratnapark and Shankhpark, respectively). Similarly in *Araucaria araucana* it ranged from 0.59 to 3.59 mg/g (at Ratnapark and Narayanthan, respectively). In all the studied common gymnosperms tree species, Total chlorophyll content was observed mostly higher in polluted sites, but at some polluted sites it was found to be reduced *Pinus roxburghii, Thuja orientalis, Cedrus deodara, and Araucaria araucana*. Tch level in *Pinus roxburghii* at Ratnapark and Shankhapark decreased significantly ($p=0.05$). Similarly, Tch concentration in *Thuja orientalis* was significantly reduced ($p=0.05$) at Shankhpark and Airport. In *Cedurus deodara* it was found to be significantly lower ($p=0.05$) at Ratnapark, and Jawalakhel while in *Araucana araucana* it was found to be lower at all sites than at a less polluted Narayanthan, a reference site.

4.1.2.2 Relative water content (RWC)

4.1.2.2. a RWC in angiosperm

RWC among the studied plants ranged from 36.93 to 96.33% (Table 10). In *Callistemon lanceolatus*, RWC ranged from 57.63 at Balaju to 93.33% at Dhumbarahi, in *Cinnamomum camphora* from 60.77 at Airport to 92.37% at Dhumbarahi, and in *Ficus religiosa* from 56.21 at Airport to 96.33% at Balaju. Similarly in *Grevillea robusta* it ranged from 36.93 at Gongbu to 72.71% at Narayanthan, in *Jacaranda mimosifolia* from 40.44 at Narayanthan to 86.09% at Banasthali, and in *Populus euramericana it ranged* from 60.03 at Gongbu to 89.65% at Dhumbarahi. Almost all studied plants, except *Grevillea robusta,* recorded more than 80% RWC at some polluted sites. In *Jacaranda mimosifolia,* RWC was significantly higher in all polluted sites as comparasion to less polluted site, but in other species this trend was not observed and RWC was found to be reduced at some polluted sites only.

4.1.2.2.b RWC in gymnosperm

Relative water content (RWC) among the studied gymnosperms ranged from 52.89 to 88.29% (Table 11). In *Pinus roxburghii* RWC ranged from 61.36 to 85.71% (at Narayanthan and Dhumbarahi, respectively), in *Thuja orientalis* it ranged from 60.20 to 81.79% (at Narayanthan and Airport, respectively) and in *Cedrus deodara* from 66.40 to 85.43% (at Narayanthan and Ratnapark, respectively). Likewise, *in Araucaria araucana* it varied from 52.89 to 88.29% at Narayanthan and Ratnapark, respectively. RWC was found significantly higher (p=0.05) in all polluted sites as compared to less polluted site, Narayanthan. Dhumbarahi and Airport had the maximum RWC for *Pinus roxburghii* and *Thuja orientalis,* respectively. However, the highest RWC for both *Cedrus deodara* and *Araucaria araucana* was recorded at Ratnapark.

4.1.2.3 Leaf extract pH

4.1.2.3.a Leaf extract pH in angiosperm

The leaf extract pH among the studied angiosperm tree species ranged from 3.53 to 6.82 (Table 10). In *Callistemon lanceolatus*, pH ranged from 3.58 to 6.45 at Airport and Dhumbarahi, in *Cinnamomum camphora* from 4.66 to 6.82 at Gongbu and Narayanthan and in *Ficus reliogisa* from 5.04 to 6.82 at Banasthali and Balaju, respectively. Similarly in *Grevillea robusta* it ranged from 5.78 to 6.76 (at Bashundhara and Narayanthan, respectively), in *Jacaranda mimosifolia* from 3.53 to 6.48 (at Airport and Bashundhara, respectively) and in *Populus euramericana* from 5.18 to 6.68 (at Gongbu and Narayanthan, respectively). The leaf extract pH of *Cinnamomum camphora, Ficus reliogisa, Grevillea robusta* and *Populus euramericana* reduced significantly (*p=0.05*) significantly in most of the polluted sites as comparasion to less polluted site, Narayanthan. In *Callistemon lanceolatus* the highest leaf extract pH was found in Dhumbarahi (6.45) and lowest in Airport (3.53). In *Jacaranda mimosifolia* the highest leaf extract pH was recorded in Basundhara (6.48) and lowest in Airport (3.53). pH range in *Callistemon lanceolatus* and *Jacaranda mimosifolia* at polluted and less polluted site were wide ranging from 3.53 to 6.48 in leaf extract, but in *Cinnamomum camphora* it was comparatively moderate and ranged from 4.66 to 6.82. pH range in *Ficus reliogisa, Grevillea robusta* and *Populus euramericana* were comparatively narrow and ranged from 5.04 to 6.82

among polluted and less polluted sites. This indicated that the species like *Ficus religiosa*, *Grevillea robusta* and *Populus euramericana* are less susceptible to air pollutants.

4.1.2.3.b Leaf extract pH in gymnosperm

pH of leaf extract among the studied gymnosperms ranged from 4.10 to 6.56 (Table 11). In *Pinus roxburghii* pH ranged from 4.29 to 6.21 (at Narayanthan and Airport, respectively), in *Thuja orientalis* from 5.30 to 6.56 (at Dhumbarahi and Ratnapark, respectively) and in *Cedrus deodara* from 4.10 to 5.76 (at Airport and Ratnapark, respectively). Similarly, in *Araucaria araucana* it ranged from 5.77 at Jawalakhel to 6.35 at Airport. The pH of leaf extract in *Pinus roxburghii* was observed considerably higher (p=0.05) at all polluted sites and that of *Cedrus deodara* leaf was higher at some polluted sites (Ratnapark and Jawalakhel) only. Whereas pH leaf *extract* decreased significantly at some polluted sites, for instance in *Thuja orientalis* at Dhumbarahi and in *Araucaria araucana* at Jawalakhel.

4.1.2.4 Ascorbic acid (AA) content

4.1.2.4.a Ascorbic acid in angiosperm

Ascorbic acid (AA) content in the studied plants ranged from 2.96 to 8.86 mg/g (Table 10). In *Callistemon lanceolatus*, AA ranged from 3.78 at Basundhara to 7.62 mg/g at Narayanthan, in *Cinnamomum camphora* from 2.96 at Gongobu to 8.6 mg/g at Airport and in *Ficus religiosa* from 3.06 mg/g at Balaju to 8.38 mg/g at Narayanthan. Similarly in *Grevillea robusta* it ranged from 4.2 mg/g to 8.52 mg/g (at Balaju and Bashundhara, respectively), in *Jacaranda mimosifolia* from 3.78 mg/g to 8.64 mg/g (at Gongbu and Narayanthan, respectively), and in *Populus euramericana* from 6.74 mg/g to 8.86 mg/g (at Basundhara and Dhumbarahi, respectively). Among six tree species, two species (*Cinnamum camphora* and *Grevillea robusta*) had highest AA content at polluted sites Airport site and Basundhara whereas in other four species (*Callistemon lanceolatus, Ficus religiosa, Jacaranda mimosifolia* and *Populus euramericana*) the highest ascorbic acid content was found at a less polluted site Narayanthan. All other polluted sites (Balaju, Banasthali, Basundhara, Gongbu and Dhumbarahi) mostly had less amount of AA content than at less polluted site.

4.1.2.4.b Ascorbic acid in gymnosperm

Ascorbic acid content (AA) in the studied gymnosperns ranged from 0.24 mg/g to 0.88 mg/g (Table 11). In *Pinus roxburghii* AA ranged from 0.44 mg/g at Dhumbarahi to 0.84 mg/g at Jawalakhel, in *Thuja orientalis* from 0.30 mg/g at Narayanthan to 0.84 mg/g at Shankhpark and in *Cedrus deodara* from 0.38 mg/g (at Airport and Narayanthan), to 0.88 mg/g (at Dhumbarahi). Similarly in *Araucaria araucana* it ranged from 0.24 mg/g at Narayanthan to 0.71 mg/g at Ratnapark. The concentration of ascorbic acid in all species was higher in most polluted areas than at Narayanthan, a considerably less polluted site. Ascorbic acid concentrations in *Thuja orientalis* at all polluted sites increased significantly (p=0.05) as compared to Narayanthan, a less polluted site. Ascorbic acid concentration also increased significantly in *Pinus roxburghii* at Jawalakhal, Ratnapark and Shankhapark.

4.1.2.5 Air pollution tolerance index (APTI)

4.1.2.5.a APTI of angiosperm

APTI values obtained for different species at different sites ranged from 7.09 to 15.67 (Table 10). In *Callistemon lanceolatus*, APTI ranged from 8.66 at Banasthali to 12.55 at Dhumbarahi, in *Cinnamomum camphora* from 8.03 at Gongbu to 12.51 at Dhumbarahi and in *Ficus religiosa* from 8.1 at Banasthali to 13.97 at Narayanthan. Similarly, in *Grevillea robusta* it ranged from 7.09 at Gongbu to 13.08 at Airport, in *Jacaranda mimosifolia* from 8.21 at Airport to 14.61 at Dhumbarahi, and in *Populus euramericana* from 10.94 at Gongbu to 15.67 at Dhumbarahi. Roadside trees showed different APTI categories at different sites of Kathmandu valley. Based on the maximum APTI values in each species recorded, the tree species from lowest to highest were in the order *Cinnamomum camphora*>*Callistemon lanceolatus*> *Grevillea robusta*>*Ficus religiosa*>*Jacaranda mimosifolia*>*Populus euramericana* and their respective highest APTI values were 12.51>12.55>13.08>13.97>14.61> 15.67. The APTI value obtained for each site was categorized according to Thakar and Mishra (2010) (Table 9). Among the studied road side trees highest APTI categories, i.e., tolerant (T) were observed at only one site in *Callistemon lanceolatus* (at Dhumbarahi), *Cinnamomum camphora* (at Dhumbarahi), *Ficus religiosa* (at Narayanthan), *Grevillea robusta* (at Airport), and *Jacaranda mimosifolia* (at

Dhumbarahi), whereas in *Populus euramericana* tolerant category was recorded at five sites (at Airport, Balaju, Bansthali, Dhumbarahi and Narayanthan).

Table 10: Physiological parameters (mean±sd, n=25-30) to determine Air pollution tolerance index (APTI) in some common road side angiosperm trees collected from different sites of Kathmandu and their APTI categories

Plants	**Sites**	**Tchl±Sd (mg/g)**	**RWC (%)**	**pH**	**AA (mg/g)**	**APTI**	**APTI categories (Thakar and Mishra, 2010)**	**APTI categories (Padmavathi *et al.*, 2013)**
Callistemon lanceolatus								
	Airport	0.14±0.02 A	70.83±10.89 BC	3.58±0.02 A	7.54±0.79 C	9.89±1.15 B	Intermediate tolerant	Sensitive
	Balaju	0.48±0.05 B	57.63±8.52 A	5.79±0.01 C	5.42±2.12 AB	9.17±1.19 AB	Sensitive	Sensitive
	Banasthali	0.61±0.10 C	62.92±11.07 AB	4.57±0.50 B	4.38±2.25 AB	8.66±2.40 AB	Sensitive	Sensitive
	Basundhara	0.61±0.10 C	62.17±10.18 AB	6.25±0.03 D	3.78±0.37 A	12.08±0.19 C	Moderately tolerant	Intermediate tolerant
	Dhumbarahi	0.61±0.08 C	93.33±4.01 E	6.45±0.21 D	4.58±1.19 AB	12.55±0.78 C	Tolerant	Intermediate tolerant
	Gongbu	0.74±0.04 D	86.65±3.82 DE	6.14±0.03 D	4.28±0.27 AB	11.61±0.50 C	Moderately Tolerant	Intermediate tolerant
	Mean APTI (Polluted sites)					10.66		
	Narayanthan	0.68±0.04 CD	75.4±8.31 C	5.57±0.34 C	7.62±1.69 C	12.26±0.66 C	Moderately tolerant	Intermediate tolerant
Cinnamomum camphora								
	Airport	0.11±0.04 A	60.77±4.64 A	5.71±0.02 B	8.6±0.53 D	11.09±0.60 BC	Moderately tolerant	Intermediate tolerant
	Balaju	0.54±0.06 B	80.85±6.6 B	5.98±0.03 CD	3.04±0.32 A	10.07±0.83 B	Intermediate tolerant	Sensitive
	Banasthali	0.66±0.02 CD	70.45±4.59 A	5.9±0.02 C	5.28±0.63 C	10.51±0.84 B	Intermediate tolerant	Sensitive
	Basundhara	0.72±0.11 D	83.15±13.60 BC	6.62±0.08 E	3.78±0.96 AB	10.87±1.35 BC	Intermediate tolerant	Sensitive
	Dhumbarahi	0.57±0.14 BC	92.37±2.65 C	6.62±0.08 E	4.56±1.04 BC	12.51±0.74 D	Tolerant	Intermediate tolerant
	Gongbu	0.2±0.05 A	66±4.66 A	4.66±0.03 A	2.96±0.11 A	8.03±0.41 A	Sensitive	Sensitive
	Mean APTI (Polluted sites)					10.51		
	Narayanthan	0.67±0.07 CD	86.08±9.32 BC	6.82±0.13 F	4.56±0.84 BC	12.03±1.20 CD	Moderately tolerant	Intermediate tolerant
Ficus religiosa								
	Airport	0.2±0.09 A	56.21±6.70 A	5.94±0.01 D	8.38±0.34 C	10.77±0.65 BC	Intermediate tolerant	Sensitive
	Balaju	0.79±0.37 B	96.33±2.13 D	6.82±0.02 F	3.06±0.23 A	11.96±0.35 C	Moderately tolerant	Intermediate tolerant
	Banasthali	0.4±0.09A	62.92±23.7 AB	5.04±0.03 B	3.32±0.35 A	8.1±2.35 A	Sensitive	Sensitive
	Basundhara	0.81±0.32 B	75.8±18.13	6.37±0.0	3.96±0.65	10.43±1.35	Intermediat	Sensitive

		B	5 B	A	A	e tolerant	
Dhumbarahi	0.71±0.02 B	94.35±0.77 C D	6.63±0.03 E	3.7±0.80 A	12.15±0.62 C	Moderately tolerant	Intermediate tolerant
Gongbu	0.8±0.01 B	69.13±9.53 AB	5.63±0.06 C	5.22±0.74 B	10.27±1.26 B	Intermediate tolerant	Sensitive
Mean APTI (Polluted sites)					10.61		
Narayanthan	1.17±0.11 C	73.08±6.47 BC	6.78±0.09 F	8.38±0.65 C	13.97±0.54 D	Tolerant	Intermediate tolerant
Grevillea robusta							
Airport	1.12±0.01 E	69.21±7.08 C	6.24±0.03 C	7.28±0.73 C	13.08±0.61 C	Tolerant	Intermediate tolerant
Balaju	0.42±0.01 A	43.95±2.53 AB	6.02±0.03 B	4.2±1.60 AB	7.1±1.15 A	Sensitive	Sensitive
Banasthali	1.13±0.02 E	69.21±7.08 CD	6.56±0.04 D	5.34±2.21 B	11.03±2.07 B	Moderately tolerant	Intermediate tolerant
Basundhara	1.14±0.02 E	47.78±3.17 B	5.78±0.05 A	8.52±00.40 C	10.67±0.33 B	Intermediate tolerant	Sensitive
Dhumbarahi	0.6±0.05 B	75.19±4.06 D	6.08±0.02 B	5.04±0.74 B	10.88±0.73 B	Intermediate tolerant	Sensitive
Gongbu	0.79±0.03 C	36.93±10.47 A	6.08±0.01 B	4.94±0.34 AB	7.09±1.02 A	Sensitive	Sensitive
Mean APTI (Polluted sites)					9.97		
Narayanthan	1.03±0.05 D	72.71±13.26 CD	6.76±0.12 E	5.54±0.32B	11.59±1.23 BC	Moderately tolerant	Intermediate tolerant
Jacaranda mimosifolia							
Airport	0.31±0.08 A	57.25±2.72 B	3.53±0.03 A	6.28±2.06 BC	8.21±0.82 A	Sensitive	Sensitive
Balaju	1.65±0.00 E	58.59±14.11 B	5.34±0.13 D	6.52±1.68 BC	12.32±1.74 BC	Moderately tolerant	Intermediate tolerant
Banasthali	0.93±0.35 BC	86.09±1.52 C	4.9±0.25 B	4.95±1.74 AB	8.65±2.57 A	Sensitive	Sensitive
Basundhara	1.25±0.21 D	58.96±13.09 B	6.48±0.05 F	7.86±0.20 CDE	12.01±1.20 BC	Moderately tolerant	Sensitive
Dhumbarahi	0.93±0.11 BC	85.76±5.50 C	6.15±0.03 E	8.36±0.58 DE	14.61±0.30 D	Tolerant	Intermediate tolerant
Gongbu	1.12±0.059 B	85.843±3.45 C	4.76±0.03 B	3.78±0.38 A	10.79±0.52 B	Intermediate tolerant	Sensitive
Mean APTI (Polluted sites)						11.09	
Narayanthan	0.79±0.07 A	40.44±2.77 A	5.14±0.01 C	8.64±0.35 E	9.02±0.24 A	Sensitive	Sensitive
Populus euramericana							
Airport	0.58±0.07 A	73.9±5.46 BC	6.24±0.03 B	8.35±0.51 B	13.09±0.77 BC	Tolerant	Intermediate tolerant
Balaju	0.71±0.02 AB	74.03±3.05 BC	6.32±0.18 BC	8.32±0.3 B	13.26±0.56 BC	Tolerant	Intermediate tolerant
Banasthali	0.71±0.02AB	71.6±17.43 ABC	6.42±0.05 BC	8.6±0.60 B	13.54±0.61 C	Tolerant	Intermediate tolerant
Basundhara	1.4±0.08 D	66.32±6.25 AB	6.59±0.38 BC	6.74±2.01 A	11.97±1.49 AB	Moderately tolerant	Intermediate tolerant
Dhumbarahi	0.92±0.04 C	89.65±7.04 D	6.64±0.15 C	8.86±0.38 B	15.67±0.75 D	Tolerant	Intermediate tolerant
Gongbu	0.69±0.30 AB	60.03±9.81 A	5.18±0.63 A	8.35±0.67 B	10.94±1.09 A	Moderately tolerant	Sensitive
Mean APTI (Polluted sites)						13.07	

Narayanthan	0.7±0.11 AB	73.39±11.68 BC	6.68±0.10 C	8.15±0.76 B	13.36±0.97 BC	Tolerant	Intermediate tolerant
				Mean APTI±SD of all species = 10.90±1.56			

Same letter followed after mean±sd in a column for a particular species denote no significant difference at *p=0.05* by Duncan’s multiple range test followed after one way ANOVA

4.1.2.5.b APTI of gymnosperm

Air pollution Tolerance Index (APTI) in the studied gymnosperm species ranged from 5.40 to 8.96 (Table 11). In *Pinus roxburghii* APTI ranged from 6.89 at Jawalakhel to 8.94 at Dhumbaraahi, in *Thuja orientalis* from 6.34 at Narayanthan to 8.44 at Airport, and in *Cedrus deodara* from 6.66 at Airport to 8.90 at Jawalakhel. Similarly, in *Araucaria araucana* it ranged from 5.40 at Jawalakhel to 8.96 at Shankpark. APTI values were found to be greater at all polluted sites than at a less polluted site, Narayanthan. The APTI value obtained for each site was categorized according to Thakar and Mishra (2010) (Table 9). Among the studied road side trees, the plant with highest APTI considered as tolerant (T) category was observed in *Araucaria araucana* at three study sites (Airport, Ratnapark and Shanpark), in *Pinus roxburghii* at two study sites (Airport and Dhumbarahi), and in *Cedrus deodara* at one site (Jwalakhel) only. *Thuja orientalis* scored only upto moderately tolerant APTI category.

Table 11: Physiological parameters (Mean±sd, n=25-30) to determine Air pollution tolerance index in some common road side gymnosperm trees collected from different sites of Kathmandu and their APTI categories

Plants	**Sites**	**Tchl (mg/g)**	**RWC (%)**	**pH**	**AA (mg/g)**	**APTI**	**APTI Categories (Thakar and Mishra, 2010)**	**APTI categories (Padmavathi *et al.*, 2013**
Araucari araucana								
	Airport	0.81±0.13 A	86.29±4.23 C	6.35±0.14 B	0.64±0.13 B	8.86±0.69 C	Tolerant	Sensitive
	Dhumbarahi	2.75±0.35 B	85.49±5.46 C	6.26±.00 B	0.64±0.13 B	7.02±1.41 B	Intermediate Tolerant	Sensitive
	Jawalakhel	0.80±0.04 A	72.70±7.33 B	5.77±0.25 A	0.30±0.06 A	5.40±0.58 A	Sensitive	Sensitive
	Ratnapark	0.59±0.20 A	88.29±4.23 C	6.24±0.02 B	0.71±0.05 B	8.64±0.80 C	Tolerant	Sensitive
	Shankhapark	0.65±0.19 A	86.89±2.99 C	6.24±0..02 B	0.64±0.13 B	8.96±0.77 C	Tolerant	Sensitive
Mean APTI (Polluted sites)						7.77		

Narayanthan	3.59±0.61 C	52.89±2.38 A	6.33±0.18 B	0.24±0.50 A	5.57±0.51 A	Sensitive	Sensitive
Cedrus deodara							
Airport	3.12±0.46 C	72.13±3.36 AB	4.10±0.05 A	0.38±0.04 A	6.67±1.07 A	Intermediate tolerant	Sensitive
Dhumbarahi	3.23±0..41 C	79.16±6.98 CD	4.12±0.07 A	0.88±0.04 C	7.85±1.29 AB	Moderately tolerant	Sensitive
Jawalakhel	1.08±0.13 A	84.82±4.97 CD	5.66±0.37 C	0.80±0.06 B	8.90±0.80 B	Tolerant	Sensitive
Ratnapark	0.67±0.05 A	85.43±4.12 D	5.76±0.14 C	0.81±0.04 B	8.70±0.41 B	Moderately tolerant	Sensitive
Shankhapark	3.47±0.24 C	78.16±6.98 BC	4.12±0.07 A	0.88±0.04 C	7.87±1.28 AB	Moderant tolerant	Sensitive
Mean APTI (Polluted sites)					7.99		
Narayanthan	2.16±0.96 B	66.40±2.00 A	4.480±0.26 B	0.38±0.04 A	7.09±0.55 A	Intermediate tolerant	Sensitive
Pinus roxburghii							
Airport	3.53±0.21 B	83.47±8.90 CD	6.21±0.19 C	0.46±0.05 A	8.80±0.94 B	Tolerant	Sensitive
Dhumbarahi	3.73±0.61 B	85.71±5.95 D	4.63±0.12 B	0.44±0.05 A	8.94±0.57 B	Tolerant	Sensitive
Jawalakhel	3.64±0.11 B	72.71±4.743 B	6.16±0.08 C	0.84±0.08 B	6.89±0.32 A	Intermediate tolerant	Sensitive
Ratnapark	0.62±0.28 A	77.16±4.83 BC	6.16±0.08 C	0.72±0.01 B	7.50±0.80 A	Moderately tolerant	Sensitive
Shankhapark	0.68±0.47 A	77.57±5.51 BC	6.16±0.08 C	0.83±0.06 B	7.82±0.94 A	Moderately tolerant	Sensitive
Mean APTI (Polluted sites)					9.49		
Narayanthan	3.55±0.58 B	61.36±5.42 A	4.29±0.16 A	0.48±0.16 A	7.53±0.37 A	Moderately tolerant	Sensitive
Thuja orientalis							
Airport	2.37±0.12 AB	81.79±5.52 C	6.320±0.18 B	0.47±0.06 B	8.45±0.64 C	Moderately tolerant	Sensitive
Dhumbarahi	3.56±0.58 C	76.82±4.16 BC	5.30±0.28 A	0.42±0.85 B	8.09±0.46BC	Moderately tolerant	Sensitive
Jawalakhel	3.66±0.18 BC	72.39±5.18 B	6.46±0.25 B	0.71±0.14 C	8.14±0.57 BC	Moderately tolerant	Sensitive
Ratnapark	3.37±0.17 C	72.39±5.28 B	6.56±0.03 B	0.69±0.06 C	7.59±0.64ABC	Intermediate tolerant	Sensitive
Shankhapark	0.59±0.23 A	73.82±6.45 B	6.51±0.04 B	0.84±0.05 D	6.94±2.11 AB	Intermediate tolerant	Sensitive
Mean APTI (Polluted sites)					7.84		
Narayanthan	3.29±0.58 BC	60.20±1.96 A	6.52±0.05 B	0.30±0.09 A	6.34±0.54 A	Sensitive	Sensitive

Mean APTI±SD of all species = 7.69±1.039

Same letter followed after mean±sd in a column for a particular species denote no significant difference at *p*=0.05 by Duncan’s multiple range test followed after one way ANOVA

4.1.3 Metal content

4.1.3.1 Metal content in angiosperm

Heavy metal (Zn, Cu and Pb) content in the leaves of common road side angiosperm trees like *Callistemon lanceolatus, Cinnamomum camphora, Ficus reliogisa, Grevillea robusta, Jacaranda mimosifolia* and *Populus euramericana* were measured from different sites in the present study and the results obtained are given in (Table 12).

4.1.3.1.a Zinc (Zn)

Zinc is an essential element for plant growth and its mean concentrations in the studied tree leaves collected from different sites ranged from 1.27 mg/kg (in *Cinnamomum camphora*) to 132.38 mg/kg (in *Populus euramericana*). Zinc accumulation ranged from 4.22 to 43.76 mg/kg in *Callistemon lanceolatus*, 1.27 to 23.23 mg/kg in *Cinnamomum camphora*, 1.54 to 22.37 mg/kg in *Ficus reliogisa*, 12.01 to 22.093 mg/kg in *Grevillea robusta*, 9.6 to 34.98 mg/kg in *Jacaranda mimosifolia*, and 14.46 to 132.38 mg/kg in *Populus euramericana.* On the basis of maximum Zn accumulation value *Populus euramericana* and *Callistemon lanceolatus* have been found to be good accumulator of Zn. Zn accumulation in most of the plants were significantly higher in most of the polluted sites than at a less polluted site Narayanthan (Table 12).

4.1.3.1.b Copper (Cu)

Copper is an essential element for plant growth and it’s mean accumulation in the studied road side tree leaves ranged from 0.89 m/kg *in Populous euramericana* to 20.27 mg/kg *in Jacaranda mimosifolia.* similarly Cu accumulation varied from 0.93 to 7.56 mg/kg in *Callistemon lanceolatus*, 3.39 to 13.93 mg/kg in *Cinnamomum camphora,* 0.90 to 15.28 mg/kg in *Ficus reliogisa*, 2.96 to 9.29 mg/kg in *Grevillea robusta,* 2.45 to 20.27 mg/kg in *Jacaranda mimosifolia*, and in *Populus euramericana,* 0.89 to 11.93 mg/kg. On the basis of mean value *Jacaranda mimosifolia* has been found to be good accumulator of Cu. Cu accumulation in most of the plants were significantly higher (p=0.05) at most of the polluted sites except in *Ficus reliogisa* and *Cinnamomum camphora* (Table 12).

4.1.3.1.c Lead (Pb)

Lead is a non-essential element for the plants and its mean accumulation in the studied road side tree leaves ranged from 0.50 to 40.33 mg/kg. Pb accumulation ranged from 0.61 to 40.33 mg/kg. in *Callistemon lanceolatus,* 0.83 to 32.06 mg/kg in *Cinnamomum camphora,* 0.50 to 39.61 mg/kg in *Ficus reliogisa,* 4.97 to 37.8 mg/kg in *Grevillea robusta*, 1.40 to 29.37 mg/kg in *Jacaranda mimosifolia*, and in *Populus euramericana,* 0.59 to 27.51 mg/kg. On the basis of mean value *Callistemon lanceolatus* and *Ficus reliogisa*, have been found to be good accumulator of Pb. Lead accumulation in most of the plants were found significantly higher (p=0.05) at the polluted sites than at a comparatively less polluted site Narayanthan (Table 12).

4.1.3.1.d Metal accumulation index (MAI)

Mean MAI value in the studied tree leaves ranged from 7.34 (in *Cinnamomum camphora*) to 20.44 (in *Populus euramericana*).MAI was observed mostly higher in all the studied tree species growing along the road side than that of less polluted site,Narayanthan. On the basis of MAI mean value the sequence of plant species from lower to higher are in the order- *Cinnamomum camphor<Ficus reliogisa<Grevillea robusta<Callistemon lanceolatus<Jacaranda mimosifolia<Populus euramericana.* Based on the highest MAI value scored by the tree species at different study sites, it can be said that the species like *Jacaranda mimosifolia* and *Populus euramericana* have more heavy metal accumulation ability than the other species among the angiosperms (Table 12).

Table 12: Metal content in the leaves (mean±sd, n=21) and the Metal Accumulation Index (MAI) in different plants at different sites (Angiosperm)

Plants	Sites	Zn mg/kg	Cu mg/kg	Pb mg/kg	MAI
Callistemon lanceolatus	Airport	15.54±0.42 C	5.41±0.62 EF	40.33±1.84 E	20.09
	Balaju	38.46±0.91 F	5.87±0.30 F	32.41±0.93 C	32.09
	Banasthali	43.76±3.23 G	7.56±0.38 G	0.83±0.67A	11.65
	Bsundhara	12.15±0.68 B	3.63±0.25 CD	0.73±0.49 A	11.25
	Dhumbarahi	20.83±1.13 DE	2.39±0.53 B	17.10±1.04 B	13.09
	Gongbu	21.53±0.84 E	0.93±0.42 A	37.56±1.49 D	17.61
	Mean MAI (Polluted sites)				17.63
	Narayanthan	4.22±1.51A	4.81±0.78 E	0.61±0.37 A	3.54
Cinnamomum camphora	Airport	23.23±2.85 D	8.79± 0.90 CD	13.53±1.60B	8.78

	Balaju	15.69±1.38 B	5.23±1.04 AB	32.06±2.16 D	10.42
	Banasthali	1.40±0.59 A	7.99±0.88 CD	0.83±0.58 A	4.29
	Basundhara	18.07± 2.25 BC	3.39±2.25 A	24.99±4.28 C	5.11
	Dhumbarahi	20.11± 2.13 C	9.29±1.54 D	2.86±1.62 A	5.74
	Gongbu	20.03±0.99 C	6.51±0.91 BC	21.87±1.85 C	13.05
	Mean MAI (Polluted sites)				7.89
	Narayanthan	1.27± 0.64 A	13.93±1.79 E	0.84± O.38 A	3.99
Ficus reliogisa	Airport	16.02±1.71 D	9.13±0.78 D	21.42±1.47 C	11.90
	Balaju	22.20±2.42 E	7.41±1.40 C	39.61±2.39 E	10.33
	Bansthali	22.37±2.27 E	11.09±1.67 E	36.65±2.73 D	9.98
	Basundhara	8.79±1.06 B	0.90±0.09 A	0.053±0.01 A	7.06
	Dhumbarahi	12.71±1.12 C	7.91±0.96 CD	3.82±0.76 B	8.17
	Gongbu	13.13± 1.62 C	3.93±0.23B	35.83±2.06 D	14.18
	Mean MAI (Polluted sites)				10.27
	Narayanthan	1.54±0.51 A	15.28±1.49 F	0.50±0.10 A	5.95
Grevillea robusta	Airport	22.09±2.86 C	9.29±1.01 D	19.83±1.51 D	10.03
	Balaju	15.06±3.42 AB	6.20±0.72 BC	37.8±1.56F	12.40
	Banasthali	13.49±0.90 AB	7.58±1.39 CD	16.92±1.41 C	10.80
	Basundhara	14.03±0.91 AB	2.96± 0.50 A	12.84± 2.12 B	9.11
	Dhumbarahi	15.2±2.43 AB	5.14±0.69 B	24.34±2.99 E	7.28
	Gongbu	12.01±2.59 A	2.97±0.89 A	36.67±1.15 F	13.23
	Mean MAI (Polluted sites)				10.47
	Narayanthan	12.45±2.21 A	8.16±1.61 CD	4.97± 2.25 A	4.31
Jacaranda mimosifolia	Airport	34.98±1.56 E	19.93±1.68 E	25.06±1.38 E	17.47
	Balaju	31.18±1.28 D	20.27±0.64 E	3.70±1.37 A	19.52
	Banasthali	22.68±1.33 B	5.72± 0.43 B	29.37±1.67F	15.97
	Basundhara	23.34±2.00 BC	13.83±1.35 D	19.42±0.64 D	17.37
	Dhumbarahi	25.54±0,84 C	11.72±0.71 C	11.58±1.21 B	18.78
	Gongbu	22.54±0.84 B	11.61±1.12 C	15.52±1.04 C	17.44
	Mean MAI (Polluted sites)				17.75
	Narayanthan	9.60± 0.45 A	2.45± 0.47 A	1.40±0.56 A	3.28
Populus euramericana	Airport	104.27±1.02 E	11.31±0.63 D	0.93±0.59 A	40.69
	Balaju	69.06±3.32 D	8.38±0.53 C	27.51±1.74B	17.37
	Banasthali	132.38±3.71 F	11.93±0.60 D	0.83±0.58A	18.96

	Basundhara	57.89±1.01 BC	0.89±0.10A	1.66±1.52 A	25.71
	Dhumbarahi	57.09±2.71 BC	1.86±1.06 AB	1.54±0.99 A	8.14
	Gongbu	65.61±1.30 CD	1.26±1.07 AB	2.32±0.84 A	18.12
	Mean MAI (Polluted sites)				21.49
	Narayanthan	14.46±0.47 A	2.41± O.86 B	0.59±0.06A	14.13

Same letter followed after mean±sd in a column for a particular species denote no significant difference at p=0.05 by Duncan's multiple range test followed after one way ANOVA

4.1.3.2 Metal content in gymnosperms

Heavy metal (Zn, Cu and Pb) content in the leaves of road side gymnosperms like and *Pinus roxburghii, Thuja orientalis, Cedrus deodara* and *Araucaria araucana* were measured in the present study and the results obtained are given in (Table 13).

4.1.3.2.a Zinc (Zn)

Zinc is an essential element for plant growth and it's mean concentrations in the collected leaves from different sites ranged from 12.28 mg/kg (in *Araucaria araucana*) to 54.52 mg/kg (in *Cedrus deodara*). Zinc accumulation ranged from 12.28 to 54.52 mg/kg in *Araucaria araucana,* 18.25 to 54.52 mg/kg in *Cedrus deodara,* 13.89 to 34.32 mg/kg in *Pinus roxburghii,* and 14.23 to 39.49 in *Thuja orientalis*. On the basis of maximum Zn accumulation, *Cedrus deodara* (54.52 mg/kg) and *Thuja orientalis* (34.94 mg/kg) can be considered as good accumulator of Zn. Zn accumulation in most of the plants were significantly higher (p=0.05) at most of the polluted sites than at comparatively a less polluted site, Narayanthan (Table 13).

4.1.3.2.b Copper (Cu)

Copper is an essential elements for the plant growth and its mean accumulation value of all studied sites in the tree leaves ranged from 1.48 (in *Araucaria araucana*) to 20.16 mg/kg (in *Thuja orientalis*). In *Araucaria araucana* copper accumulation ranged ranged from 1.48 to 9.92 mg/kg, in *Cedrus deodara* from 1.96 to 11.85 mg/kg, in *Pinus roxburghii* from 1.94 to 8.79 mg/kg, and in *Thuja orientalis* from 2.86 to 20.16 mg/kg. On the basis of maximum Cu accumulation values, *Thuja orientalis* and *Cedrus deodara* were found to be good accumulator of Cu (Table 13). Copper accumulation in the gymnosperm leaves were significantly (*p=0.05*) higher at most of the polluted sites.

4.1.3.2.c Lead (Pb)

Lead is a non-essential element for plants and it's accumulation in the studied gymnosperm leaves along the road side ranged from 0.88 (in *Araucaria araucana* to 8.85 mg/kg (in *Pinus euramericana*). In *Araucaria araucana* lead accumulation ranged from 0.88 to 3.62 mg/kg, in *Cedrus deodara* from 0.96 to 8.58 mg/kg, in *Pinus euramericana* from 1.52 to 8.85 mg/kg, and in *Thuja orientalis* it ranged from 2.04 (at Narayanthan) to 4.84 mg/kg (at Ratnapark). On the basis of maximum accumulation value, *Pinus roxburghii* and *Cedrus deodara* were found to be good accumulator of Pb (Table 13). Lead accumulation in leaves of gymnosperms was significantly higher at most of the polluted sites.

4.1.3.2.d Metal accumulation index (MAI)

Mean MAI in the studied gymnosperm leaves ranged from 10.78 (*Pinus roxburghii*) to 31.27 (*Thuja orientalis*). MAI in the leaves of gymnosperms were mostly higher at polluted sites than at a less polluted sites (Narayanthan) (Table 11). On the basis of MAI mean value, the sequence of plant species from lower to higher are in the order- *Pinus roxburghii<Araucaria araucana<Cedrus deodara<Thuja orientalis.* Based on the highest mean value of MAI scored by the tree species at different study sites, it can be said that the species like *Thuja orientalis* and *Cedrus deodara* have more heavy metal accumulation ability than the other species among gymnosperms (Table 13).

Table 13: Metal content in the leaves (mean±sd, n=15) and the Metal Accumulation Index (MAI) in different plants at different sites (Gymnosperm)

Plants	Sites	Zn mg/kg	Cu mg/kg	Pb mg/kg	MAI
Araucari araucana	Airport	24.18±0.63 D	5.15±0.51 BC	3.62±0.56 C	18.37
	Dhumbarahi	18.24±2.51 C	9.92±1.06 D	1.37±0.57 A	19.03
	Jawalakhel	22.55±1.18 D	4.58±0.40 B	0.88±0.03 A	19.73
	Ratnapark	28.22±0.51 E	4.92±0.54 B	2.68±0.13 B	28.35
	Shankha park	16.02±1.96 B	1.48±0.57 A	1.72±0.47 A	14.42
	Mean MAI(Polluted sites)				19.98
	Narayanthan	12.28±0.51 A	6.62±1.19 C	1.49±0.24 A	11.90

Cedrus deodara	Airport	26.79±0.95 BC	4.33±0.79 C	8.58±1.17 C	13.68
	Dhumbarahi	54.52±1.20 D	3.94±0.07 BC	3.42±0.17 B	40.77
	Jawalakhel	18.25±0.51 A	9.26±0.64 D	3.15±0.56 B	18.72
	Ratnapark	28.70±0.99 BC	11.85±1.06 E	2.70±0.56 B	44.99
	Shankha park	23.57±0.59 ABC	2.99±0.37 AB	1.98±0.02 AB	44.54
	Mean MAI (Polluted sites)				32.54
	Narayanthan	21.25±5.69 AB	1.96±0.06 A	0.96±0.08 A	16.75
Pinus roxburghii	Airport	18.27±0.59 A	5.36±0.65 A	3.21±0.58 A	14.86
	Dhumbarahi	34.32±0.94 D	8.79±0.87 C	1.52±0.30 A	17.31
	Jawalakhel	28.32±9.29BCD	6.66±1.15 BC	2.16±0.54 A	4.27
	Ratnapark	30.64±5.20 CD	7.20±1.52 BC	8.85±2.65 C	4.65
	Shankha park	20.41±1.24 ABC	2.80±0.09 A	4.31±5.11 AB	16.08
	Mean MAI(Polluted sites)				11.43
	Narayanthan	13.89±4.06 A	1.94±0.96 A	3.29±1.59 AB	7.51
Thuja orientalis	Airport	26.21±0.62D	20.16±0.48 D	3.29±0.13 A	36.39
	Dhumbarahi	18.75±1.01B	2.95±0.36 A	2.31±0.22 A	12.44
	Jawalakhel	39.49±0.55 E	8.44±0.61 C	3.31±0.51 A	30.53
	Ratnapark	37.95±0.52 E	5.92±0.04 B	4.84±0.61 B	75.78
	Shankha park	21.15±1.12 C	2.86±0.23 A	2.43±0.09 A	19.85
	Mean MAI (Polluted sites)				34.99
	Narayanthan	14.23±0.54A	5.25±0.60 B	2.04±0.80 AB	12.63

Same letter followed after mean±sd in a column for a particular species denote no significant difference at $p=0.05$ by Duncan's multiple range test followed after one way ANOVA

4.1.4 Correlation between metal content and SLA in leaves of angiosperm

Correlations between heavy metal content (Zn, Cu, and Pb) and SLA decrease in the studied leaves of some common angiosperms trees along the road side are shown in Table 14. Coorelation of percentage decrease in SLA and Zn was mostly negative ($p<0.005$) and significant in most of the species, except *Ficus religiosa* and *Grevillea robusta*. The correlation between percentage decline in SLA and Cu content were mostly positive and significant, but in *Jacaranda mimosifolia* it was negatively significant. Similarly the coorelation of percatage decrese in SLA and Pb content was positive in all species. Decrease in SLA percatage showed a significant negative correlation with Zn content in *Populus euramericana*, while it showed a significant positive correlation with Pb content. Percentage SLA decrease in *Ficus religiosa*, showed positive correlation with Pb and Cu but in *Jacaranda mimosifolia*, percentage decrease in SLA showed negative correlation with Zn and Cu. In, percentage decrease in SLA showed positive correlation with Pb in *Ficus religiosa*, *Populus euramericana* and *Grevillea robusta.* In *Cinnamomum camphora,* the percentage decrease in SLA was observed a positive correlation with Cu and negative correlation with Zn.

Table 14: Correlations between percentage decrease SLA and heavy metal content (Zn, Cu, and Pb) in leaves of some angiosperms trees

Plants	**% decrease SLA-Zn**	**% decrease SLA-Cu**	**% decrease SLA-Cu**
Callistemon lanceolatus	-0.72**	0.28	-0.50
Cinnamomum camphora	-0.76**	0.61*	-0.35
Ficus religiosa	-0.31	0.53*	0.59*
Grevillea robusta	-0.38	0.19	0.66**
Jacaranda mimosifolia	-0.65**	-0.76**	0.11
Populous euramericana	-0.46*	0.09	0.73**

Significant at **p<0.05 and **p<0.01*

4.1.5 Correlations between metal content and APTI parameters

4.1.5.1 Correlations in angiosperm

The correlation between different metal (Zn, Cu and Pb) content and different biochemical parameters (RWC, AA, pH, Tchl) and APTI) of the studied six tree species of angiosperms are given in Table 15. Correlation between RWC and zinc content was mostly negative in all studied tree species and was significant (p=0.05) in *Callistemon lanceolatus.* Similarly, correlation of Cu content and RWC was positively significant (p=0.05) in *Grevillea robusta,* but negatively significant (p<0.01) with *Callistemon lanceolatus* and *Jacaranda mimosifolia*. In *Grevelliea robusta,* significant correlation was observed between Pb content in leaves and RWC. Cumulative effect of Pb+Cu+Zn accumulation was found to be negatively correlated with RWC in all plants and was significant (p<0.05) in *Grevillea robusta* and *Jacaranda mimosifolia.* The correlations between AA content and Zn accumulation in the studied tree leaves were mostly negative and was significant (p<0.05) in *Jacaranda mimosifolia*. Significant positive correlation was observed between Cu accumulation and AA in *Ficus reliogisa*. Pb accumulation mostly negative correlation with AA and was significant in *Cinnamomum camphora* but in *Jacaranda mimosifolia* it was positively significant (*p*<0.01). Cumulative effect of Pb+Cu+Zn accumulation was found to be significantly (*p*<0.05) negatively correlated with AA in *Grevillea robusta.* The correlation between leaf extract pH and Zn content were mostly negative, and was significant (*p*<0.05) in *Cinnamomum camphora* and *Jacaranda mimoisifolia.* Mostly positive correlation was found between Cu content and leaf extract pH in most of the tree species and was significant (*p*<0.01) in *Cinnamomum camphora* but significant negative correlation found in *Callistemon lanceolatus and Jacaranda mimosifolia*. Pb content in leaf showed negative correlation with pH in most trees and was significant (*p*<0.01) in *Grevillea robusta, Cinnamomum camphora* and *Jacaranda mimoisifolia.* Cumulative effect of Pb+Cu+Zn accumulation was found to be mostly negatively correlated with pH in most trees and was significant (*p*<0.05) in *Grevillea robusta* and *Cinnamomum camphora.* Zinc accumulation in leaves showed negative correlation with total chlorophyll in most of the trees and was significant (*p*<0.01) in *Ficus reliogisa, Jacaranda mimosifolia* and *Populus euramericana*. Cu content in leaves was significantly (*p*<0.01) negatively correlated with Tchl in *Jacaranda mimosifolia.* Similarly, Pb content in leaf showed significant (*p*<0.05) negatively correlated with Tchl in *Ficus reliogisa.* Cumulative effect of Pb+Cu+Zn content in leaves showed negative correlation in most of the studied plants and was significant in *Ficus reliogisa* and *Jacaranda mimosifolia.* APTI was found to be negatively correlated with Zinc accumulation in most trees and was significant (*p*<0.01) in *Callistemon lanceolatus* and *Jacaranda mimosifolia.* Cu content in leaves showed negative correlation with APTI in some studied tree species and was significant (p<0.05) in *Ficus reliogisa, Callistemon lanceolatus* and *Jacaranda mimoisifolia* but in *Cinnamomum camphora* it was significantly (*p*<0.01) positive. Pb content showed negative correlation with APTI in most studied tree species and was significant (*p*<0.01) in *Ficus reliogisa, Populus euramericana, Cinnamomum camphora* and *Grevillea robusta*. Cumulative effect of Pb+Cu+Zn accumulation was found to be mostly significant negatively correlated in all studied plants and was significant

($p<0.05$) in *Grevillea robusta*, *Cinnamomum camphora*, *Callistemon lanceolatus* and *Jacaranda mimosifolia*.

Table 15: Pearson correlation of metals with RWC, AA, pH, Tch and APTI (Angiosperm)

Parameters	***Callistemon lanceolatus***	***Cinnamomum camphora***	***Ficus reliogisa***	***Grevillea robusta***	***Jacaranda mimosifolia***	***Populus euramericana***
	(n=50)	(n=35)	(n=35)	(n=50)	(n=40)	(n=45)
RWC-Zn	-0.40**	-0.18	-0.30	-0.11	-0.15	-0.06
RWC-CU	-0.64**	0.16	-0.23	0.34*	-.05**	0.12
RWC-Pb	0.08	-0.12	-0.20	-0.38**	-0.15	-0.08
RWC-Zn+Cu+Pb	-0.20	-0.15	-0.29	-0.30*	-0.38*	-0.06
AA-Zn	-0.16	0.11	-0.30	-0.24	-0.36*	0.20
AA-Cu	0.19	0.27	0.50**	-0.03	-0.11	0.04
AA-Pb	-0.02	-0.37*	0.245	-0.26	0.47**	-0.21
AA-Zn+Cu+Pb	-0.08	-0.16	0.30	-0.33*	0.21	0.13
pH-Zn	-0.17	-0.41*	-0.15	0.04	-0.66**	-0.14
pH-Cu	-0.51**	0.53**	0.73	0.09	-0.58**	0.18
pH-Pb	-0.01	-0.49**	0.42	-0.40**	-0.45**	0.07
pH-Zn+Cu+Pb	-0.14	-0.48**	0.30	-0.33*	-0.08	-0.09
Tchl-Zn	0.07	-0.24	-0.75**	0.04	-0.78**	-0.33*
Tchl-Cu	-0.15	0.08	0.09	0.17	-0.82**	-0.03
Tchl-Pb	0.01	-0.01	-0.34*	-0.13	0.03	0.26
Tchl-Zn+Cu+Pb	0.02	-0.13	-0.34*	-0.06	-0.52**	-0.24
APTI-Zn	-0.43**	-0.14	0.32	-0.21	-0.53**	0.07
APTI-Cu	-0.51**	0.43**	-0.95*	0.24	-0.66**	-0.12
APTI-Pb	-0.01	-0.46**	-0.98**	-0.47**	0.06	-0.77**
APTI-Zn+Cu+Pb	-0.28*	-0.33*	-0.48	-0.45**	-0.36*	-0.13

Significant at *$p<0.05$ and **$p<0.01$

4.1.5.2 Correlation in gymnosperm

The correlations between different metals (Zn, Cu and Pb) content with different biochemical parameters (RWC, AA, pH, Tchl and APTI) of the various tree species from different sites are given in Table 16. RWC and zinc content mostly showed positive correlation in all studied tree species and was significant (*p*=0.05) in *Pinus roxburghii* and *Araucaria araucana.* Similarly Cu was found to be significantly (*p*=0.05) positively correlated with RWC of *Pinus roxburghii, Thuja orientalis* and *Cedrus deodara.* Significant positive correlation was observed between Pb content in leaves and RWC in *Araucariai araucana.* Cumulative effect of Pb+Cu+Zn accumulation was found to be positively correlated with RWC in all plants and was significant (*p*<0.05) in *Pinus roxburghii*, *Thuja orientalis* and *Araucaria araucana.* Mostly positive correlation was observed between AA content and Zn accumulation in the studied tree leaves and was significant (*p*<0.05) in *Thuja orientalis* and *Araucaria araucana.* Significant positive correlation was observed between Cu accumulation and AA in *Cedrus deodara*. Correlation between Pb content and AA was positive and was significant in *Araucaria araucana.* Cumulative effect of Pb+Cu+Zn accumulation was found to be significantly (*p*<0.05) positively correlated with AA and was significant in *Cedrus deodara* and *Araucaria araucana.* Mostly insignificant correlation was observed between leaf extract pH and Zn content in all studied tree leaves. Cu content in leaf was mostly found to be positive correlation with pH in most of the tree species and was significant (*p*<0.01) in *Cedrus deodara*. Pb content in leaf showed positive correlation with pH in *Araucaria araucana.*Correlation between cumulative effect of Pb+Cu+Zn content and leaf extract pH were mostly positive but was insignificant. Total chlorophyll content in leaf showed no significant correlation with Zinc content but revealed positive significant (p<0.01) correlation with Cu content in *Cedrus deodara* and negative significant correlation with Pb content in *Pinus roxburghii.* Cumulative effect of Pb+Cu+Zn content in leaves showed positive correlation with Tchl but were insignificant.APTI was found to be positively correlated with Zinc accumulation in most trees and was significant (*p*<0.01) in *Araucaria araucana.* Cu content in leaves showed positive correlation with APTI in some studied tree species and was significant (*p*<0.05) in *Cedrus deodara.* A negative significant (p<0.05) correlation was observed with APTI and metal content in *Pinus roxburghii* but in *Araucaria araucana* it was significantly (*p*<0.05) positive. Cumulative effect of Pb+Cu+Zn content in leaves with APTI was found to be positively correlated and was significant (*p*<0.05) in *Thuja orientalis.*

Table 16: Pearson correlation of metals with RWC, AA, pH, Tch and APTI (Gymnosperm)

Parameters	***Araucaria araucana*** (n=30)	***Cedrus deodara*** (n=30)	***Pinus roxburghii*** (n=30)	***Thuja orientalis*** (n=30)
RWC-Zn	0.57**	0.09	0.47**	0.25
RWC-Cu	-0.15	0.66**	0.53**	0.44*
RWC-Pb	0.43*	-0.02	-0.14	0.30
RWC-Zn+Cu+Pb	0.50**	0.27	0.43*	0.40*
AA-Zn	0.43*	0.34	0.21	0.53**
AA-Cu	-0.06	0.37*	-0.07	-0.19
AA-Pb	0.04**	-0.34	0.07	0.35
AA-Zn+Cu+Pb	0.42*	0.37*	0.15	0.32
pH-Zn	-0.15	-0.34	0.12	0.31
pH-Cu	0.14	0.88**	0.11	0.16
pH-Pb	0.49**	-0.23	0.26	0.32
pH-Zn+Cu+Pb	-0.01	-0.12	0.18	0.32
Tchl-Zn	0.14	0.04	0.12	0.26
Tchl-Cu	0.08	.69**	0.30	-0.08
Tchl-Pb	0.16	-0.10	-0.49**	0.22
Tchl-Zn+Cu+Pb	0.18	0.21	0.18	0.16
APTI-Zn	0.37*	-0.06	0.32	0.28
APTI-Cu	-0.36	0.56**	0.30	0.35
APTI-Pb	.61**	-0.21	-0.49**	0.28
APTI-Zn+Cu+Pb	0.27	0.05	0.18	0.39*

Significant at **p<0.05 and **p<0.01*

4.2 Discussion

Discussion is focused mainly on the above mentioned results, i.e., the first discussion is about macro and microphological of experimental leaves, second one is about Air Pollution Tolerance Index (APTI) of the experimental plants and third one is about their metal accumulation and correlations.These are described as below.

4.2.1 Leaf morphology

4.2.1.1 Macro morphology

4.2.1.1.a Leaf area

Leaf area (cm^2) of all the studied tree leaves except *Celtis australis* significantly reduced at polluted areas compared to less polluted sites.Tiwari *et al.* (2006) observed that pollutants can cause serious leaf injury, stornatal damage, premature senescence, decrease in photosynthetic activity, disturbance in membrane permability and reduce yelding efficiency in sensitive plant species. Reducton in leaf area may be due to decreased leaf production rate and enhanced senescence. The reduced leaf area result in reduced absorbed radiatons and subsequently in reduced photosynthetic rate (Tiwari *et al.,* 2006). Kayode and Otoide (2007) also observed reduction in leaf area in *Newbouldia laevis* and *Albizia lebbek*.

4.2.1.1.b Specific leaf area (SLA)

Specific leaf area (SLA) reduced at polluted sites compared to less polluted sites in all studied tree species and was significant ($p<0.01$) in *Callistemon lanceolatus, Celtis australis, Eucalyptus globulus, Ficus elastica, Ficus religiosa, Grevillea robusta, Jacaranda mimosifolia,* and *Populus euramericana* at some or all polluted sites. Decrease in SLA of *Ligustrum qulhoul* was also reported by Zhu *et al.* (2021). Song *et al.* (2019) found that species with higher SLA could be better adapted to the urbanization environment. Abbasi *et al.* (2018) detected a decrease in specific leaf area in the *elm* leaf at the polluted sites from Azadi and Gisha Bridge of Tehran. Balasooriya *et al.* (2009) and Carreras *et al.* (1996) also reported an increase in SLA of *Taraxacum officinale* and *Ligustrum* species, respectively, in more polluted areas. SLA reduction minimizes water loss and transpiration. This might be an adaptive approach of plants to enhance stress tolerance (Xu *et al.,* 2009).

4.2.1.2 Micro morphology

4.2.1.2.a Stomata density

Stomatal density increased in polluted sites in all plants and was significant in *Cinnamomum camphora, Callistemon lanceolatus, Celtis australis, Ficus reliogisa,* *Ficus elastica*, *Grevillea robusta*, *Prunus cerasoides,* and *Populus euramericana.* Stomatal density increases and stomata size decreases in stressed plants (Balasooriya *et al.,* 2009; Kardel *et al.*, 2010). Pal *et al.* (2002) reported two-fold increase in stomatal frequency and trichome length in plant samples taken from high-traffic sites in comparison to low-traffic sites. Alves *et al.* (2008) observed that stomatal density in *Eugenia uniflora* varied between specimens collected at locations with different degrees of air pollution. Their results indicated that individuals growing at sites with the highest level of pollution had the highest stomatal density. Gostin (2009) also reported increase in stomata density in the plants growing under air pollution. Under metal stress, stomatal closure is usually reduced. Pb stress has been shown to cause changes in the inner anticlinal walls of guard cells (Ahmad *et al.*, 2005). Besides this the increase in stomatal density in polluted sites is possibly to fulfill the required gas exchange for physiological processes. Stomatal traits like stomatal density (Zhang *et al.,* 2017) and stomatal size (Liang *et al.*, 2016) have been recognized as important characters for capturing particulate matter from the polluted air. Stomatal uptake is concerned with the diffusion of gaseous pollutants from the air as a result of photosynthesis and water management processes in plants (Lawson and Blatt, 2014), although particles<2 µm can enter the stomatal cavity (Song *et al.*, 2015). Opening and closing of stomata are regulated by environmental circumstances, and removal of air pollutants could be enhanced by the selection of plant species with long opening periods. According to Grote *et al.* (2016), anisohydric species (like *Populus* and some *Quercus* species, which have more variable leaf water potential and keep their stomata open and photosynthetic rates high for longer periods, even in the presence of decreasing leaf water potential) can trap particulate matter more than the isohydric plants (like *Pinus* and *Platanus* species, which shorten their stomatal opening period in response to drought).

4.2.1.2.b Size of stomata

The size of stomata (i.e., length and breadth) reduced in all the studied plants at the polluted sites as comparasion to less polluted sites. Both length and breadth of stomata reduced significantly in *Callistemon lanceolatus, Celtis australis,* *Cinnamomum camphora, Ficus elastica, Ficus reliogisa, Grevillea robusta, Prunus cerasoides,* and *Populus euramericana.* Saadabi (2011) also reported reduction in the length and width of the leaves of *Tabernaemontana divaricata* and *Hamelia patens* in polluted sites. Kulshrestha *et al.* (1994) reported significant decrease in stomata size of epidermal cells, and trichome per unit area in *Calotropis procera* and *Nerium indicum* growing at auto exhaust polluted busy roadside. The decrease of the stomatal size may be an avoidance mechanism against the inhibitory effect of a pollutant on physiological activities such as photosynthesis (Verma *et al.*, 2006).

4.2.1.2.c Thickness of cuticle

Thickness of cuticle of leaf adaxial surface decreased in all plants at polluted sites. Maximum decrease in cuticle thickness at polluted sites was observed in *Callistemon lanceolatus* and minimum decreased in *Populus euramericana.* Cuticle is the outermost layer of leaf which is exposed to pollutants and is also a barrier between the interior and outer environment. As it is outermost layer, remains in continuous contact with the air pollutants, and this may exert stress (Baker and Hunt, 1986), as a result cuticle becomes responsive to change in such environment. The pH of leaf extract at most of the studied polluted sites decreased, which possibly is mainly due to acidic nature of the pollutants. It is well established that low pH of fall out leaches the cuticle layer and this might be another reason for its reduction at polluted sites. According to Pourkhabbaz *et al.* (2010) the cuticle covering the outer epidermal layer at polluted sites contained less cutin and high accumulation of phenolics. The accumulation of more phenolics is possibly due to heavy metal and other pollutant stress.

4.2.1.2.d Thickness of epidermis

Epidermis is usually outermost layer in leaves, with transparent cells having no chloroplast, except guard cells, and is tightly connected with each other.In present study, thickness of epidermis reduced in all plants at polluted sites. Mitu *et al.* (2019) revealed that leaves of selected roadside plants had reduced cell size with black dot like substance deposited in the epidermis at polluted sites. These deposited pollutants possibly induce several physiological processes to combat the stress, as a result of which reduction in the epidermal cells might have occurred.

4.2.2 Air pollution tolerance index (APTI)

4.2.2.1 Total chlorophyll content (Tchl)

4.2.2.1.a Total chlorophyll in angiosperm

Total chlorophyll in the same species was lower in certain contaminated areas than in Narayanthan, a less polluted site. For example, in *Ficus religiosa*, it was significantly lower at all sites than at Narayanthan, in *Jacaranda mimosifolia* it was significantly increased at all polluted sites (except Airport) than at Narayanthan, in *Callistemon lanceolatus* it was significantly less at airport and Balaju; in *Cinnamomum camphora* it was less at Airport, Gongbu; in *Grevillea robusta* it was less at Balaju, Dhumbarahi and Gongbu. Many researchers have reported reduction in chlorophyll under air pollution with increasing heavy metal content (Allen *et al.,* 1987; Chettri *et al.,* 1998; Tiwari *et al.,* 2006; Tripathi and Gautam 2007; Joshi and Swami, 2009). The total chlorophyll content in plant leaves has been reported to be also affected by gaseous pollutants (such as SO_2), high temperatures, drought, salt stress, and daylight intensity, in addition to heavy metal air and soil pollution (Karmakar, *et al.,* 2016; Zhang *et al.,* 2016). In *Populous euramericana* total chlorophyll content was more or less same in most sites in the present study and at some polluted sites like Basundhara and Dhumbarahi it was significantly high, indicating its resistance to air pollution, as is also evident with its high APTI values. The present study also supports the findings of Katiyar and Dubey (2001) and Chandawat *et al.* (2011) as suggested that the difference in the total chlorophyll content in the same species at different sites could be due to differences in pollution level as well as environmental factors. Besides this the total content is also influenced by tolerance or sensitivity of the species (Chandawat *et al.,* 2011). Total chlorophyll concentration in the plant governs the biomass growth, development and photosynthetic activity. In the present study, *Callistemon lanceolatus Cinnamomum camphora*, *Ficus religiosa* and *Populus euramerican* in Airport, i.e., at polluted sites had high total chlorophyll. Total chlorophyll increased in the present study among some plants growing in polluted sites and the possible reason for this may be due to high concentration of carbon dioxide and high temperature. The enhanced growth of plants due to higher levels of CO_2 has been reported by Poorter (1993). Plants grow faster at higher temperature if provided with adequate levels of CO_2, water, sunlight and plant nutrients. Yet another reason could be the smoke released from the vehicles that enhance the chlorophyll production in the leaves (Geeta and Namrata, 2014). Increase in total chlorophyll in *Eucalyptus camaldulensis*, *Prosopis juliflora* and *Mangifera indica* leaves from polluted site was also reported when compared with less polluted site (Tripathi and Gautam, 2007; Seyyednejad and Koochak, 2011). In the present study the polluted sites were with high traffic areas to enhance chlorophyll content on one hand and the presence of comparatively moderate to high concentrations of Zn and Pb content in their leaves. The concentrations of Zn and Pb in them possibly also helped to protect the degradation of chlorophyll in them, which was observed by Chettri *et al.* (1998) in lichens. Higher chlorophyll content has been postulated as favorable conditions for plants to withstand polluted environment and that possibly connected with tolerance of a species in contaminated environments (Pathak *et al.,* 2011; Rai and Panda, 2014; Ogunkunle *et al.,* 2015).

4.2.2.1.b Total chlorophyll content (Tchl) in gymnosperm

The current study found that the chlorophyll content in gymnosperms were higher than those of angiosperms. Gymnosperms have ability to synthesixe chlorophyll both in darkness as well as in light (Schoefs and Bertrand, 1997), whereas angiosperms can synthsize chlorophyll in light only. The ability to synthesis in darkness is linked with the presence of three genes (chlL, chlN, and chlB) in the chloroplast genome that code for subunits of the light-independent Pchlide reductase (Armstrong, 1998). Amomng the chloroplast genome of angiosperms at least two of these genes are absent and hence are unable to synthesize in darkness (Suzuki and Bauer, 1992). Variable results of chlorophyll content in the leaves of gymnosperms were recorded. Chlorophyll content varied with plant’s tolerance and sensitivity, i.e., the more sensitive the plant species (lower APTI), the higher the chlorophyll content in *Araucaria araucana.* But in *Pinus roxburghii* and *Thuja orientalis* high total chlorophyll content was recorded even at contaminated areas, which may be attributed to their tolerant nature (Beg *et al.,* 1990; Jyothi and Jaya, 2010). From these two different results of chlorophyll in Gymnosperms it appears that chlorophyll content in plant alone is not responsible to determine a plant to be a tolerant or sensitive.

4.2.2.2 Relative water content (RWC)

4.2.2.2.a RWC in angiosperm

RWC in all studied angiosperm plants were mostly higher at polluted sites than those in less polluted site. But at some place for instance *Callistemon lanceolatus* at Balaju, *Cinnamomum camphora* at Gongbu, *Ficus reliogisa* at Gongbu, *Grevillea robusta* at Gongbu, and *Populus euramericana* at Gongbu measured low RWC than at less polluted site, i.e., Narayanthan. RWC is perhaps the most suitable indicator for knowing the water status of a plant in terms of the physiological effects of cellular water deficit. It determines the present water content of the sampled leaf tissue in relation to the maximum water where it can hold at full turgidity.Based on the maximum amount of water it can hold at full turgidity. In plants, relative water content is related to protoplasmic permeability (Oleinikova, 1969) and tolerance against air pollutants in plants (Achakzai *et al.*, 2017). RWC of a plant species might increase or decrease with increasing pollution load depending upon several factors like the climate of an area, season, type of plant species, type of pollutant and the tolerance level of plant species (Sharma *et al.*, 2017). Relative water content (RWC) is a vital physiological feature which affects transpiration rate, stomatal resistance, plant water relations, and leaf water potential (Hartmann *et al.,* 2013) and so it is considered as a marker of plant water status that regulates metabolic activity in tissues. RWC is formed as a result of water loss by transpiration and uptake by roots (Georgii *et al.,* 2017).Leaf water potential is important for turgor pressure, plant survival and photosynthetic processes which is closely related to cell growth and stomatal closure (Alghabari *et al.,* 2015) and maintaining tolerance from low to moderate water stress. More RWC in plants can dilute acidity inside the leaf cell sap and also resist the drought condition in plants (Kaur and Nagpal, 2017). High water content in plants ensures the maintenance of the physiological balance under stresses condition against air pollution (Deepalakshmi *et al.*, 2013). In particular plant species, higher content of RWC supports against drought and provides tolerance capacity (Dedio, 1975). Hence, the plants with higher RWC even under polluted conditions have been suggested as tolerant species towards air pollutants (Dhankhar *et al.*, 2015). In the present study a plants like *Callistemon lanceolatus, Cinnamomum camphora, Ficus reliogisa, Jacaranda mimosifolia* and *Populus euramericana* measured RWC more than 80% in some polluted sites and hence these species can be considered as more tolerant to combat air pollutants. Water is an essential requirement for plant existence and drought tolerance in plants is favored by high RWC (Swami *et al.,* 2004).

4.2.2.2.b RWC in gymnosperm

Likewise, RWC increased significantly (p=0.05) in all the studied gymnosperms plants (*Araucaria araucana, Cedrus deodara, Thuja orientalis,* and *Pinus roxburghii*) at all polluted sites compared to Narayantan (a less polluted site). Similar results were also observed by Deepalakshmi *et al.* (2013) among the plants growing alongside busy roadways exhibited significant reduction in relative water content. In some species at polluted sites, relative water content was reduced than those at less polluted site. High water content in the studied gymnosperms possibly helped to maintain physiological balance under pollution stressed conditions, as in the drought tolerant plants (Swami *et al.,* 2004). Tolerant plant species may have high relative water content in leaves to resist pollution in polluted sites.

4.2.2.3 Leaf extract pH

4.2.2.3.a pH in angiosperm

In the present study the leaf extract pH of *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa, Grevillea robusta* and *Populus euramericana* was reduced significantly (p=0.05) at most of the polluted sites than at Narayanthan, comparatively a clean site. Scholz and Reck (1977) suggested that the cell system functions well at optimum pH but in some plants when they are exposed to acidic pollutants over a long period, reduces pH level and affects normal physiological activity in the cell (Saxena and Ghose, 2013). Leaf extract pH can help in determining the susceptibility of plants to the pollution exposure like NOx and SO_2 (Rahul and Jain, 2014). Leaf extract pH showed a varying trend in the studied leaves of different plants exposed to same sites. In *Callistemon lanceolatus, Jacaranda mimosifolia* and *Grevillea robusta,* the leaf extract pH ranged from 3.53 to 6.76; in *Cinnamomum camphora* it ranged from 4.66 to 6.82 and in *Ficus religiosa, Grevillea robusta, Jacaranda mimosifolia* and *Populus euramericana* it ranged from 5.04 to 6.82. Higher leaf extract pH of plants, have been reported to increase their tolerance level to acidic air pollutants, especially in polluted conditions (Singh *et al.,* 1991; Govindaraju *et al.,* 2012) On the basis of leaf extract pH in the present study, *Callistemon lanceolatus,* and *Jacaranda mimosifolia* can be considered as sensitive, *Cinnamomum camphora* as modereately tolerant and *Ficus reliogisa, Grevillea robusta* and *Populus euramericana* can be suggested to be tolerant species to acidic air pollutants. These tolerant species (*Ficus reliogisa, Grevillea robusta* and *Populus euramericana*) scored higher pH value more than 6.5 at polluted sites. High pH may enhance the efficiency of converting hexose sugar to ascorbic acid, whereas low leaf extract pH correlated with susceptibility to air pollution (Escobedo *et al.,* 2008). In the presence of an acidic pollutant, the pH of the leaves decreases and the decline is greater in sensitive species than in tolerant species (Scholz and Reck, 1997). Any alteration in cell sap pH towards the acid side in the presence of an acidic pollutant may lower the efficiency of hexose sugar conversion to ascorbic acid and the reducing action of ascorbic acid is dependent on pH. Plants show more resistant towards pollution when their pH is high (Joshi and Bist, 2016).

4.2.2.3.b pH in gymnosperm

Pinus roxburgii exhibited a significant increase in the leaf extract pH at polluted sites than that of other studied gymnosperms trees as compared to the less polluted site, Narayanthan while other plants showed small pH changes or reductions, indicating the presence of acidic contaminations in the environment. There are several factors that affect plant tolerance. Plants with low pH values are considered as sensitive, and those of between 7 and 8 pH values are more resistant (Bakiyaraj and Ayyappan, 2014). On the basis of this *Cedrus deodara* (pH ranges from 4.1 to 5.76) can be considered to be more vulnerable, while other Gymnosperms (having pH range from 4.29 to 6.56) can be considered to be more tolerant.

4.2.2.4 Ascorbic acid content (AA)

4.2.2.4.a Ascorbic acid in angiosperm

Ascorbic acid is an antioxidant present in high concentrations in all growing plant parts that promotes resistance to a variety of stress conditions. Ascorbic acid provides tolerance to plants by quickly interacting with superoxide, singlet oxygen, ozone, and hydrogen peroxide, therefore participating in the elimination of reactive oxygen species (ROS) generated during aerobic metabolism and exposure to certain pollutants (Sharma *et al.,* 2012; Poljsak and Rok, 2014). Ascorbic acid plays an important role in the development and growth of plants and it is associated to a variety of physiological responses (El-Hariri, 2010). Ascorbic acid is also a well- known anti-oxidant and cellular reductant with a close and complex role in the response of plants to O_3 (Conklin and Barth, 2004). Thus, plant tolerance is determined by the amount of ascorbate present in plant cellular components, which confers resistance to plants against the negative effects of oxidative pollutants. Ascorbic acid content in the studied angiosperm plants was found to be higher. Higher ascorbic acid content may act as an antioxidant and enhance tolerance of plants to unfavourable environmental conditions such as air pollution (Keller and Schwager, 1977; Lima *et al,* 2002). In this study *Populus euramericana,* mostly had high ascorbic acid content (more than 8/mg/g) in most of the studied sites. Ascorbic acid (AA) is a powerful reducing agent that drives several defence mechanisms and physiological functions. Its reducing power is proportional to concentration (Raza and Murthy, 1988). Conklin (2001) stated that ascorbic acid being a strong reducing agent is essential for cell wall formation, defense, and cell division. In other species like *Cinnamomum camphora* at Gongobu and *Ficus religiosa* at Banasthali scored low ascorbic acid content with low leaf extract pH value. Tanee and Albert (2013) also reported reduction in the content of ascorbic acid in plant samples collected at polluted sites when compared to less polluted site. According to them, lower ascorbic acid contents were associated with lower pH of the leaves of plant samples. A shift in cell sap pH towards the acid zone in the presence of acidic pollutants might decrease the efficiency of conversion of hexose sugar to ascorbic acid (Agrawal, 1988). Ascorbic acid content in tree leaves of *Ficus religiosa, Populus euramericana, Grevillea robusta* and *Jacaranda mimosifolia* were more at some other polluted sites than in less polluted site. Plants with a high ascorbic acid content are more resistant to stress and can protect their physiological and molecular functions even when stressed. According to Chen and Gallie (2006), ascorbic acid is the most abundant antioxidant in plants and is a major contributor to the cell redox state.

4.2.2.4.b Ascorbic Acid in gymnosperm

The current findings revealed that ascorbic acid concentrations in polluted areas were higher than in less polluted sites in all the investigated gymnosperms (*Araucaria araucana, Thuja orientalis, Cedrus deodara*, and *Pinus roxburghii*). The increased rate of production of reactive oxygen species (ROS) during photo-oxidation of SO_2 to

SO_3 (Chaudhary and Rao, 1997) and other pollutants may contribute to an increase in ascorbic acid. These acids have the potential to bind harmful metals in high quantities and help in defence mechanisms (Yannawar and Bhosle, 2013). Thus, the plant with high ascorbic acid level might be interpreted as a measure of its resistance to contaminants.

4.2.2.5 Air pollution tolerance index (APTI)

4.2.2.5.a APTI in angiosperm

Air pollution tolerance index (APTI) is used for screening the susceptibility of the diverse plants group growing at the different or same pollutants level (Nadgorska-Socha *et al.*, 2017). The plants with higher APTI value are considered as a tolerant species and have been suggested for plantation to mitigate air pollution, whereas plants with lower APTI are sensitive species and have been suggested to use for bio-indicator (Singh and Rao, 1983). Depending on the APTI value, Padmavathi *et al.* (2013) grouped the plants as sensitive (with APTI value<11), intermediate (with APTI value 12 to16) and tolerant (with APTI value ≥ 17). Among the plants in the present study maximum APTI value of trees *Callistemon lanceolatus*, *Cinnamomum camphora, Ficus reliogisa, Grevillea robusta, Jacaranda mimosifolia* and *Populus* e*uramericana* could score between 12 and 15 at some polluted sites, hence these species can be grouped under the intermediate categories of APTI to combat the stress. Thakar and Mishra (2010) also categorized the plants in to different groups as tolerant (T), intermediate tolerant (IT), moderately tolerant (MT), and sensitive (S) on the basis of mean APTI values and standard deviation scored by the plants occurring at the same study sites. Among studied plant species *Populus euramericana* scored APTI tolerant (T) categories at five study sites (at Airport, Balaju, Banasthali, Dhumbarahi and Narayanthan) indicating its high tolerance to air pollutants. The tree species like *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa Grevillea robusta* and *Jacaranda mimosifolia* showed tolerant (T) categories at only one study sites, i.e., at Dhumbarahi, Dhumbarahi, Narayanthan, Airport and Dhumbarahi, respectively. This indicated that *Callistemon lanceolatuts, Cinnamomum* *camphora, Ficus religiosa, Grevillea robusta* and *Jacaranda mimosifolia* are comparatively less tolerant than *Populus euramericana.*

4.2.2.5.b APTI in gymnosperm

Though APTI value in most of gymnosperms was found to be higher at control site, but the plants like *Pinus roxburghii, Thuja orientalis, Cedrus deodara,* and *Araucaria araucana* could survive for years against air pollution along the polluted road side at different places in the city of Kathmandu. Among the studied gymnosperms, alteration in their physiology by increasing their RWC and ascorbic acid have been found to be the strategy to survive under air pollution stressed condition. The total chlorophyll content at some polluted sites was found to be reduced but at many other polluted sites were not reduced and showed inconsistent results. These inconsistent results of total chlorophyll at polluted sites could be due to their ability to synthesize chlorophyll both in dark as well as in light (Schoefs and Bertrand, 1997), which is different than in angiosperms. This could be the reason for scoring less APTI among gymnosperms. APTI tolerant (T) categories at three study sites (at Airport, Ratnapark,Shankhpark and Balaju) and *Pinus roxburghii* also scored APTI tolerant at two study sites at (Airport and Dhumbarah) whereas *Cedrus deodara* at one site (at Jawalakhel) and, *Thuja orientalis* with only moderant APTI categories. Hence *Araucaria araucana* and *Pinus roxburghii* are considered to have higher tolerance to air pollutants than *Cedrus deodara* and *Thuja orientalis.* According to Padmavathi *et al.* (2013) APTI value less than 11 were categorized as sensitive and in the present study all the gymnosperms scored less than 9, hence according to them all studied gymnosperms fall under sensitive category. But when APTI categories were calculated according to Thakar and Mishra (2010), *Araucaria araucana, Cedrus deodara* and *Pinus roxburghii* scored up to tolerant (T) APTI category at some sites. From this it is evident that simply following Padmavathi *et al.* (2013) for APTI categorization may mislead the research outcome. Padmavathi *et al.* (2013) calculated and categorized APTI among crop plants and it is not justified to categorize the same APTI value for road side trees, as the growing conditions provided for crops and road side trees are not same.

4.2.3 Metal content

Both essential and non-essential heavy metals are present in environment. Among these heavy metals, the metals like Co, Cu. Cr, Fe, Mg, Mn, Mo, Ni, Se, and Zn are essential and required in very small amounts for the growth and development of plants (Alloway, 2013). These essential heavy metals play important biochemical and physiological role in plants, especially as a constituent of many cellular enzymes and actively take part in numerous oxidation-reduction reactions (Emamverdian *et al.,* 2015). Results of the metal accumulation in road side angiosperm and gymnosperm tree leave in the present study are discussed below.

4.2.3.1 Metal content in angiosperm leaves

4.2.3.1.a Zinc (Zn)

Zn is required for several biochemical processes in plants, like auxin, chlorophyll, glucose, and protein synthesis (Broadley, 2007). Among the studied road site plants *Populus euramericana* accumulated the highest amount of Zn (132.38 mg/kg) and ranged from 14.46 at unpolluted sites to 132.38 mg/kg at polluted site (Banasthali). Similar results for Zinc accumulation in *Populus euramericana* was observed in Greece (Sawidis *et al.,* 1995) and ranged from 26.1 mg/kg at unpolluted areas to 139 mg/kg at contaminated areas which showed that plants growing in urban areas accumulated more Zn than other metals. In plants, Zn concentrations commonly range from 10 to 150 mg/kg (Padmavathiamma and Li, 2007; Hu *et al.,* 2014) but considered at toxic level when it’s concentration ranges from 300 to 400 mg/kg and differs according to the plants species. Various species of *Populus* have also been reported as a good accumulator of Zn by other researchers. Hermle *et al.* (2007) reported higher Zn concentrations in *Populus tremula* leaves than in *Salix viminalis* leaves on the calcareous subsoil, when treated with heavy metal in the laboratory using open-top chambers (OTCs) at Switzerland. Tőzsér *et al.* (2023) observed significantly Zn accumulation in leaves, stems and roots in poplar plants and found higher zinc metal accumulation in leaves than roots and stems.plants. Todeschini *et al.* (2011) reported that the movement of zinc in poplar from the soil was observed with high degree into roots and then into the above ground parts asuch as leaf. But Benyó *et al.* (2016) reported with low movement of zinc from soil in to roots in *Populus deltoides* and *Populus canadensis* which might be due to accumulation and restrained of most of the metals growing in Cu/Zn multi contaminated media. Renninger *et al.* (2013) observed significantly higher Zn concentration in leaves stems, and roots,of *Populus deltoides* from highly polluted soils as comparasion to moderately and slightly contaminated ones. Fernàndez *et al.* (2012) reported a constant positive relationship between soil and tissue concentrations of Zn while testing in two poplar clones. All these study suggest *Populus* to be a good accumulator of Zn.

4.2.3.1.b Copper (Cu)

Copper is a vital trace element for plants. Many enzymes are affected by deficiency and excess concentrations of Cu that takes part in catalyzing oxidation- reduction reactions (Celik *et al.,* 2005; Doganlar and Atmaca, 2011). Cu concentrations from 3 to 30 mg/kg in plants are a covential range (Kabata and Pendias, 2010) but it's phytotoxic concentration in plants ranges from 20 mg/kg to 100 mg/kg (Padmavathiamma and Li, 2007). In the studied plants, Cu concentration was found to be varied from 0.89 mg/kg to 20.27 in *Populus euramericana* and *Jacaranda mimosifolia,* respectively. Maximum concentration of Cu content was noted in *Jacaranda mimosifolia* at Balaju (20.27 mg/kg). Olowoyo *et al.* (2010) evaluated Cu accumulation in *Jacaranda mimosifolia,* a common tree from ten different locations during two sampling periods in Tshwane City of South Africa, and noted Cu concentrations ranging from 68.4 to 490 μg/g, which were comparatively more than that in present study. The possibility of Cu accumulation in plants, have been suggested to be from automobile emission (Olowoyo *et al.,* 2010) From these study it is evident that *Jacaranda mimosifolia* can be regarded as a good accumulator of Cu.

4.2.3.1.c Lead (Pb)

The normal or permissible concentration of Pb in trees range between from 0.1 to 5 mg/kg while it's toxic concentrations varies from 10 to 100 mg/kg (Kabata and Pendias , 2010). Pb accumulation was found to be within normal limits in all of the studied tree leaves growing along the less polluted road side while it was observed higher at polluted sites. The maximum Pb content was noticied in *Callistemon lanceolatus* (40.33 mg/kg) at Airport in the current study, and was followed by *Ficus religiosa* at various places. Tañgan (2007) studied the lead and cadmium contents in the leaves of *Callistemon viminalis* and *Ficus benjamina* grown in the polluted and unpolluted areas of Baguio City in the Philippines and showed more accumulations of

Cd and Pb in their leaves compared to the other plant species. From this it is clear that *Callistemon lanceolatus* and *Ficus religiosa* can be regarded as good accumulator of Pb.

4.2.3.1.d Metal accumulation index (MAI)

Metal Accumulation Index (MAI) depends upon a variety of factors such as sample height (tree), sampling time, local atmospheric chemistry, weather, and plant features (Hu *et al.,* 2014). Plants with higher MAI values have good accumulation capacities and also regarded as tolerant species (Hu *et al.,* 2014). In the present study plants like *Populus euramericana, Jacaranda mimosifolia* and *Callistemon lanceolatus,* which also accumulated high amount of Zn, Cu and Pb, respectively, also showed high MAI value. Hu *et al.* (2014) reported that MAI values in shrubs (like *Sabina chinensis* and *Juniperus formosana*) showed higher than trees (like *Salix matsudana var. matsudana*) and *Ailanthus altissima* growing in Yan'an city, China and also suggested that the shrubs have better heavy metal accumulation properties than trees while growing in polluted environments near the ground. Due to high biomass and large canopy cover, trees have been thought to be the most efficient in plant group for removing and screening pollutants from polluted air (Serbula *et al.,* 2012; Mok *et al.,* 2013). Large amounts of pollutants like dry aerosol particles deposited on the leaf surfaces are quickly absorbed by the plant, and get accumulated in their tissue as a result the concentrations of heavy metals increase in plants (Kleckerová and Dočekalová, 2014). Based on different metal accumulation, metal accumulation index was calculated and was compared with the micro-morphological measurements to ascertain if the hypothesis one made, i.e., ‘the trees with large leaf surface area, thin cuticle and large stomatal pore, will absorb and accumulate heavy metals in leaves’ was accepted or rejected. Metal accumulation index was found less (9.65) even with largest mean leaf area (137.19 cm^2) in *Ficus religiosa* whereas it was found just reverse in *Callistemon lanceolatus* with smallest leaf area (3.83 cm^2) but had the high metal accumulation index (15.16). The cuticle thickness in C*allistemon lanceolatus* was high (6.94 µm) but scored less metal accumulation index (15.61) whereas *Cinnamomum camphora* with thin cuticle (4.20 µm) scored high metal accumulation index (7.34) (Table 13. Likewise largest stomatal pore size (length-25.44 µm and breadth-13.23 µm) in *Ficus reliogisa* had less metal accumulation index (9.65) where as *Callistemon lanceolatus* with small stomatal pore (Length-11.45 µm and Breadth-

8.51 µm) scored high metal accumulation index (15.16). Thus from above it is evident that leaf area, cuticle thickness and size of stomatal pore did not play the role in metal accumulation so the first hypothesis stated was rejected from this study.

4.2.3.2 Metal content in gymnosperm leaves

4.2.3.2.a Zinc (Zn)

Zinc is considered as a vital plant micronutrient because it is crucial for normal cell metabolism and plant growth (Dhankhar *et al.*, 2012). The highest Zn accumulation was found in *Cedrus deodara* (54.52 mg/kg) at Dhumbarahi in the current study and was within the normal range. According to Onder and Dursun (2006), the highest Zn concentrations were detected in *Cedrus libani* needles in an industrial area (Konya city), Turkey. In the present study Zn accumulations in *Thuja orientalis* at Jawlakhel (39.49 mg kg$^{-1)}$ were followed next highest after *Cedrus deodara*. Esfandiari *et al.* (2020) reported the maximum Zn accumulation in *Thuja orientalis* leaves was 127.46 mgkg^{-1}at 35 m away and the minimum concentration was 15.02 mg kg^{-1} at 170 m away from Yazd Highway green belt, Iran.

4.2.3.2.b Copper (Cu)

The maximum increase in Cu content was noted in *Thuja orientalis* at Airport (20.16 mg/kg) in the present study. Though the mean value of Cu content (14.15 mg/kg) was within the normal range, but at some places the concentration of Cu was found to be at phytotoxic range (Above 20 mg/kg). Esfandiari *et al.* (2020) reported the highest amount of Cu (14.78 mg/kg), in the tree leaves of *Thuja orientalis* at 35 m away from the highway and its lowest value (1.05 mg/kg) was detected in the barks of the cypress tree at a distance of 170 m away from the Yazd Highway green belt, Iran.

4.2.3.2.c Lead (Pb)

In the current study, Pb concentration in all the studied plants and sampling points were found to be within normal limits and were found higher at polluted sites as compared to less polluted site. *Pinus roxburghii* at Ratnapark showed the highest accumulation of Pb (8.85 mg/kg). Kord and Kord (2011) measured Pb content in the leaves of *Pinus eldarica* from Tehran City and reported the highest Pb content at highway sites (87.22 ppm) and lowest at the control site (19.74 ppm). The chemical form of Pb within the plant has critical importance and acts as a factor in movements, translocation, and the toxic effectiveness.At local scale Pb pollution is caused by emissions from motor vehicles (Viard *et al*. 2004; Yilmaz and Zengin 2004) while vehicles using leaded gasoline emit lower value of 3 ppm which is considered as normal or natural level for plants (Allen, 1989). The close relationship between traffic intensity and lead concentrations has been studied in detail by many authors (Gromov and Emelina, 1994; Wong and Li *et al.* 2004; Viard *et al.,* 2004).

4.2.3.2.d Metal accumulation index (MAI)

Mean MAI value in the studied tree leaves of common gymnosperm ranged from 10.78 to 31.27. Minimum MAI was observed in *Pinus roxburghii* (4.27) at Jawlakhel and maximmun MAI was recorded in *Thuja orientalis* (75.78) at Ratnapark. Mostly MAI values were higher at polluted sites than at Narayanthan, i.e., less polluted site. The sequence of MAI means value in plant species from lower to higher were in the order- *Pinus roxburghii<Araucaria araucana<Cedrus deodara<Thuja orientalis.* Based on the highest mean value of MAI scored by the tree species at different study sites, it can be said that the species like *Thuja orientalis* and *Cedrus deodara* showed high heavy metal accumulation ability than the others species among gymnosperm. Evergreen conifers like *Pinus* may not be useful for removing air pollution because most of these plants keep their needles for a long period of time, so no way for recycling of accumulated pollutants Beckett *et al.* (2000). Esfandiari *et al.* (2020) reported very high metal accumulation index of 1973.16 mg/kg in *Thuja orientalis* leaves from Yazd urban area and was also able to absorb different heavy metals simultaneously along with very high concentrations of Fe and Mn. Coniferous trees have been suggested to be more efficient than broad-leaved trees in accumulating particulate matter (PM), which could be due to the presence of resin (Beckett *et al.,* 1998). Needle-shaped leaves like *Pine* species provide a relatively larger surface area, with the thick epicuticular wax on leaf surfaces, enhance the capturing capacity of PM during the winter season when pollutants are at high concentration (Kaupp *et al.,* 2000; Jouraeva *et al.,* 2002). Dzieranowski *et al.* (2011) mentioned that conifers are less tolerant towards high concentrations of traffic-related pollution so they are not usually recommended for roadside plantation. Based on the metal accumulation index (which is calculated from all studied metals measured) and APTI value in both angiosperms and gymnosperms, it was observed that the metal accumulation index and APTI value in the same species differed due to differences in metal accumulation

at different polluted and less polluted sites. From this the second hypothesis was accepted, which stated that the differences in metal accumulation in the leaf of same plant species exposed at different pollution level will have different APTI value.

4.2.4 Correlation between SLA and metal content in leaves of angiosperm

Among the studied angiosperm tree leaves from the polluted site, *Cinnamomum camphora, Callistemon lanceolatus, Populus euramerican* and *Jacaranda mimosifolia* showed a significant negative correlation between the percentage decrease in SLA and Zn accumulation. In these plants Zn accumulation was found to be lesser than it's phytotoxic level as recommended by Chaney (1993), and reduction percentage in SLA varied from 10 to 32% in *Cinnamomum camphora,* 9 to 50% in *Populus euramericana,*13 to 73% in *Jacaranda mimosifolia,* and 21 to 78% in *Callistemon lanceolatus.* This reduction in SLA might be due to stress situation and lack of some other vital elements in plants by which concentrations of one toxic metal increased (Sharma and Chettri, 2012). *Cinnamomum camphora* and *Ficus religiosa,* had low Cu contents but observed a significant positive relationship between the percentage decrease in SLA and Cu accumulation. Cu is needed at low concentrations for a number of physiological processes such as electron transport, mitochondrial respiration, regulatory protein structure, and oxidative stress responses (Raven *et al.,* 1999) The percentage decrease in SLA ranged from 10-32% in *Cinnamomum camphora* to 25-53% in *Ficus religiosa* and the positive correlation was observed between them which might be due to other factors that control SLA but no toxicity of Cu. Correlation between Cu accumulation and percentage reduction in SLA was found to be a significant negative in *Jacaranda mimosifolia*. The highest Cu accumulation was found in *Jacaranda mimosifolia* in the current study, and similar results were observed in *Jacaranda mimosifolia* by Banu *et al.* (2012).The negative relationship between SLA and Cu accumulation in *Jacaranda mimosifolia* might be due to Cu concentrations, reaching up to at phytotoxic level (20.60 mg/kg) at contaminated sites (Padmavathiamma and Li, 2007). The significant positive correlation was observed between Pb content and percentage reduction in SLA which might be due to toxicity of Pb. Toxic concentration of Pb in trees ranges from 10 to 100 mg/kg (Kabata and Pendias , 2010). In the studied tree leaves growing along the road side, Pb concentrations reached up to 39.61 mg/kg in *Ficus religiosa,* 37.8 mg/kg in *Grevillea robusta* and 27.51 mg/kg in *Populus euramericana,* in polluted areas and these concentrations were found to be at the phytotoxic level in these plants .Toxic concentration of Pb in trees ranges from 10 to 100 mg/kg (Kabata and Pendias, 2010). As a result, Pb toxicity could be the cause of the decrease in SLA with increasing Pb concentrations.

4.2.5 Correlations between metal content and APTI parameters

4.2.5.1 Correlations in angiosperm

Mostly the significant negative correlations were observed between RWC and all metal accumulation in most of the species, except *Grevillea robusta*. The excess concentrations of the heavy metals possibly affected plant water status and this might have changed RWC in the plants. The most intensive effect on the RWC of plants was observed with Cd, less intensive by Cu and Zn and the least intensive by Pb in sunflower (Kastori *et al.,* 1992). This might be the reason for showing insignificant relation of Pb accumulation in most of the trees except *Grevillea robusta*. Lead accumulation in *Lupinus* root has been shown to enhance the degree of vacuolization in meristem cells (Przymusinski and Wozny 1985) and cortical parenchyma (Gzyl *et al.,* 1997), suggesting that the metal had no effect on the water status of these cells. Therefore, Pb induced vacuolization in *Lupinus luteus* was correlated with high values of RWC, suggesting that water might be deposited in the vacuoles in response to metal stress (Rucińska-Sobkowiak, 2016). The response of RWC in *Grevillea robusta* to Pb accumulation in the present study was significantly negative, which indicates that the Pb toxicity prevailed in the leaves. From this it can be suggested that the Pb localization in the cell and its toxicity might not be same in all plants. The correlation of RWC and Cu accumulation was found to be positively significant in *Grevillea robusta.* This might be due to low concentration of Cu (below 10 mg/kg), which is an essential element and mostly not toxic below 15-20 mg/kg (Pahlsson, 1989). Correlation between AA and Zn accumulation in *Jacaranda mimosifolia* was significantly negative. Smith *et al.* (1989) reported the decrease in ascorbic acid in roots and shoots of pigeon pea with increasing concentrations of Zn. Correlation of AA content with Cu accumulation in *Ficus religiosa* and Pb accumulation in *Jacaranda mimosifolia* were significantly positive. Copper is an essential element and its maximum accumulation in *Ficus religiosa* was 15.28 mg/kg in less polluted site, indicating nontoxic level of Cu accumulation in most of the sites, which might have resulted in positive correlation. Though Pb is not an essential element, but plant absorbs Pb and mostly accumulates in the cell wall as granules (Chettri *et al.,* 2000). In the present study Pb accumulation in *Jacaranda mimosifolia* leaves reached up to 29.79 mg/kg, and showed positive correlation with ascorbic acid, which might be due to accumulation of Pb on cell wall and not interfering with the metabolic functions. But in *Cinnamomum camphora* Pb accumulation and AA showed negative correlation. Tolerance capacity of plants against various air pollutants at different sites vary due to the variation in ascorbic acid content,which might be one of the factors (Aghajanzadeh *et al.,* 2016; Agular-Silva *et al.,* 2016). In the present study ascorbic acid content mostly increased with increase in heavy metal content to combat their toxicity which also supports the findings of other researchers (Govindaraju *et al.,* 2012; Rai and Panda, 2014). Correlations of pH with most of the metal content in leaf were found negatively significant, except Cu content in *Cinnamomum camphora*. This might be be due to decrease of leaf pH value in the presence of heavy metals and an acidic pollutant (Scholz and Reck, 1977), and also suggested that the reducing rate in sensitive plants is more as compared to tolerant plant species. The positive correlation of pH with Cu content in *Cinnamomum camphora* is mainly due to the fact that both Cu content and leaf pH in Narayanthan is significantly high in this site than in other studied sites. Besides, the Cu is an essential elements and its content in *Cinnamomum camphora* is below the toxic level as suggested by Pahlsson (1989). Correlations between total chlorophyll and heavy metal content were negatively significantly in most cases. Heavy metals such as (Hg, Cu, Cr, Cd, and Zn) have been shown to reduce chlorophyll concentration in several plants (Aggarwal *et al.,* 2012). This decrease in photosynthetic pigments is most likely due to the inhibition of reductive steps in biosynthesis pathways due to synthetic pigments due to high redox potential of many heavy metals (Chettri *et al.,* 1998; Aggarwal *et al.*, 2012) Among the studied plants, *Callistemon lanceolatus, Cinnamomum camphora* and *Grevillea robusta* showed insignificant correlation of total chlorophyll with all metals. In the case of *Cinnamomum camphora* and *Grevillea robusta* also showed insignificant relation which might be due to less metal content as indicated by mean metal accumulation index (MAI) in them. In the case of *Callistemon lanceolatus* though the MAI value is 15.61, the accumulation of Zn and Cu is within the threshold limit and below the toxic level. Besides this Pb accumulation in leaves are up to 40 mg/kg but negative correlation with total chlorophyll was not observed, indicating no effect of Pb on chlorophyll content. The possible reasons for this may be their localization in the cell. Ultrastructural studies have revealed that Pb is deposited mainly in the intercellular space, cell wall and vacuoles (Wierzbicka and Antosiewicz, 1993; Chettri *et al.,* 2000) and this possibly inhibit Pb to interfere with the cell physiology. A small deposit of Pb has also been reported to be seen in the ER, dictyosomes and dictyosome derived vesicles (Wierzbicka and Antosiewicz, 1993). Correlation between APTI and Zinc content in most of the studied tree leaves were found to be negative and were significant in *Callistemon lanceolatus,* and *Populus euramericana.* Correlation of Cu content with APTI showed significant negative relation in all studied trees except *Cinnamomum camphora.* Pb content was also found to be significant negative correlated with APTI of *Cinnamomum camphora, Ficus reliogisa, Grevillea robusta* and *Populus euramericana.* Similarly, cumulative effect of Pb+Cu+Zn accumulation also showed significant negative correlation in *Callistemon lanceolatus, Cinnamomum camphora*, *Grevillea robusta* and *Jacaranda mimosifolia.* From these results it is evident that significant correlations of APTI with all metal content were mostly negative in all cases except with Cu content in *Cinnamomum camphora*. APTI is predicted on the basis of four major biochemical properties of leaves that are ascorbic acid, relative water content, total chlorophyll and leaf pH (Singh and Rao, 1983). APTI varies with these parameters because all these parameters are influenced by the increase or decrease in metal content.

4.2.5.2 Correlation in gymnosperm

Correlation between RWC and Zn content were found significantly positive in *Araucaria araucana* and *Pinus roxburghii.* Similarly Cu content in leaves of *Cedrus deodara*, *Pinus roxburghii* and *Thuja orientalis* also showed positive significant with RWC. Similarly Pb content and RWC in *Araucaria araucana* were also found to be positively significant. Generally high RWCs in most of the plants growing at polluted sites in the present study and were also reported by Rai *et al.* (2013). The significant positive relation between the metal content and RWC in above cases could be due to greater relative water content with more metal accumulation at polluted areas (Kumar *et al.,* 2019). High RWC has been suggested to be responsible for maintaining the normal physiological function at polluted sites where intake of metal is high (Kumar *et al.,* 2019; Rai *et al.,* 2013). Correlation between AA and Zn accumulation in

Araucaria araucana and *Thuja orientalis* was significantly positive. Similarly, correlation of AA content with Cu accumulation in *Cedrus deodara* was significantly positive. Ascorbic acid acts as an antioxidant in plants and develops the mechanism to resist against the adverse atmospheric conditions (Keller and Schwager, 1997). The metal accumulation in plants at polluted sites might have accelerated Ascorbic acid synthesis to combat against the toxicity of accumulated heavy metals, resulted increase in AA at polluted sites. Increase of both AA and metal content in plants at polluted sites could be responsible for their positive correlation. Mostly the correlation of AA with Pb content were insignificant, but was significantly positive with *Araucaria*. Though Pb is not an essential element, but plants absorb Pb and mostly accumulate in the cell wall as granules (Chettri *et al.*, 2000) and possibly not interfere with the metabolic functions. Aghajanzadeh *et al.* (2016) reported that variation in ascorbic acid content could vary the tolerance level of the plants against different air pollutants, growing at pollutd sites. In the present study, ascorbic acid content increased mostly with increasing heavy metal content, which is consistent with the results of Rai and Panda (2014) and Govindaraju *et al.* (2012). Correlations of pH with most of the metal content in leaf were mostly insignificant, but only in *Cedrus deodara* with Cu content, and *Arucaria* with Pb content were positively significant. Generally the presence of heavy metals and an acidic pollutant reduces leaf pH value (Scholz and Reck, 1977), and the reducing rate is more in sensitive plants compared to that in tolerant plant species. Correlations between total chlorophyll and heavy metal content in most cases were insignificant but were found positively significantly in *Cedrus deodara* (with Cu) and negatively significant in *Pinus roxburghii* (with Pb content). Generally heavy metals such as (Hg, Cu, Cr, Cd, and Zn) have been shown to reduce chlorophyll concentration in several plants (Aggarwal *et al.,* 2012). The positive correlation in *Cedrus* with Cu content (11.85 mg/kg) is possibly due to their normal concentrations within the range of essential elements. The negative correlation of *Pinus* with Pb content could be due to reduction of chlorophyll with Pb accumulation at polluted sites. Mostly positive insignificant correlation was observed between APTI and metal content but was significant in *Araucaria araucana* with Zn content, in *Cedrus deodara* with Cu content and in *Araucaria araucana* with Pb content. In both *Araucaria araucana and Cedrus deodara*, the APTI and metal content increased at polluted sites, which led to positive correlation. Along with metal accumulation the RWC in the plants also increased at polluted sites, which must have helped to combat the toxicity of metals at polluted sites (Kumar *et al.*, 2019; Rai *et al.*, 2013).

CHAPTER 5

5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the macro and micro morphological studies it can be concluded that there is reduction of thickness of the leaf cuticle and epidermis with air pollution. The stomatal density (i.e., thenumber of stomata per unit area) increased with air pollution but the length and breadth of individual stoma decreased. The comparison of APTI parameters in angiosperm plants between polluted sites (at Airport, Balaju, Banasthali, Basundhara, Dhumbarahi, Gongbu) and less polluted (Narayanthan) revealed that the relative water content was high at most of the polluted sites, but no trend was observed for Ascorbic acid at polluted sites. The leaf extract pH and total chlorophyll levels in leaves of angiosperm plants were inconsistent at polluted sites. The APTI value was found to be increased at polluted sites among both studied gymnosperms and angiosperms plants. Based on the maximum APTI value scored by each species, the evaluated common tree species with the lowest to highest APTI value were *Cinnamomum camphora>Callistemon lanceolatus>Grevillea robusta> Ficus religiosa>Jacaranda mimosifolia>Populus euramericana*. As the APTI levels of these angiosperm species ranged between 12 and 16, based on Padmavathi (2013), all were categorized as intermediate or moderately tolerant. Among gymnosperms, *Pinus roxburghii, Thuja orientalis, Araucaria araucana, Cedrus deodara,* and *Thuja orientalis* had APTI values less than 11 and hence based on APTI values according to Padmavathi *et al*. (2013), APTI categories all these gymnosperms falls under the APTI sensitive category. But according to Thakar and Mishra's APTI categories *Populus euramericana* scored tolerant (T) categories at 5 sites (Airport, Balaju, Banasthali, Dhumbarahi and Narayanthan), and other species scored tolerant category atleast at one site - *Callistemon lanceolatus* at Dhumbarahi, *Ficus religiosa* at Narayanthan, *Grevillea robusta* at Airport and *Jacaranda mimosifolia* at Dhumbarahi. Tree species *Populus euramericana* scored tolerant (T) APTI categories in most of the sites as comparasion to others showing more tolerance than other species. In gymnosperms, relative water content and Ascorbic acid were found to be greater at polluted sites than in less polluted sites. The leaf extract pH and total chlorophyll content decreased at some of the polluted sites only. The APTI tolerant (T) category was scored in *Araucaria araucana, Cedrus deodara* and *Pinus roxburghii* among the studied gymnosperms (calculated as per Thakar and Mishra, 2010), indicating that these three species had more tolerance to air pollutants than *Thuja orientalis,* which scored APTI up to moderately tolerant (MT) only.

Based on the mean concentrations of each metal accumulation, *Populus euramericana* and *Jacaranda mimoisifolia* are identified as good accumulators of Zn, *Populus euramericana*, *Jacaranda mimoisifolia*, and *Grevelliea robusta* are good accumulators of Cu; *Populus euramericana, Jacaranda mimoisifolia*, and *Grevillea robusta* are good accumulators of Pb. Among gymnosperm, *Cedrus deodara* and *Thuja orientalis* are good accumulator of Zn and Cu, and *Pinus roxburghii and Cedrus deodara* are good accumulator of Pb. *Populus euramericana, Jacaranda mimoisifolia,* and *Callistemon lanceolatus* have been suggested as ideal plants for the roadside plantation due to their high MAI value. Despite growing in the same polluted site as others, *Cinnamomum camphora, Grevillea robusta,* and *Ficus reliogisa* obtained low MAI values, indicating a metal avoidance mechanism in them. Similarly, among gymnosperm *Thuja orientalis* and *Cedrus deodara* scored high MAI values indicating their metal accumulation ability. Hypothesis one which stated that the trees with large leaf surface area, thin cuticle and large stomatal pore, will absorb and accumulate heavy metals in leaves was rejected as the metal accumulation index was found high with low leaf surface area, thick cuticle and small stomatal pore size. Second hypothesis which stated that the differences in metal accumulation in the leaf of same plant species exposed at different pollution level will have different APTI value was accepted, as the metal accumulation index and APTI value in the same species differed due to differences in metal accumulation at different polluted and less polluted sites.

5.2 Recommendations

1. Both APTI and MAI of plants should be considered while developing green belt to mitigate air pollution.

2. More than one APTI categories need to be tested to ascertain a plant as sensitive, moderately tolerant or tolerant. Simply one APTI Categories of Padmavathi *et al.* (2013) may not be enough to ascertain pollution tolerance of a plant species growing at different sites having different level of air pollution.

3. The study was mainly focused at Kathmandu valley so plants like *Populus euramericana*, *Jacaranda mimoisifolia,* and *Callistemon lanceolatus* with high APTI and MAI value are recommended for plantation along the roadside among angiosperms.

4. Similarly, among studied gymnosperms *Cedrus deodara* and *Thuja orientalis* with high MAI and also tolerant to moderately tolerant value are suggested for plantation along the roadside.

5. Evergreen trees such as *Callistemon lanceolatus, Cinnamomum camphora, Pinus roxxburghii, Cedrus deodar* and *Thuja orientalis* are recommended for plantation on roadside along with deciduous trees especially to mitigate pollution during winter season.

6. Further research on impact of dust accumulation on physiology, growth and metal content of road side trees is required.

CHAPTER 6

6. Summary

Due to rapid unplanned urbanization and various infrastructure development activities over the last few decades, air pollution in Kathmandu valley is increasing rapidly. Kathmandu valley is a bowl-like structure, surrounded by mountain ranges of Phulchowki (3132 m) in the south west, Shivapuri (2713 m) in the north, Champa Devi (2400 m) in the south west, and Nagarjun (2100 m) in the west. The pollutants are trapped in the valley and the air pollution problem is further aggravated by the "thermal inversion". The overall objectives of this work was to investigate the impact of air pollution on macro and micro morphology of leaves, their physiological parameters and metal accumulation abilities of both angiosperm and gymnosperm trees. Based on these parameters, tolerant and sensitive species were identified and ultimately the tolerant species were suggested for plantation while developing green belt areas.

To fulfill these objectives leaf samples of different trees were collected from different biotopes of Kathamndu valley (Airport, Balaju, basundhara, Chabhil, Dhumbarahi, Gangabu, Samakhusi, Sankhapark, Swyambhu, Tinkune, Ratnapark, Jawlakhel, and Lainchaur) as polluted sites, and Narayanthan (Budhanilkantha) a considerably less polluted site. Fully matured leaf samples were collected in the morning from above 3m height and immediately brought to the laboratory, where they were cleaned and then analyzed for micro morphology, APTI, and metal analyses. For the macro and micromorphological study, leaf Samples of tree leaves (*Callistemon lanceolatus, Celtis australis, Cinnamomum camphora, Eucalyptus globulus, Ficus elastica, Ficus religiosa, Grevillea robusta, Jacaranda mimosifolia, Populus euramericana, Prunus cerasoides*) were collected from Airport, Balaju, Dhumbarah (polluted site) and Narayanthan (Less polluted site). Macro and Micro morphological characteristics like specific leaf area, stomata density, length and breadth of guard cells were measured from abaxial leaf surface, and thickness of epidermis and cuticle were measured from adaxial leaf surface by lasting impression technique. Photographs of anatomical slides were taken and the measurements were taken using Image J computer program.To evaluate APTI, common roadside leaves of trees were collected from the Airport, Balaju, Banasthali, Basundhara, Dhumbarahi, Gongbu (polluted sites) and

Narayanthan areas (Less polluted site). Six angiosperm tree species were collected from polluted and less polluted sites, while four gymnosperm tree species were selected from Airport, Dhumbarahi, Jawalakhel, Ratnapark, Shankpark, area (polluted sites), and Narayanthan (Less polluted site). Air pollution tolerance index (APTI) values of the trees were calculated considering the biochemical parameters, i.e., relative water content, total leaf chlorophyll, ascorbic acid and leaf extract pH by using standard method. The biochemical parameters like Chlorophyll content, ascorbic acid and leaf extract pH of fresh leaves were measured on the same day of sample collection. For relative water content, fresh leaf weight was taken immediately after cleaning the dust from the collected leaf samples and further steps were continued later. After the subsequent measurement of RWC, Tch, AA, and leaf extract pH, APTI values of plants were calculated by using the equation of Singh and Rao (1983). Leaves of common road side trees like *Callistemon lanceolatus, Cinnamomum camphora, Ficus reliogisa, Grevillea robusta, Jacaranda mimosifolia, Populus euramericana Araucaria araucana*, *Cedrus deodara, Pinus roxburghii*, and *Thuja orientiales* collected from different sites were used for the measurement of heavy metal content. Representative samples of each species at each site were prepared for metal analysis. Each of the representative leaf samples were wet digested and then metal content in the filtrate was measured according to US EPA (1996) using Perkin Elmer (2380) Atmospheric Absorption Spectrometer (AAS). To evaluate the overall metal accumulation performance of the plants, the metal accumulation index (MAI) of each species was calculated according to Liu *et al.* (2007). The data obtained from the macro and micro morphological study was statistically analyzed by using one sample T test. Similarly, data of various biochemical parameters were statistically analysed using one-way ANOVA followed by Duncan's multiple range test. Data from biochemical measurements (for APTI) and concentrations of different heavy metals (Zn, Cu, and Pb) were again subjected to Pearson's correlations test to determine the relationship between biochemical parameters and heavy metal content.

The results of macro and micro morphological study revealed that leaf area, specific leaf area, cuticle thickness, epidermal thickness, stomatal size (i.e., length and width of guard cells) decreased at polluted sites while stomatal density increased at all polluted sites. Maximum decrease in leaf area and specific leaf area was found in *Celtis australis* and *Ficus elastica,* respectively. Maximum decrease in cuticle thickness at polluted sites was observed in *Callistemon lanceolatus* and minimum decrease in cuticle was in *Populus euramericana.* At polluted sites, thickness of epidermis was found to be reduced in all plants and was significant (*p=0.05*) in *Callistemon lanceolatus, Celtis australis, Cinnamomum camphora, Eucalyptus globulus, Ficus elastica, Ficus reliogisa, Gravelliea robusta, Populus euramericana Prunus cerasoides.* The deposited pollutants might have interrupted several physiological processes resulting in a reduction in epidermal cells. Stomatal density increased significantly (*p=0.05*) in *Callistemon lanceolatus, Celtis australis, Cinnamomum camphora, Ficus elastica, Ficus reliogisa, Graveliea robusta, Populus euramericana* and *Prunus cerasoides* at polluted sites except *Eucalyptus globulus*. The decrease in stomatal size in all plants at polluted sites may be due to a pollutant's inhibitory effect on physiological activities such as photosynthesis (Verma *et al.,* 2006). Besides this the increase in stomatal density in polluted sites is possibly to fulfill the required gas exchange for physiological processes.

To calculate APTI, four physiological parameters like AA, Tchl, pH and RWC were measured. Ascorbic acid (AA) content in the majority of the studied sites, increased (more than 8mg/g) in *Cinnamomum camphora, Ficus religoisa, Populus euramericana,* and *Jacaranda mimosifolia*, but it was low in other species like *Callistemon lanceolatus* and *Gravillea robusta*. Ascorbic acid content of *Ficus religiosa, Cinnamomum camphora, Gravillea robusta,* and *Jacaranda mimosifolia* tree leaves was found to be higher in polluted sites than in less polluted site. Similarly, ascorbic acid content was found to be lower in the leaves of gymnosperms such as *Thuja orientalis, Cedrus deodara, Araucaria araucina* and *Pinus roxburgii* than in angiosperm tree species. Plants with a high ascorbic acid content are more resistant to stress and can protect their physiological and molecular functions even when stressed. Total chlorophyll (TChl) content in the same species were reduced in some polluted sites than in Narayanthan (a less polluted site). Total chlorophyll was found to be lower in *Ficus religiosa* and *Araucaria araucana* than in less polluted site. In addition to heavy metal the total chlorophyll content in the plant leaves has been reported to be affected by soil pollution, gaseous pollutants (such as SO_2), high temperatures, drought, salt stress, and daylight intensity (Keller and Schwager, 1997; Zhang *et al.*, 2016). In contrary to this, *Cinnamomum camphora*, *Grevillea robusta*, *Jacaranda mimosofolia*, *Populus euramericana*; *Callistemon lanceolatus* including the leaves of gymnosperm plants *like Pinus roxburghii*, *Thuja orientalis, Cedrus deodara* had high total chlorophyll at some polluted sites than the less polluted site. Most of these heavy traffic areas had greater CO_2 to enhance chlorophyll content on the one hand, and also the presence of comparatively moderate to higher Zn and Pb content in their leaves possibly helped to protect chlorophyll degradation in them (Chettri *et al.*, 1998).

The pH of leaf extracts of *Cinnamomum camphora, Ficus reliogisa, Grevillea robusta*, and *Jacaranda mimosifolia* was significantly lower (p=0.05) in most of the polluted sites than in Narayanthan (Less polluted site). However, leaf extracts pH in gymnosperm like *Pinus roxburghii*, *Thuja orientalis, Cedrus deodara*, and *Araucaria arucana*, was found to be lower in less polluted site than at polluted sites. On the basis of pH of leaf extract, *Cinnamomum camphora, Ficus reliogisa, Grevillea robusta,* and *Jacaranda mimosifolia* can be regarded sensitive to acidic air pollutants, whereas *Callistemon lanceolatus* and *Populus euramericana* can be as considered as tolerant. Similarly, among gymnosperms, *Araucaria arucana* and *Thuja orientalis* were found to be sensitive, whereas *Pinus roxburgii* and *Cedrus deodara* were tolerant.

Most of the studied plants showed increased relative water content (RWC) at polluted sites, but in some species it was reduced at polluted sites than that of less polluted site. RWC is probably the most appropriate measure to know the status of water in plants in terms of the physiological consequence of cellular water deficit. In the present study at less polluted site plants like *Callistemon lanceolatus, Cinnamomum camphora, Ficus reliogisa, Grevillea robusta,* and *Populus euramericana* measured low RWC than at polluted site. Similarly in gymnosperms plants *like* in *Pinus roxburghii, Thuja orientalis, Cedrus deodara*, and *Araucaria arucana* measured low RWC at less polluted site compared to the polluted site. RWC of a plant species might increase or decrease with increasing pollution load depending upon several factors such as local climate of an area, season, plant species type, pollutant type, and the tolerance level of plant species (Sharma *et al.*, 2017). More RWC in plants can dilute acidity inside the leaf cell sap and also resist the drought condition in plants (Kaur and Nagpal, 2017). High water content in plants ensures the maintenance of the physiological balance under stresses condition against air pollution (Deepalakshmi *et al.*, 2013). Hence, plants with higher RWC under polluted conditions have been suggested as tolerant to air pollutants (Dhankhar *et al.*, 2015). In the present study, plants like *Callistemon lanceolatus, Cinnamomum camphora, Ficus reliogisa, Jacaranda mimosifolia, Pinus roxburghii, Cedrus deodara, Araucaria arucana,* and *Thuja orientalis* measured RWC more than 80% in some polluted areas, indicating more resistant to air pollutants.

Padmavathi *et al.* (2013) grouped the plants as sensitive (with an APTI value of<11), intermediate (with an APTI value of 12 to 16), and tolerant (with an APTI value ≥ 17). In the present study trees like *Callistemon lanceolatus, Cinnamomum camphora, Ficus reliogisa, Grevillea robusta, Jacaranda mimosifolia,* and *Populus euramericana* scored APTI value between 12 and 15 at some polluted sites, so these species can be grouped under the intermediate categories of APTI. Thakar and Mishra (2010) categorized the plants as tolerant (T), moderately tolerant (MT), intermediately tolerant (IT), and sensitive (S) based on the mean APTI values of a species and standard deviation scored by the same species at the same study sites. *Populus euramericana* scored APTI tolerant (T) category at five study sites (Airport, Balaju, Banasthali, Dhumbarahi and Narayanthan), showing a high tolerance to air pollutants at more sites as compared to other tree species whereas *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa, Grevillea robusta* and *Jacaranda mimosifolia* scored tolerant (T) category at only one study site, i.e., at Dhumbarahi, Dhumbarahi, Narayanthan, Airport, and Dhumbarahi, respectively. This indicated that *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa, Jacaranda mimosifolia* and *Grevillea robusta* were less tolerant than *Populus euramericana*. Tree species like *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa, Jacaranda mimosifolia* and *Grevillea robusta* scored mostly moderately tolerant APTI categories at many sites indicating that they are less tolerant to air pollutants than the *Populus euramericana*. Likewise in most of the gymnosperms, higher APTI values were observed than at the less polluted site which indicated that the studied plants (like *Pinus roxburghii, Thuja orientiales, Cedrus deodara*, and *Araucaria araucana*) could survive against air pollution along the roadside in Kathmandu valley. APTI tolerant (T) category was recorded in *Araucaria araucana* and *Pinus roxburghii* among the studied gymnosperm, indicating that these species had better tolerance to air pollution than *Thuja orientialis* and *Cedrus deodara* (which scored APTI up to moderately tolerant only). Padmavathi *et al.* (2013) stated that APTI values less than 11 were categorized as sensitive, and in the present study, all gymnosperms scored less than 9, therefore they were grouped into the sensitive category. But when APTI categories were calculated according to Thakar and Mishra (2010), *Araucaria araucana* and *Pinus roxburghii* scored up to tolerant (T) APTI category at most of the sites. From this it is evident that simply following Padmavathi *et al.* (2013) for APTI categorization may mislead the research outcome. Padmavathi *et al.* (2013) calculated and categorized APTI among crop plants and it is not justified to use the same APTI value for road side trees, as the growing conditions provided for crops and road side trees are not same.

Heavy metals (Zn, Cu and Pb) accumulation were measured in the leaves of road side trees, Maximum Zn accumulation in leaves were observed in high traffic areas like Airport, Balaju, Banasthali, Basundhara, Dhumbarahi, Gongbu, Shank park, Jawalakhel, Ratnapark, in the present study, which might be due to tailpipe emissions, brake and tire wear. Zinc accumulation in the leaves of *Cinnamomum camphora, Ficus religiosa*, and *Grevillea robusta* was low (23.23 mg/kg) in comparison to *Populus euramericana* (132.38 mg/kg). *Cedrus deodara* had the highest Zn accumulation among studied gymnosperm plants whereas *Araucaria arucana* had the lowest. In comparison to angiosperms, Zn accumulation in gymnosperms was comparatively low, which possibly is due to their thick cuticle. Zn concentrations in the leaves in the present study were below the phytotoxic level hence toxicity among roadside trees was not expected. Roadside trees like *Populus euramericana, Callisatemon lanceolatus,* and *Cedrus deodara* showed good accumulations of Zn in leaves, hence are regarded as good accumulator of Zn among the studied trees. Cu accumulation in *Jacaranda mimosifolia* at Balaju (Cu 20.27 mg/kg) and *Ficus religiosa* at Narayanthan (Cu 15.28 mg/kg) was slightly high and showed phytotoxic effect like significant reduction in total chlorophyll content. High amount of Cu accumulation in polluted sites may be related to tailpipe emissions, brake and tire wear, but in the less polluted area (Narayanthan), it may be associated to the carpet and handycraft industries, which utilize different copper-rich woollen dyes and paints. Copper accumulation in the studied gymnosperms varied from 1.48 mg/kg (in Araucaria) to 20.16 mg/kg (in *Thuja orientalis*). The mean value of Cu accumulation in all studied sites was lowest in *Araucaria araucana* and highest in *Thuja.* Copper accumulation was significantly higher in *Thuja orientalis* at Airport, and *Pinus roxburgii* at Dhumbarahi and Ratnapark.Concentrations of Pb in tree leaves ranged from 0.61 to 40.33 mg/kg in *Callistemon laneolatus* 0.83 to 32.06 mg/kg in *Cinnamomum camphora* 0.05 to 39.61mg/kg in *Ficus religiosa,* 4.97 to 37.8 mg/kg in *Grevillea robusta* 1.40 to 29.37 mg/kg in *Jacaranda mimosifolia* and 0.59 to 27.51 mg/kg in *Populus euramericana.* Lead accumulation in the leaves of *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa, Grevillea robusta, Jacarand mimosifolia* and *Populus euramericana* were mostly found higher at polluted sites. Similarly, lead accumulation in *Thuja orientalis* at Airport and *Pinus roxburgii* at Ratnapark, was significantly higher. Pb in *Pinus roxburgii* showed higher than at less polluted site, Narayanthan. Based on the present study, it is evident that the soil and/or air of our ambient environment are not free from lead, even the Government of Nepal is importing unleaded gasoline but the remaining of the lead still exist in our surroundings.

Plants with high MAI values have good accumulation capacities and also regarded as tolerant species (Hu *et al.*, 2014). On the basis of MAI value obtained it can be said that the species like *Callistemon lancolatus, Jacaranda mimosifolia* and *Populus euramericana* have more heavy metal accumulation ability than the others and the species like *Grevillea robusta, Ficus religiosa* and *Cinnamomum camphora* among the angiosperms, and both gymnosperms (*Pinus roxburgii* and *Araucaria araucana*) had the least.

Significant negative Correlation of APTI with all metal content in all cases (except with Cu content in *Cinnamomum camphora*) indicated that when the metal accumulation is high, APTI value decreases. As APTI is determined by four biochemical parameters and all these parameters are also influenced by the increase or decrease in metal content. Though APTI values of all studied angiosperms were within intermediate range, but because of high MAI value, *Populus euramericana, Jacaranda mimoisifolia* and *Callistemon lanceolatus* have been identified as the suitable species for the plantation near the road side.Despite growing in the same polluted environment as others, *Cinnamomum camphora*, *Grevillea robusta*, and *Ficus reliogisa* scored less MAI values, indicating a metal avoidance mechanism in them. Based on the findings, it can be stated that both APTI and MAI should be considered while selecting plants to mitigate air pollution problem in urban areas. Similarly, based on the mean value of each metal accumulation, *Populus euramericana, Jacaranda mimosifolia,* and *Callistemon lanceolatus,* showed good accumulation of Zn whereas *Jacaranda mimosifolia* and *Ficus reliogisa* showed good accumulation of Cu, and plants like *Callistemon lanceolatus, Ficus reliogisa* and *Grevelliea robusta* showed good accumulation of Pb. In gymnosperms *Cedrus deodara, Thuja orientalis* and *Pinus roxburgii* showed good accumulation of Zn. Copper accumulation efficiency was observed in *Cedrus deodara* and *Thuja orientalis* while Pb accumulation efficiency was good in *Pinus roxburgii* and *Cedrus deodara.* On the basis of this study, to mitigate air pollution problem and reduce toxic heavy metals from the air, it is recommended to establish green belt having diversity of plant species (mixing both angiosperms and gymnosperms) with high metal accumulation ability, rather than to grow a single species. Thus near the road, monocultures of any one tree species or only deciduous trees should be avoided or supplemented by introducing other tolerant and good accumulator species.

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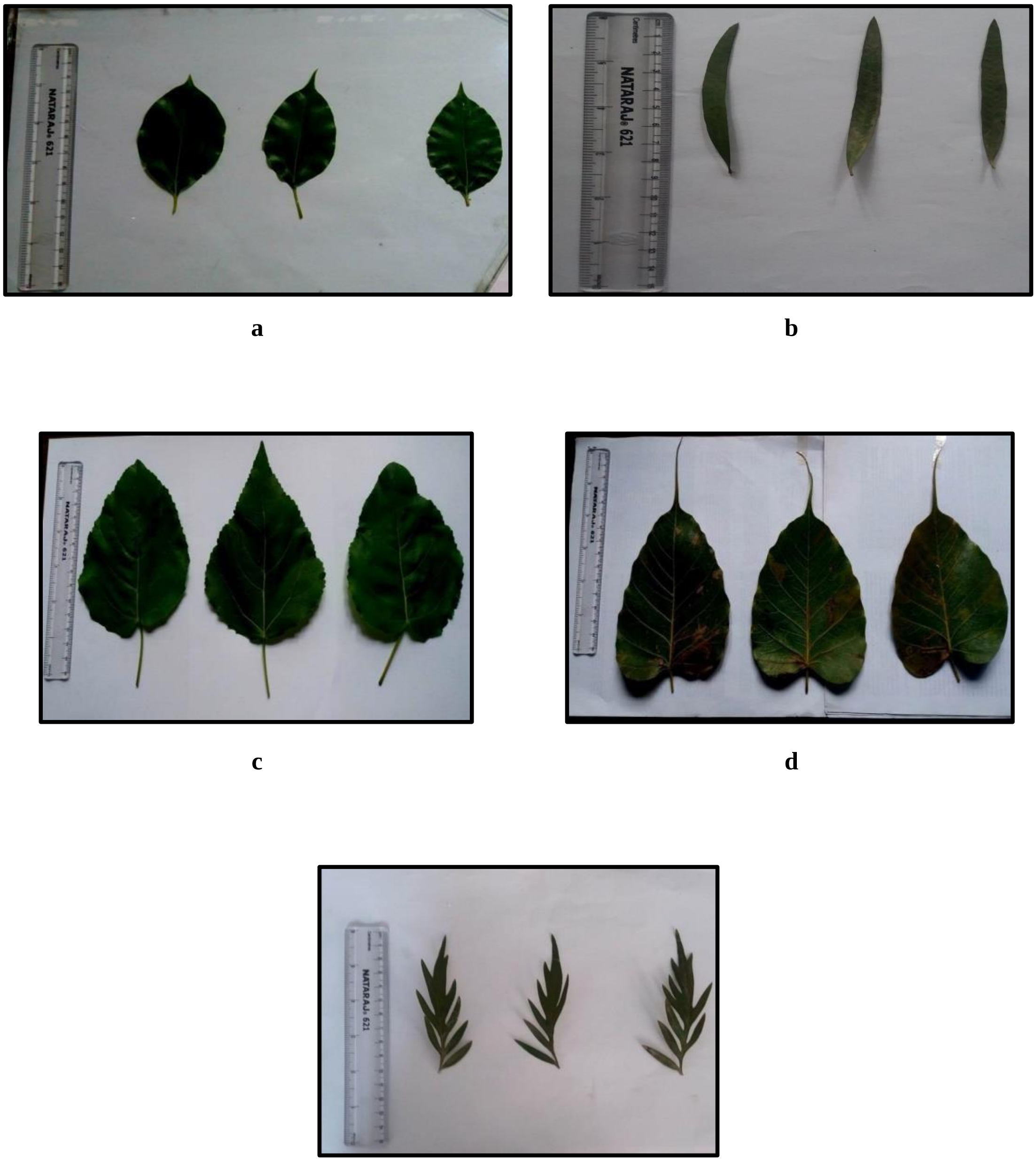
APPENDICES

Appendix 1: Images for measurments of SLA and Leaf area



a

b

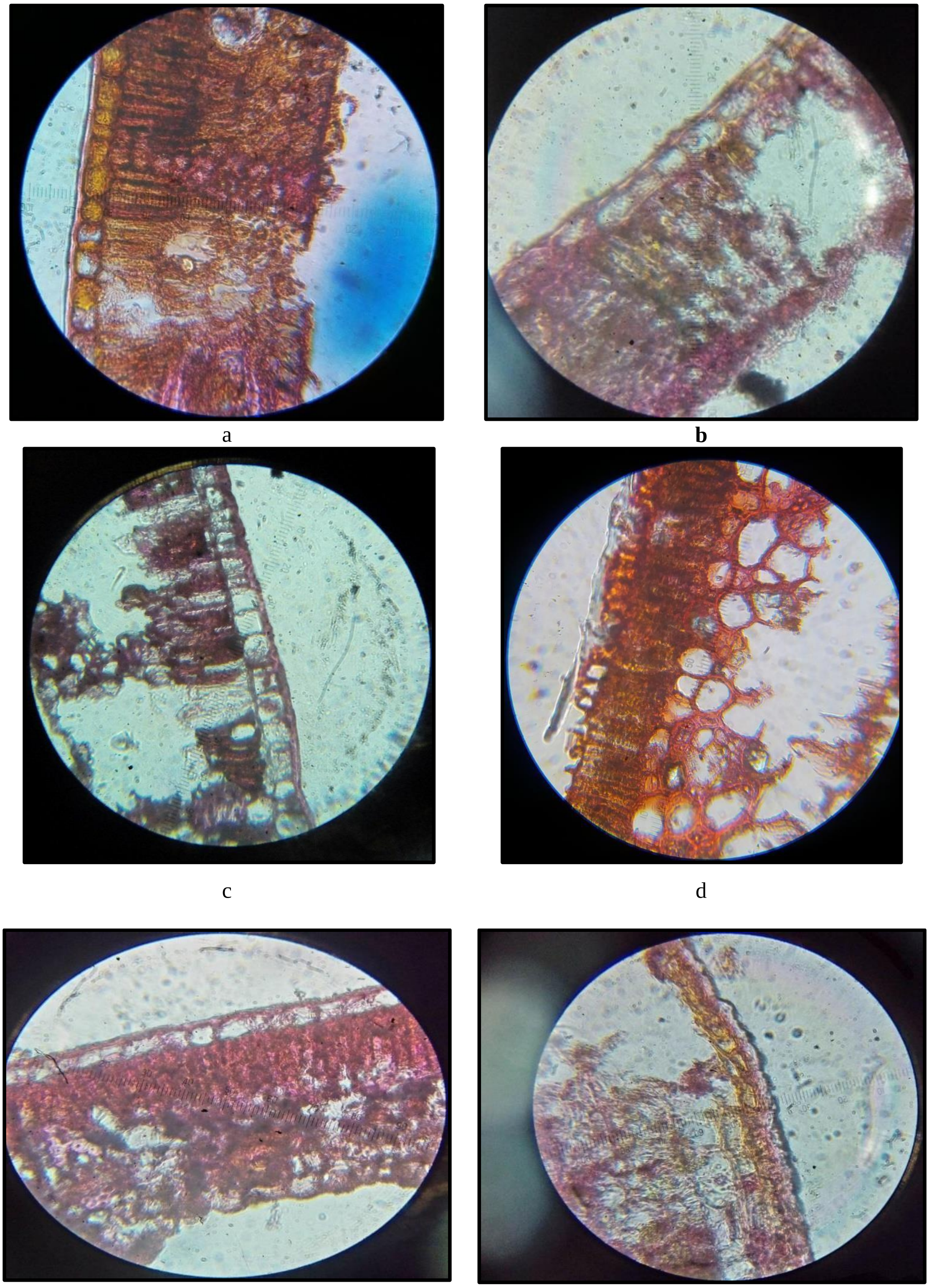
c

d

E

a. *Cinnamomum camphora* b. *Callistemon lanceolatus* c. *Populus euroamericana* d. *Ficus religiosa* e. *Grevillea robusta*

2) Images for measurement of micro morphological characters of leaf



a

b

c

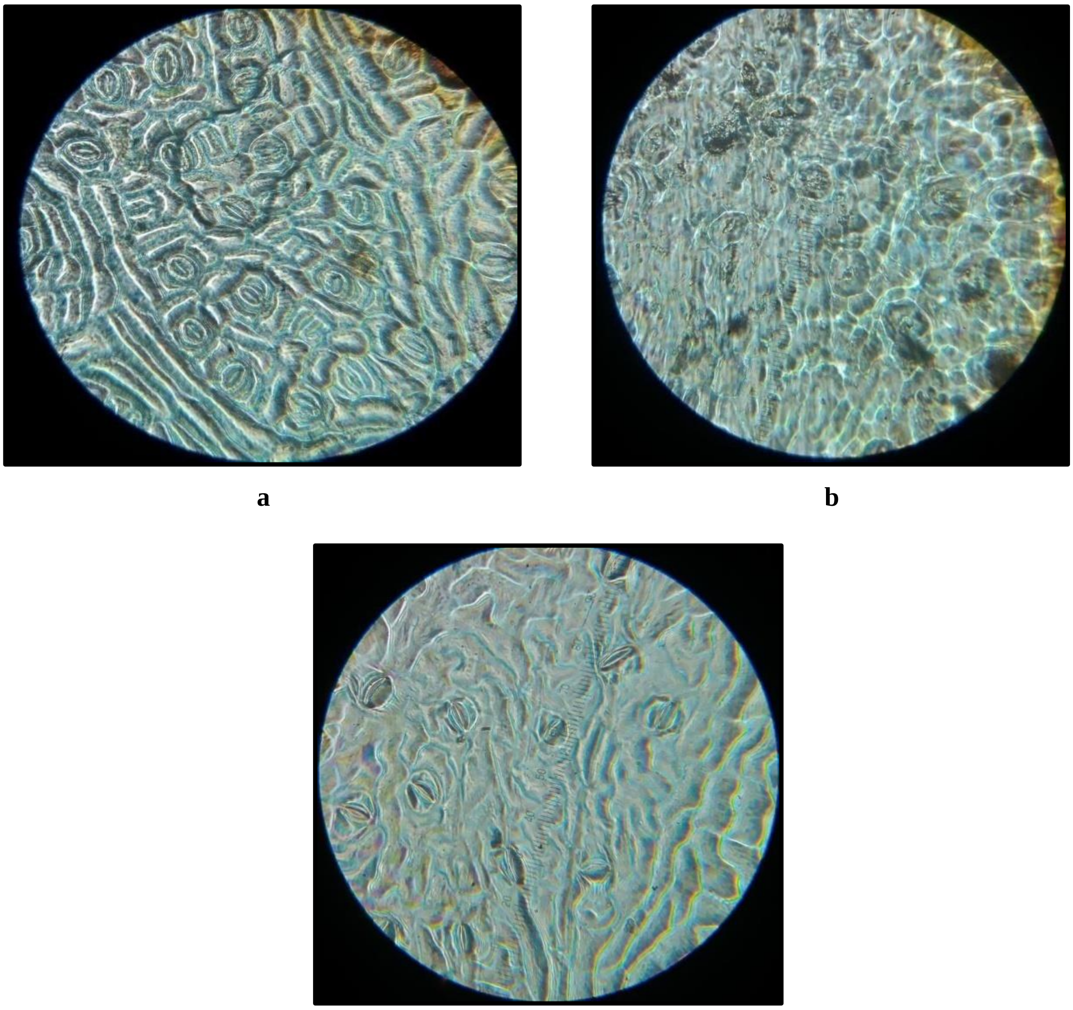
d

e

f

a. *Cinnamomum camphora* (Polluted site) b. *Cinnamomum camphora* (Less polluted site) c. *Callistemon lanceolatus* d. *Grevillea robusta* (Less polluted site)e. *Grevillea robusta* (Polluted site) f. *Ficus elastica* (Polluted site)

3) Image for measurment of stomatal length, breadth and density



a **b**

c

a. *Cinnamomum camphora* b. *Callistemon lanceolaouts* c. *Populus euramericana*

4) Photos of sample collection, lab work and conference

a

b

c

a.Sample collected at less polluted site b. Mesuring chlorophyll and ascorbic acid at Amrit Campus, Botany Lab c. Attended International Conference

Appendix 2: List of publications

1. Air pollution tolerance index of some selected gymnosperm species along the road side of Kathmandu valley, Nepal. *ECOPRINT, 24:* 13-19, 2017. www.nepjol.info/index.php/eco; www.ecosnepal.com

2. Screening of some road side trees for plantation in polluted urban areas of Kathmandu, Nepal. *Pollution Research, 40* (May Suppl. Issue): S45-S53, 2021

3. Impact of heavy metals and biochemical parameters on specific leaf area of roadside trees in Kathmandu, *Ecology Environment and Conservation. 28* (3):1108-1118, 2022. DOI No.: http://doi.org/10.53550/EEC.2022.v28i03.005

ECOPRINT **24:** 13-19, 2017 ISSN 1024-8668
DOI: http://dx.doi.org/10.3126/eco.v24i0.20642
Ecological Society (ECOS), Nepal
www.nepjol.info/index.php/eco; www.ecosnepal.com

AIR POLLUTION TOLERANCE INDEX OF SOME SELECTED GYMNOSPERM SPECIES ALONG THE ROAD SIDE OF KATHMANDU VALLEY, NEPAL

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ABSTRACT

Response of plants towards air pollution is assessed by air pollution tolerance index (APTI). Four species of Gymnosperms (*Thuja orientiales, Cedrus deodara, Pinus roxburghii* and *Araucaria bidwillii)* were evaluated for APTI. Leaves were collected during winter season from polluted sites (Airport, Dhumbarahi, Jawalakhel, Ratnapark, and Sankhapark) and less polluted site (Narayanthan) of Kathmandu valley. Of four gymnosperm species collected from road side, all species (*Cedrus deodara, Araucaria bidwillii, Thuja orientiales* and *Pinus roxburghii*) showed high value of APTI (i.e., more than 8), indicating their resistance to air pollution.

Key words: Total chlorophyll content, Relative water content, Total ascorbic acid, Leaf extract, and pH.

INTRODUCTION

Kathmandu has a pollution load in the air containing various pollutants like sulphur oxide, oxide of nitrogen, hydrocarbon, and particulates (Shrestha 2001). Rapid urbanization, industrialization, poor maintenance of road, poorly maintained vehicle deteriorate the air quality in Kathmandu valley (MaYa 2014). Bowl-shaped topography of Kathmandu valley restricts air movement and traps pollutants, and accelerates air pollution.

There are approximately 755,546 vehicles registered in the Bagmati Zone (DOTM 2014). The number of registered vehicles is rapidly increasing in Kathmandu, particularly, in the last five years accompanied with the rapid increase of urban population and economic development. The motorcycle has increased at an alarming rate of more than 20% in the past five years (JICA 2012).

According to the Pollution Index Rate 2016 published by numbeo.com, a user-generated cost-of-living statistics website, Kathmandu was ranked at the 3rd position with 96.60 pollution index (The Kathmandu Post, March 19, 2016). From the above it is clear that air of Kathmandu contains high amount of dust particles and causes adverse impacts on human health. Impacts of air pollution and dust on plants have largely been neglected. Therefore, in this study an attempt has been made to understand the impacts of pollution on different physiological parameters of some road side gymnosperms and their air pollution tolerance index.

MATERIALS AND METHODS

Kathmandu valley is located between 27°37'30" N and 27°45'0" N latitude, and 85°15'0" E and 85°22'30" E longitude with 340 sq km area. The valley is bowl-like structure surrounded by high hills and the altitude of valley floor varies between 1300 m and 1400 m. The prominent boundary features of the valley are Phulchowki Hill (2782 m) in South east, Shivapuri (2713 m) in North, Champa Devi (2400 m) in South West and Nagarjun (2100 m) in West.

A sub-tropical type of climate prevails in Kathmandu valley. The mean annual temperature in the Kathmandu valley is 18°C. The coldest month is January with a mean temperature of 10°C. The warmest months are July and August, with an average temperature of 24°C. The valley has an annual rainfall of 1343 mm. The wettest month is July with an average rainfall of about 378 mm. December is the driest month, the average rainfall is less than 2 mm (www.nepal.climatemps.com/precipitation.php).

Polluted and less polluted sites were selected on the basis of frequency of movement of traffic vehicles. Experimental sites with high traffic areas like Airport, Dhumbarahi, and Sankhapark are along the ring road whereas experimental sites like Ratnapark and Jawalakhel are located inside ring road. Narayanthan was selected as a control site because of less traffic and less air pollution.

Leaf samples of *Thuja orientiales*, *Cedrus deodara*, *Pinus roxburghii* and *Araucaria bidwillii* were collected from different polluted and less polluted sites in winter (December-January). Fully matured leaves were collected in the morning around 9 to 10 am and brought to laboratory for physiological analysis.

Fig. 1. **Map of Study site: Kathmandu valley**
1) Airport **2) Dhumbarahi,**
3) Jawalakhel **4) Ratnapark,**
5) Shankhapark **6) Narayanthan**

Estimation of relative water content (RWC)

RWC was determined according to Barrs and Weatherly (1962). RWC is a ratio of the amount of water in the leaf tissue at sampling to that present when fully turgid. A composite sample of leaf discs was taken and the fresh weight was taken and then leaf was floated on water for up to 24 h. The turgid weight was then recorded, and the leaf tissue was subsequently oven-dried to a constant weight at about 85°C for 24 h. RWC is calculated by using the following formula:

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

Chlorophyll

Chlorophyll estimation was conducted according to Barnes *et al*. (1992). 0.05 g of leaves were cut in smaller pieces and placed in test tubes containing 5 ml DMSO. Test tubes were incubated in a water bath at 60-65ºC for an hour. From preliminary studies this time was judged satisfactory for the full decolorisation of tissues. Cooling at room temperature was followed for 30 min, filtration and absorption measured at 665 nm and 648 nm being the final stages. Blank determination was carried out with DMSO. Absorption measurement was carried out with a Spectrophotometer.

Chlorophyll concentration (a, b and total) was expressed as mg/g fresh weight and determined by the following formulae[16] (Barnes *et al.* 1992).

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

Chlorophyll b (mg/g F.W) = (25.48 A_{665} – 7.36 A_{648}) (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

Least extract pH

Leaf-extract pH was determined with pH meter. Five g 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).

Ascorbic acid (AA)

Ascorbic acid content (expressed in mg/g) was measured using spectrophotometric method (Bajaj and Kaur 1981). One g of the fresh foliage was taken, 4 ml oxalic acid EDTA extracting solution was added to it, then one ml of Orthophospheric acid to it. 1 ml 5% tetraoxosulphate acid was added to this mixture, and then 2 ml of ammonium molybdate and 3 ml of water was added. The solution is then allowed to stand for 15 min after which the absorbance at 760 nm is measured with a spectrophotometer. The concentrations of ascorbic acid in the sample then extrapolated from a standard ascorbic acid curve.

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.

Statistical analysis

The data obtained from the above experiments were statistically analyzed using SPSS version 16. ANOVA followed by Duncan’s Multiple range test was conducted to understand significant differences (at P = 0.05) among different sites.

RESULTS

Total Chlorophyll in all four species (*Pinus roxburghii*, *Thuja orientialis, Cedrus deodara* and *Araucaria bidwillii*) showed reduction at one or two sites. Total chlorophyll content reduced significantly in *Pinus* at Ratnapark and Shankhapark. Similarly in total chlorophyll (TCH) was reduced in *Thuja* significantly at Shankhpark and Airport. In case of *Cedurus* TCH was reduced significantly (P = 0.05) at Jawalakhel and Ratnapark area. total chlorophyll in *Araucaria* reduced in all sites than in Narayanthan, a control site.

Relative water content was significantly higher (P = 0.05) at all polluted sites than in Narayanthan (Table 1). Almost similar pattern for RWC were observed in all species. In *Pinus* and *Thuja* highest RWC was observed at Dhumbarahi and Airport, respectively. But highest RWC was recorded at Ratnapark for both *Cedrus* and *Araucaria*.

pH of leaf extract was found to be increased significantly (P = 0.05) in *Pinus roxburghii* at all studied polluted sites. In *Thuja* pH reduced significantly at Dhumbarahi. Inconsistent result was seen in *Cedrus* leaf pH, in some sites (Ratnapark and Jawalakhel). At Airport, Dhumbarahi and Shankhapark significant decrease was observed in pH than in Narayanthan. In *Araucaria*, significant decrease in pH of leaf extract was observed at Jawalakhel.

Ascorbic Acid concentration in all species increased at most of the polluted sites than in less polluted site Narayanthan. Concentration of

Ascorbic acid in *Thuja orientialis* increased significantly at all sites than in Narayanthan (control site). In *Pinus*, significant increase in Ascorbic Acid was recorded at Jawalakhal, Ratnapark and Shankhapark.

APTI values at all polluted sites were found to be higher than in less polluted site, Narayanthan. Among the studied gymnosperms, APTI value was recorded more than 8 in all gymnosperms, indicating their ability to withstand pollution.

Table 1. Mean value ± standard deviation of total chlorophyll (TCH in mg/g), Relative water content (RWC in %), pH, Ascorbic acid (AA in mg/g) and Air pollution tolerance index (APTI) of (A) *Pinus roxburghii*, (B) *Thuja orientialis*, (C) *Cedrus deodara* and (D) *Araucaria bidwillii* at different sampling sites of Kathmandu valley.

(A) Pinus roxburghii

Sites	TCH±SD	RWC	pH	AA	APTI
Airport	3.530±0.216 B	83.470±8.909 CD	6.210±0.197 C	0.464±0.053 A	8.801±0.943 B
Dhumbarahi	3.736±0.617 B	85.717±5.950 D	4.632±0.120 B	0.445±0.057 A	8.941±0.571 B
Jawalakhel	3.645±0.111 B	72.71±4.743 B	6.160±0.089 C	0.843±0.088 B	6.891±0.320 A
Ratnapark	0.629±0.283 A	77.161±4.831 BC	6.160±0.089 C	0.725±0.015 B	7.505±0.804 A
Shankhapark	0.684±0.47 A	77.57±5.518 BC	6.160±0.089 C	0.832±0.065 B	7.827±0.940 A
Narayanthan	3.555±0.586 B	61.36±5.428 A	4.294±0.164 A	0.485±0.160 A	7.537±0.376 A

(B) ***Thuja orientialis***

Sites	TCH±SD	RWC	pH	AA	APTI
Airport	2.364±0.1234AB	81.799±5.510C	6.320±0.17889B	0.465±0.053B	8.447±0.637 C
Dhumbarahi	3.551±0.579C	76.814±4.152BC	5.300±0.27386A	0.424±0.848B	8.093±0.459BC
Jawalakhel	3.665±0.181BC	72.393±5.189B	6.464± 0..25938B	0.716±0.1406C	8.138±0.577BC
Ratnapark	3.378±0.177C	72.393±5.28B	6.564±0.03578B	0.695±0.067C	7.597±0.641ABC
Shankhapark	0.599±0.236A	73.824±6.45B	6.516±0.00894B	0.843±0.0516D	6.939±2.109AB
Narayanthan	3.295±0.585BC	60.205±1.960A	6.520±0.05814B	0.305±0.098A	6.347±0.542A

(C) ***Cedrus deodara***

Sites	TCH±SD	RWC	pH	AA	APTI
Airport	3.128±0.461C	72.136±3.361AB	4.104±0.058A	0.383±0.041A	6.677±1.079A
Dhumbarahi	3.232±0..413C	79.168±6.984CD	4.128±0.071A	0.882±0.041C	7.856±1.291AB
Jawalakhel	1.085±0.132A	84.827±4.976CD	5.664±0.371C	0.803±0.067B	8.907±0.800B
Ratnapark	0.672±0.0598A	85.435±4.128D	5.764±0.147C	0.814±0.049B	8.705±0.414B
Shankha park	3.474±0.242C	78.168±6.984BC	4.128±0.071A	0.882±0.041C	7.876±1.283AB
Narayanthan	2.169±0.960B	66.403±2.002A	4.480±0.268B	0.384±0.042A	7.096±0.556A

(D) *Araucaria bidwillii*

Sites	TCH±SD	RWC	pH	AA	APTI
Airport	0.812±0.138 A	86.292±4.233C	6.356±0.149B	0.644±0.130B	8.861±0.694C
Dhumbarahi	2.759±0.352 B	85.492±5.461C	6.260±.000B	0.644±0.130B	7.026±1.417B
Jawalakhel	0.8011±0.043A	72.709±7.337B	5.776±0.251A	0.302±0.064A	5.404±0.581A
Ratnapark	0.599±0.207 A	88.292±4.233C	6.248±0.026B	0.711±0.052B	8.648±0.805C
Shankha park	0.650±0.196 A	86.892±2.993C	6.248±0..026B	0.644±0.130B	8.963±0.777C
Narayanthan	3.596±0.611 C	52.898±2.389A	6.336±0.187B	0.245±0.509A	5.576±0.514A

Identical letters following the mean value ± SD in the vertical column (for each plant species) denote no significance difference among sites at P=0.05 by Duncan's multiple range test followed after ANOVA.

DISCUSSION

Air pollution tolerant index (APTI) is an index that shows capability of a plant to tolerate air pollution. Plants which have higher index value are tolerant to air pollution and can be used to with stand pollution, while plants having low index value show less tolerance and can be used to indicate levels of air pollution (Singh and Rao 1983). All gymnosperm plants under present study showed high APTI value at polluted sites indicating their ability to combat air pollution. To understand APTI value, different physiological parameters like chlorophyll content, ascorbic acid, relative water content and pH were considered. To understand the physiological phenomena related with air pollution tolerance in plant, the above mentioned parameters are discussed below.

Chlorophyll content of plants determines its photosynthetic activity as well as the growth and development of biomass. The chlorophyll content of plant differs from species to species with the age of leaf and also with the pollution level (Katiyar and Dubey 2001). Present study showed that chlorophyll content in all the plants varies with the pollution status of the area. It also varies with the tolerance as well as sensitivity of the plant species, i.e., higher the sensitive nature of the plant species lower the chlorophyll content. High total chlorophyll content was observed in *Pinus roxburghii* even in polluted site and these may be due to its tolerance nature (Beg *et al*. 1990, Jyothi and Jaya 2010).

Ascorbic acid is regarded as an antioxidant found in plants, and influences resistance to a adverse environmental condition including air pollution (Keller and Schwager 1977, Lima *et al*. 2000). Ascorbic acid (AA) is a strong reducing agent and it activates many physiological and defence mechanism. Its reducing power is directly proportional to its concentration (Raza and Murthy 1988, Lewis 1976). Being a very important reducing agent, ascorbic acid also plays a vital role in cell wall synthesis, defense and cell division (Conklin 2001). Present study showed increase in the concentration of ascorbic acid in polluted with respect to the control site in all studied gymnosperms- *Araucaria bidwillii*, *Thuja orientialis, Cedrus deodar* and *Pinus roxburghii.* Increase in ascorbic acid may be due to the increased rate of production of reactive oxygen species (ROS) during photo-oxidation of SO_2 to SO_3 (Chaudhary and Rao 1977) or other pollutants. These acids may bind toxic metals in large quantity and help in defence mechanism. Thus higher ascorbic acid content of the plant can be considered as a sign of its tolerance against the pollutants.

Leaf extract pH increased at different sites in the present study. High pH may increase the efficiency of conversion from hexose sugar to Ascorbic acid, while low leaf extract pH showed good correlation with sensitivity to air pollution

(Escobedo *et al*. 2008, Pasqualini *et al*. 2001). Scholz and Reck (1977) have reported that in presence of an acidic pollutant, the leaf pH is lowered and the decline is greater in sensitive species. A shift in cell sap pH towards the acid side in presence of an acidic pollutant might decrease the efficiency of conversion of hexose sugar to ascorbic acid. However, the reducing activity of ascorbic acid is pH dependent being more at higher and lesser at lower pH. Hence increase in the leaf extract pH gives tolerance to plants against pollution.

Only *Pinus roxburghii* showed significant increase in leaf extract pH level as compare to less polluted site Narayanthan. Other studied plants showed insignificant difference or decrease in pH indicating possibility of presence of acidic pollutants in the atmosphere. There are so many factors controlling tolerance in plants. Plants with lower pH are more susceptible, while those with pH around 7 are more tolerant (Bakiyaraj and Ayyappan 2014). From this statement *Cedrus deodara* is more susceptible and others are comparatively more tolerant species.

Relative water content in *Pinus roxburghii, Thuja orientiales*, *Cedrus deodara*, and *Araucaria bidwillii* increased significantly (P = 0.05) in all polluted sites than in less polluted site, Narayanthan. Water is crucial prerequisite for plant life. High RWC favours drought resistance in plants (Swami *et al*. 2004). High water content within plant body possibly helps to maintain its physiological balance under stress condition such as exposure to air pollution when the transpiration rates are usually high. It also serves as an indicator of drought resistance in plants. Relative water content (RWC) of a leaf is the water present in it relative to its full turgidity. Relative water content is associated with protoplasmic permeability in cells causeing loss of water and dissolved nutrients, resulting in early senescence of leaves (Agrawal and Tiwari 1997). High relative water content in plants under polluted condition might be an strategy of tolerant species of plants to withstand pollutants.

From the study it can be concluded that *Pinus roxburghii, Thuja orientiales, Cedrus deodara* and *Araucaria bidwillii* have high APTI and hence they can withstand air pollution along the roads in Kathmandu. These plants mostly adjusted their physiology by increasing total chlorophyll content, ascorbic acids content, pH and relative water content.

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SCREENING OF SOME ROAD SIDE TREES FOR PLANTATION IN POLLUTED URBAN AREAS OF KATHMANDU, NEPAL

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ABSTRACT

Kathmandu valley is facing air pollution problem because of rapid increase in vehicular emissions. Some common road side trees like *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa, Grevillea robusta, Jacaranda mimosifolia* and *Populus euroamericana* were evaluated for their air pollution tolerance index (APTI) and metal accumulation index (MAI). These two indices were measured during winter season at different polluted sites (Airport, Balaju, Banasthali, Basundhara Dhumbarahi and Gongbu) and less polluted site (Narayanthan) in Kathmandu. Air pollution tolerance Index (APTI) ranged from 12.51±0.74 to 15.67±0.75. Highest APTI value was observed in *Populus euroamericana* (15.67±0.75) at Dhumbarahi. The mean value of metal accumulation index (MAI) values ranged from 7.34 to 20.44 and highest MAI value was observed in *Populuseuroa mericana* (20.44). Correlation of APTI and heavy metal (Cu, Pb and Zn) accumulation in most of the studied tree leaves were negative showing their inverse relationship, which indicated the heavy metal avoidance mechanism among them for their survival. *Populus euroamericana, Jacaranda mimosifolia and Callistemon lanceolatus* with high MAI and intermediate APTI range are recommended for plantation on road side of Kathmandu.

KEY WORDS : Metal Accumulation Index (MAI), Urban area, Avoidance mechanism, APTI

INTRODUCTION

Kathmandu valley, ranging from 1200 to 1400 m above sea level in subtropical zone, has bowl shaped topography, which makes it more prone to air pollution (Pradhanet *et al*., 2012; Shrestha, 2001). Rapid urbanization, industrialization, poor maintenance of road, poorly maintained vehicle deteriorate the air quality in Kathmandu valley (MaYa, 2014) and particulate matter are being one of the major pollutants (Shrestha, 2001). Vehicle numbers in Kathmandu Valley has rapidly increased in the last 15 years. Data have shown that in the fiscal year 2000/2001, number of registered vehicles was 24,003 and by 2015/2016 it has increased to 7,79,822. This shows an increment by more than 32 times in the last one and a half decade (Saud and Paudel, 2018).

Biochemical parameters like leaf pH, relative water content, chlorophyll content, and ascorbic acid content collectively suggested as the best index of the susceptibility levels of plants known as air pollution tolerance index (APTI) (Kuddus *et al.*, 2011; Rai *et al.,* 2013). The sensitivity or tolerance of a plant depends upon these parameters (Liu nad Ding, 2008; Singh and Verma, 2007). Trees with high air pollution tolerance index (APTI) values can tolerate as well as withstand to air pollution to a greater extent than those with less APTI value. Resistance of heavy metals in a plant is performed either by avoidance or tolerance mechanism. Avoiders group of plants can protect themselves by stopping metal ions through entering their cellular cytoplasm while tolerant plants can detoxify metal ions through crossed organelle biomembranes or internal the plasma membrane (Millaleo *et al*., 2010). It is not clear if the plants with high APTI value also have high bio-accumulation ability or APTI are inversely related with metal accumulation. Hence to ascertain this, APTI and metal accumulation in six common roadside tree species growing in high vehicle traffic (polluted) and less vehicle traffic (less polluted) areas were studied in Kathmandu.In the present study, based on the high APTI and MAI value the common road side tree species will be screened for plantation, as the screened plants will be able to accumulate more heavy metals and also will have high tolerance to withstand pollution.

MATERIALS AND METHODS

Leaf samples of six common road side trees like *Callistemon lanceolatus, Cinnamomum camphora, Ficus religiosa, Grevillea robusta, Jacaranda mimosifolia and Populus euroamericana* were collected from high traffic areas (Airport, Balaju, Banasthali, Basndhara, Gongobu Dhumbarahi) and less traffic area (Narayanthan, a control site) (Fig. 1) on the same day around 9.00 to 10:00 AM for (A) APTI and (B) metal analysis.

Fig. 1. Map of Study site: Kathmandu showing sampling sites -1. Airport 2. Balaju 3. Banasthali 4. Basundhara 5. Dhumbarahi 6. Gongbu and 7. Narayanthan

(A) APTI : All the collected leaves were cleaned thoroughly in dry condition using brush and cotton before the processing for APTI. APTI was obtained by using the parameters like ascorbic cacid content, leaf extract pH, total chlorophyll content and relative water content according to Singh and Rao (1983).

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

(Where A is the ascorbic acid content (mg g^{-1}) of fresh weight; T is the total chlorophyll (mg g^{-1}) of fresh weight; P is the pH of leaf extract and R is the relative content of water (%.).

Ascorbic acid (AA) and total chlorophyll (TC) content of leaf samples was determined using spectrophotometer according to Bajaj and Kaur (1981) and Barnes *et al.* (1992) respectively. Relative water content (RWC) and leaf extract pH was measured according to Barrs *et al*. (1962) and Apriyantono *et al*. (1989) respectively.

(B) Metal Analysis: For metal analysis, the leaves were washed with running tap water, rinsed with double deionized water and then dried at room temperature (20 °C) about 24-48 hours. Again dried at 60 °C for 48 hours. The dried leaf samples were grinded using mortar and pestle to prepare representative samples.1 g DW of the representative sample was dipped in 8 ml concentrated HNO_3 (Merck). These were left overnight at room temperature. On the next day, the mixture was warmed for 2 h at 50 °C and subsequently heated at 160 °C for 4 h. The cooled extracts were filtered through Ashless filter paper (Whatman 589^3) and were diluted to prepare 25 ml with double deionized water (Sawidis *et al.*, 1995c). The filtrates were then analyzed for Cu, Pb and Zn by using Perkin Elmer (2380) Atomic Absorption Spectrometer (AAS) at wavelength 324.7 nm for Cu, 283.3nm for Pb and 213.9nm for Zn (Welz, 1985). Two plant materials of National Bureau of Standard (USA) each with Nos. 1537 (Tomato leaves) and 1575 (Pine needles) were analyzed following the same procedure and the metal recoveries obtained were 95.5%, 94.2% and 97.5% for Cu, Pb and Zn respectively. The relative standard deviation of the measurements was 2.7% for Cu. 7.5% for Pb and 3.9% for Zn. Ultimately metal accumulation index (MAI) was calculated by the formula given by Liu *et al.* (2007).

(C) Statistical Analysis: All the data analysis was conducted using SPSS program version 17.0. To evaluate the significant differences of various biochemical parameters (for the APTI) and heavy metals (Zn, Cu, Pb, Cr and Ni) concentrations among the study sites in the same species, the data obtained were analyzed statistically using one-way ANOVA followed by Duncan's multiple range test. Data of APTI parameters and heavy metals (Zn, Cu and Pb) were further subjected to Pearson's correlations test to assess the relationship between them.

RESULTS

Ascorbic acid, total chlorophyll, relative water content, potentiality of hydrogen pH and Air Pollution Tolerance Index of different plants at different sites of Kathmandu are given in Table 1.

Ascorbic acid (AA) content in the studied plants ranged from 2.96±0.11 to 8.86±0.38 mg g^{-1}. Out of six tree species two species (*Cinmomnaum camphora* and *Grevellia robusta*) had highest AA content in Airport site and the rest four species (*Callistemon lanceolatus, Ficus religiosa, Jacaranda mimosifolia* and *Populus euroamericana)* had highest ascorbic acid content in Narayanthan, a less polluted site. All other polluted sites (Balaju, Banasthali, Basndhara, Gongobu and Dhumbarahi) mostly had less amount of AA content than in control site.

In all the studied tree species, except *Ficus religiosa*, total chlorophyll (TC) was found to bemostly significantly increased in polluted sites than at control site. TC in all the studied plants, except *Grevillea robusta*, were lowest in Airport. Highest TC in *Cinnamomum camphora, Grevillea robusta* and *Populus euroamericana* was recorded in Basundhara, a polluted site. Similarly, highest TC content in *Callistemon lanceolatus* and *Jacaranda mimosifolia* was also recorded in polluted sites, Gongobu and Balaju, respectively.

Relative water content (RWC) in the studied plants ranged from 36.93±10.47to 96.33±2.13%. Almost all studied plants, except *Grevillea robusta*, recorded more than 80% RWC in some polluted sites (Table 1). In *Jacaranda mimosifolia,* RWC was significantly higher in all polluted sites than in control site, but in other species this trend was not observed.

The leaf extract pH of *Cinnamomum camphora, Ficus reliogisa, Grevillea robusta* and *Populus euroamericana* reduced significantly (p=0.05) in most of the polluted sites than at Narayanthan (Control site). In *Callistemon lanceolatus* the highest leaf extract pH was found in Dhumbarahi (6.45±0.21) and lowest in Airport (3.53±0.03). In *Jacaranda mimosifolia* the highest leaf extract pH was recorded in Basundhara (6.48±0.05) and lowest in Airport (3.53±0.03). pH range in *Callistemon lanceolatus* and *Jacaranda mimisifolia* at polluted and control sites were wide ranging from 3.53 to 6.48 in leaf extract, but in *Cinnamomum camphora* it was comparatively moderate and ranged from 4.66 to 6.82. pH range in *Ficus reliogisa, Grevillea robusta* and *Populus euroamericana* were comparatively narrow and ranged from 5.04 to 6.82 among polluted and less polluted sites (Table 1). From this it is evident that the species like *Ficus religiosa, Grevillea robusta* and *Populus euroamericana* are more tolerant to air pollutants.

APTI values obtained by different species at different sites ranged from 7.09 (in *Grevillea*) to 15.67 (in *Populus*). Based on the maximum APTI value scored, the plant species from lowest to highest were in the order *Cinnamomum camphora* > *Callistemon lanceolatus* > *Grevillea robusta* > *Ficus religiosa* > *Jacaranda mimosifolia* > *Populus euroamericana* and their respective APTI values were 12.51±0.74 >12.55±0.78>13.08±0. > 13.97±0.54 > 14.61±0.30> 15.67±0.75 (Table 1).

Heavy metal content and Metal Accumulation Index (MAI) in the leaves

Metal (Zn, Cu and Pb) concentrations in different leaf samples at different sites are given in Table 2. All metals accumulation in the polluted sites were mostly significantly (p= 0.05) higher than the control site (Narayanthan). Zinc accumulation was highest in *Populus euroamericana* in most of the sites, Cu accumulation was highest in *Jacaranda mimosifolia* at most sites and Pb accumulation was recorded highest in *Callistemon lanceolatus* at the Airport. Accumulation of metals in different plants collected from the same area differed (Table 2).

Among six tree species MAI values (40.69) was highest recorded in the leaves of *Populus euroamericana* collected from Airport site. The mean MAI value obtained from low to high were in the order 7.34>9.59>9.65>15.61>15.69>20.44 for *Cinnamomum camphora*> *Grevillearobusta* > *Ficus religiosa* > *Callistemon lanceolatus*> *Jacaranda mimosifolia* > *Populus euroamericana* (Table 2)

Correlations of metals with the physiological parameters of APTI

The correlation of different metal (Zn, Cu and Pb) content and different biochemical parameters (RWC, AA, pH, TC) and APTI) of the studied six tree species are given in Table 3. RWC and zinc content mostly showed negative correlation in all studied tree species and was significant (p=0.05) in *Callistemon lanceolatus.* Similarly Cu was found to be positively significantly (p=0.05) correlated with RWC of *Grevelliea robusta* and negatively significant (p<0.01) with *Callistemon lanceolatus* and *Jacaranda mimosifolia*. Significant negative correlation was observed between Pb content in leaves and RWC in *Grevelliea.* Cumulative effect of Pb+Cu+Zn accumulation was found to be negatively correlated with RWC in all plants and was significant (p<0.05) in *Grevelliea robusta* and *Jacaranda mimosifolia.*

Mostly negative correlation was observed between AA content and Zn accumulation in the

Table 1. The physiological parameters- (A) Ascorbic Acid, (B) Total chlorophyll, (C)Relative water content (D) potentiality of hydrogen pH and (E)Air Pollution Tolerance Index of different plants at different sites of Kathmandu

Physiological parameters	**Plants**	Sites						
		Airport	Balaju	Banasthali	Basundhara	Dhumbarahi	Gongbu	Narayanthan
Ascorbic Acid (mg/g)	*Callistemon lanceolatus*	7.54±0.79C	5.42±2.12 AB	4.38±2.25 AB	3.78±0.37 A	4.58±1.19 AB	4.28±0.27 AB	7.62±1.69 C
	Cinnamomum camphora	8.6±0.53 D	3.04±0.32 A	5.28±0.63 C	3.78±0.96 AB	4.56±1.04 BC	2.96±0.11 A	4.56±0.84 BC
	Ficusreliogisa	8.38±0.34 C	3.06±0.23 A	3.32±0.35 A	3.96±0.65 A	3.7±0.80 A	5.22±0.74 B	8.38±0.65 C
	Grevillearobusta,	7.28±0.73 C	4.2±1.60 AB	5.34±2.21 B	8.52±00.40 C	5.04±0.74 B	4.94±0.34 AB	5.54±0.32 B
	Jacaranda mimosifolia	6.28±2.06 BC	6.52±1.68 BC	4.95±1.74 AB	7.86±0.20 CDE	8.36±0.58 DE	3.78±0.38 A	8.64±0.35 E
	Populuseuroa mericana	8.35±0.51 B	8.32±0.3 B	8.6±0.60 B	6.74±2.01 A	8.86±0.38 B	8.35±0.67 B	8.15±0.76 B
Total chlorophyll (mg/g)	*Callistemon lanceolatus*	0.14±0.02 A	0.48±0.05 B	0.61±0.107 C	0.61±0.10 C	0.61±0.08 C	0.74±0.04 D	0.68±0.041 CD
	Cinnamomum camphora	0.11±0.04 A	0.54±0.06 B	0.66±0.02 CD	0.72±0.11 D	0.57±0.14 BC	0.2±0.05 A	0.67±0.07 CD
	Ficusreliogisa	0.2±0.09 A	0.79±0.37 B	0.4±0.09 A	0.81±0.32B	0.71±0.02 B	0.8±0.01 B	1.17±0.11 C
	Grevillearobusta,	1.12±0.01 E	0.42±0.01 A	1.13±0.02 E	1.14±0.02 E	0.6±0.05 B	0.79±0.03 C	1.03±0.05 D
	Jacaranda mimosifolia	0.31±0.08 A	1.65±0.00 E	0.93±0.35 BC	1.25±0.21 D	0.93±0.11 BC	1.12±0.059 B	0.79±0.07 A
	Populuseuroa mericana	0.58±0.07A	0.71±0.02 AB	0.71±0.02 AB	1.4±0.08 D	0.92±0.04 C	0.69±0.30 AB	0.7±0.11 AB
Relative Water content (%)	*Callistemon lanceolatus*	70.83±10.89 BC	57.63±8.52 A	62.92±11.07 AB	62.17±10.18 AB	93.33±4.01E	86.65±3.82 DE	75.4±8.31 C
	Cinnamomum camphora	60.77±4.64 A	80.85±6.6 B	70.45±4.59A	83.15±13.60BC	92.37±2.65 C	66±4.66 A	86.08±9.32 BC
	Ficusreliogisa	56.21±6.70 A	96.33±2.13 D	62.92±23.7 AB	75.8±18.13 B	94.35±0.77 C D	69.13±9.53 AB	73.08±6.47 BC
	Grevillearobusta,	69.21±7.08C	43.95±2.53 AB	69.21±7.08 CD	47.78±3.17 B	75.19±4.06 D	36.93±10.47 A	72.71±13.26 CD
	Jacaranda mimosifolia	57.25±2.72 B	58.59±14.11 B	86.09±1.52C	58.96±13.09 B	85.76±5.50 C	85.843±3.45C	40.44±2.77 A
	Populuseuroa mericana	73.9±5.46 BC	74.03±3.05 BC	71.6±17.43ABC	66.32±6.25 AB	89.65±7.04 D	60.03±9.81A	73.39±11.68 BC
Potentiality of hydrogen pH	*Callistemon lanceolatus*	3.58±0.02 A	5.79±0.01 C	4.57±0.50 B	6.25±0.03 D	6.45±0.21D	6.14±0.03 D	5.57±0.34 C
	Cinnamomum camphora	5.71±0.02 B	5.98±0.03 CD	5.9±0.02 C	6.62±0.08E	6.62±0.08 E	4.66±0.03 A	6.82±0.13 F
	Ficusreliogisa	5.94±0.01 D	6.82±0.02 F	5.04±0.03 B	6.37±0.05 B	6.63±0.03 E	5.63±0.06 C	6.78±0.09 F
	Grevillearobusta,	6.24±0.03 C	6.02±0.03 B	6.56±0.04 D	5.78±0.05 A	6.08±0.02 B	6.08±0.01 B	6.76±0.12 E
	Jacaranda mimosifolia	3.53±0.03 A	5.34±0.13 D	4.9±0.25B	6.48±0.05 F	6.15±0.03 E	4.76±0.03 B	5.14±0.01 C
	Populuseuroa mericana	6.24±0.03 B	6.32±0.18 BC	6.42±0.05 BC	6.59±0.38B C	6.64±0.15 C	5.18±0.63 A	6.68±0.10 C
Air pollution Tolerance Index (APTI)	*Callistemon lanceolatus*	9.89±1.15 B	9.17±1.19 AB	8.66±2.40 AB	12.08±0.19 C	12.55±0.78 C	11.61±0.50 C	12.26±0.66 C
	Cinnamomumcamphora	11.09±0.60 BC	10.07±0.83 B	10.51±0.84 B	10.87±1.35 BC	12.51±0.74 D	8.03±0.41 A	12.03±1.20 CD
	Ficusreliogisa	10.77±0.65 BC	11.96±0.35 C	8.1±2.35 A	10.43±1.35 A	12.15±0.62 C	10.27±1.26 B	13.97±0.54 D
	Grevillea robusta,	13.08±0.61 C	7.1±1.15 A	11.03±2.07281 B	10.67±0.33 B	10.88±0.73 B	7.09±1.02 A	11.59±1.23 BC
	Jacaranda mimosifolia	8.21±0.82 A	12.32±1.74 BC	8.65±2.57 A	12.01±1.20 BC	14.61±0.30 D	10.79±0.52 B	9.02±00.24 A
	Populuseuro americana	13.09±0.77 BC	13.26±0.56 BC	13.54±0.61 C	11.97±1.49 AB	15.67±0.75 D	10.94±1.09 A	13.36±0.97 BC

Different capital letter along the row after mean ± sd indicates significant difference at p=0.05obtained from Duncan's multiple range test after one way ANOVA

Table 2. Metal content in the leaves and the Metal Accumulation Index (MAI) in different plants at different sites

Plants	Sites	Zn mg/kg	Cu mg/kg	Pb mg/kg	MAI
Callistemon lanceolatus				Mean	15.61
	Airport	15.54±0.42 C	5.41±0.62 EF	40.33±1.84 E	20.09
	Balaju	38.46±0.91 F	5.87±0.30 F	32.41±0.93 C	32.09
	Banasthali	43.76±3.23 G	7.56±0.38 G	0.83±0.67 A	11.65
	Bsundhara	12.15±0.68 B	3.63±0.25 CD	0.73±0.49 A	11.25
	Dhumbarahi	20.83±1.13 DE	2.39±0.53 B	17.10±1.04 B	13.09
	Gongbu	21.53±0.84 E	0.93±0.42 A	37.56±1.49 D	17.61
	Narayanthan	4.22±1.51 A	4.81±0.78 E	0.61±0.37 A	3.54
Cinnamomum camphora				Mean	7.34
	Airport	23.23±2.85 D	8.79± 0.90 CD	13.53±1.60 B	8.78
	Balaju	15.69±1.38 B	5.23±1.04 AB	32.06±2.16 D	10.42
	Banasthali	1.40±0,59 A	7.99±0.88 CD	0.83±0.58 A	4.29
	Basundhara	18.07± 2.25 BC	3.39±2.25 A	24.99±4.28 C	5.11
	Dhumbarahi	20.11± 2.13 C	9.29±1.54 D	2.86±1.62 A	5.74
	Gongbu	20.03±0.99 C	6.51±0.91 BC	21.87±1.85 C	13.05
	Narayanthan	1.27± 0.64 A	13.93±1.79 E	0.84± O.38 A	3.99
Ficus religiosa				Mean	9.65
	Airport	16.02±1.71 D	9.13±0.78 D	21.42±1.47 C	11.90
	Balaju	22.20±2.42 E	7.41±1.40 C	39.61±2.39 E	10.33
	Bansthali	22.37±2.27 E	11.09±1.67 E	36.65±2.73 D	9.98
	Basundhara	8.79±1.06 B	0.90±0.09 A	0.053±0.01 A	7.06
	Dhumbarahi	12.71±1.12 C	7.91±0.96 CD	3.82±0.76 B	8.17
	Gongbu	13.13± 1.62 C	3.93±0.23 B	35.83±2.06 D	14.18
	Narayanthan	1.54±0.51 A	15.28±1.49 F	0.50±0.10 A	5.95
Grevillea robusta				Mean	9.59
	Airport	22.093±2.86 C	9.29±1.01 D	19.83±1.51 D	10.03
	Balaju	15.06±3.42 AB	6.20±0.72 BC	37.8±1.56 F	12.40
	Banasthali	13.49±0.90 AB	7.58±1.39 CD	16.92±1.41 C	10.80
	Basundhara	14.03±0.91 AB	2.96± 0.50 A	12.84± 2.12 B	9.11
	Dhumbarahi	15.2±2.43 AB	5.14±0.69 B	24.34±2.99 E	7.28
	Gongbu	12.01±2.59 A	2.97±0.89 A	36.67±1.15 F	13.23
	Narayanthan	12.45±2.21 A	8.16±1.61 CD	4.97± 2.25 A	4.31
Jacaranda mimosifolia				Mean	15.69
	Airport	34.98±1,56 E	19.93±1.68 E	25.06±1.38 E	17.47
	Balaju	31.18±1.28 D	20.27±0.64 E	3.70±1.37 A	19.52
	Banasthali	22.68±1.33 B	5.72± 0.43 B	29.37±1.67 F	15.97
	Basundhara	23.34±2.00 BC	13.83±1.35 D	19.42±0.64 D	17.37
	Dhumbarahi	25.54±0,84 C	11.72±0.71 C	11.58±1.21 B	18.78
	Gongbu	22.54±0.84 B	11.61±1.12 C	15.52±1.04 C	17.44
	Narayanthan	0.96± 0.45 A	2.45± 0.47 A	1.40±0.56 A	3.28
Populus euroamericana				Mean	20.44
	Airport	104.27±1.02 E	11.31±0.63 D	0.93±0.59 A	40.69
	Balaju	69.06±3.32 D	8.38±0.53 C	27.51±1.74B	17.37
	Banasthali	132.38±3.71 F	11.93±0.60 D	0.83±0.58 A	18.96
	Basundhara	57.89±1.01 BC	0.89±0.10 A	1.66±1.52 A	25.71
	Dhumbarahi	57.09±2.71 BC	1.86±1.06 AB	1.54±0.99 A	8.14
	Gongbu	65.61±1.30 CD	1.26±1.07 AB	2.32±0.84 A	18.12
	Narayanthan	14.46±0.47 A	2.41± O.86 B	0.59±0.06A	14.13

Different capital letter after mean±sd along the column indicates significant difference at p=0.05 obtained from Duncan's multiple range test after one way ANOVA

studied tree leaves and was significant ($p<0.05$) in *Jacaranda mimosifolia*. Significant positive correlation was observed between Cu accumulation and AA in *Ficus religiosa*. Pb accumulation mostly negative correlation with AA and was significantin *Cinnamomum camphora* but in in *Jacaranda mimosifolia* it was positively significant ($p<0.01$). Cumulative effect of Pb+Cu+Zn accumulation was found to be significantly ($p<0.05$) negatively correlated with AA in *Grevelliea robusta.*

Mostly negative correlation was observed between leaf extract pH and Zn content, and was significant (*$p<0.05$) in *Cinnamomum camphora* and ($p<0.01$) *Jacaranda mimoisifolia.* Cu content in leaf was mostly found to be positive correlation with pH in most of the tree species and was significant ($p<0.01$) in *Cinnamomum camphora* but in *Callistemon lanceolatus* and *Jacaranda mmoisifolia* it was found to be significantly ($p<0.01$) negatively correlated. Pb content in leaf showed negative correlation with pH in most trees and was significant ($p<0.01$) in *Grevillea robusta, Cinnamomum camphora* and *Jacaranda mmoisifolia.* Cumulative effect of Pb + Cu + Zn accumulation was found to be mostly negatively correlated with pH in most trees and was significant ($p<0.05$) in *Grevelliea robusta* and *Cinnamomum camphora.*

Zinc accumulation in leaves showed negative correlation with total chlorophyll in most of the trees and was significant ($p<0.01$) in *Ficus religiosa, Jacaranda mimoisifolia* and *Populus euroamericana*. Cu content in leaves was significantly ($p<0.01$) negatively correlated with TC in *Jacaranda mimoisifolia* whereas Pb content in leaf showed significant ($p<0.05$) negatively correlated with TC in *Ficus reliogisa.* Cumulative effect of Pb+Cu+Zn content in leaves were negatively correlation in most of the studied plants and was significant in *Ficus reliogisa* and *Jacaranda mimoisifolia.*

APTI was found to be negatively correlated with Zinc accumulation in most trees and was significant ($p<0.01$) in *Callistemon lanceolatus* and *Jacaranda mimoisifolia.* Cu content in leaves showed negative correlation with APTI in some studied tree species and was significant ($p<0.05$) in *Ficus reliogisa, Callistemon lanceolatus* and *Jacaranda mimoisifolia* but in *Cinnamomum camphora* it was significantly ($p<0.01$) positive. Pb content showed negative correlation with APTI in most studied tree species and was significant ($p<0.01$) in *Ficus religiosa, Populus euroamericana, Cinnamomum camphora and Grevillea robusta*. Cumulative effect of Pb+Cu+Zn accumulation was found to be mostly significant negatively correlated in all studied plants and was significant ($p<0.05$) in *Grevillea robusta*, *Cinnamomum camphora*, *Callistemon lanceolatus* and *Jacaranda mimoisifoli.*

DISCUSSION

Depending on the APTI value, Padmavathi *et al.*

Table 3. Correlations of metals with RWC, AA, pH, TC AND APTI

Parameters	*Ficus religiosa* (n=35)	*Gravelliea robusta* (n=50)	*Cinnamomum-camphora* (n=35)	*Callistemon lanceolatus* (n=50)	*Jacaranda mimosifolia* (n=40)	*Populus euroamericana* (n=45)
RWC-Zn	-0.309	-.110	-0.180	-0.404**	-0.159	-0.065
RWC-CU	-0.232	0.344*	0.166	-0.643**	-.0552**	0.126
RWC-Pb	-0.200	-0.389**	-0.125	0.089	-0.158	-0.087
RWC-Zn+Cu+Pb	-0.293	-0.305*	-0.158	-0.208	-0.387*	-0.064
AA-Zn	-0.307	-0.243	0.111	-0.160	-0.369*	0.204
AA-Cu	0.508**	-0.034	0.276	0.199	-0.118	0.046
AA-Pb	0.245	-0.261	-0.376*	-0.023	0.472**	-0.219
AA-Zn+Cu+Pb	0.303	-0.338*	-0.162	-0.083	0.219	0.137
pH-Zn	-0.152	0.049	-0.411*	-0.172	-0.667**	-0.145
pH-Cu	0.732	0.095	0.530**	-0.513**	-0.580**	0.184
pH-Pb	0.427	-0.409**	-0.497**	-0.010	-0.457**	0.071
pH-Zn+Cu+Pb	0.304	-0.330*	-0.486**	-0.140	-0.089	-0.090
Tchl-Zn	-0.754**	0.047	-0.240	0.070	-0.780**	-0.337*
Tchl-Cu	0.097	0.173	0.008	-0.155	-0.827**	-0.030
Tchl-Pb	-0.346*	-0.137	-0.011	0.001	0.031	0.264
Tchl-Zn+Cu+Pb	-0.346*	-0.060	-0.137	0.025	-0.529**	-0.245
APTI-Zn	0.329	-0.212	-0.144	-0.436**	-0.530**	0.078
APTI-Cu	-0.953*	0.243	0.438**	-0.514**	-0.665**	-0.127
APTI-Pb	-0.983**	-0.475**	-0.469**	-0.018	0.061	-0.775**
APTI-Zn+Cu+Pb	-0.482	-0.451**	-0.338*	-0.286*	-0.368*	-0.135

Significance *$p<0.05$ ** $p<0.01$ ***$p<0.00$ significant ($p=0.05$)

(2013) grouped the plants as sensitive (with APTI value <11), intermediate (with APTI value 12 to16) and tolerant (with APTI value e" 17). In the present study all the studied tree species scored APTI value between 12 and 16, hence they were in the intermediate categories of APTI.

Metal Accumulation Index (MAI) depends on various factors like, local atmospheric chemistry, meteorology,sampling height (tree), time of sampling and plant characteristics (Hu *et al.,* 2014). Plants with higher MAI values have good accumulation capacities and also regarded as tolerant species. Hu *et al.,* (2014). From the present study plants like *Populus euroamericana, Jacaranda and Callistemon* with high MAI value are suitable tree species that can be suggested to be planted for greenbelt area along the road side.

The significant correlation between RWC and all metal accumulation was mostly negative, except Cu accumulation and RWC in *Grevillea robusta*. The excess concentrations of the heavy metals significantly affected plant water status, causing water deficit and subsequent changes in the plants. The most intensive effect on the plants was observed with Cd, less intensive by Cu and Zn and the least intensive by Pb in sunflower (Kastori *et al.* 1992). This might be the region for showing insignificant relation of Pb accumulation in most of the trees except *Grevillea robusta*. Lead accumulation in root of *Lupinus* has been reported to increase the degree of vacuolization in the meristem cells (Przymusin´ski and Woz´ny 1985) and cortex parenchyma (Gzyl *et al.,* 1997), which may suggest that the water status of these cells was not affected by the metal. Thus, Pb-induced vacuolization in *Lupinusluteus* was correlated with high values of RWC, suggesting that water might be stored in the vacuoles in response to metal stress (Rucin´ska-Sobkowiak *et al.,* 2013). The response of RWC in *Grevillea robusta* to Pb accumulation in the present study was significantly negative, which indicates that the Pb toxicity prevailed in the leaves. From this it can be suggested that the Pb localization in the cell and its toxicity might not be same in all plants. The correlation of RWC and Cu accumulation was found to be positively significant in *Grevelliea robusta.* This might be due to low concentration of Cu (below 10 mg/kg), which is an essential element and mostly not toxic below 15-20 mg/kg (Pahlsson, 1989).

Correlation between AA and Zn accumulation in *Jacaranda mimoisifolia* was significantly negative. Smith *et al.* (1989) reported the decrease in ascorbic acid in roots and shoots of pigeon pea with increasing concentrations of Zn. Correlation of AA content with Cu accumulation in *Ficus religiosa* and Pb accumulation in *Jacaranda mimoisifolia* were significantly positive. Copper is an essential element and its maximum accumulation in *Ficus* was 15.28 mg/kg in control site, indicating nontoxic level of Cu accumulation in most of the sites, which might have resulted in positive correlation. Though Pb is not an essential element, but plant absorb Pb and mostly accumulate in the cell wall as granules (Peterson, 1978; Kabata-Pandias and Pandis, 1985; Chettri *et al.,* 2000). In the present study Pb accumulation in *Jacaranda mimoisifolia* leaves reached up to 29.79 mg/kg, and showed positive correlation with Ascorbic acid, which might be due to accumulation of Pb on cell wall and not interfering with the metabolic functions. But in Camphor Pb accumulation and AA showed negative correlation. Variation in Ascorbic acid content is one of the factors, which may be held responsible for differential tolerance capacity of plants against various air pollutants at different sites (Agular-Silva *et al.,* 2016; Aghajanzadeh *et al.,* 2016). In the present study Ascorbic acid content mostly increased with increase in heavy metal content and also in accordance with other findings of Rai and Panda (2014) and Govindaraju *et al.* (2012).

Correlations of pH with most of the metal content in leaf were negatively significant, except Cu content in *Cinnamomum camphora*. This must be due to reduction of leaf pH value in the presence of heavy metals and an acidic pollutant (Scholz and Reck, 1977), and also suggested that the leaf pH is reduced and the reducing rate is more in sensitive plants compared to that in tolerant plant species. The positive correlation of pH with Cu content in Camphor is mainly due to the fact that both Cu content and leaf pH in Narayanthan is significantly high in this site than in other studied sites. Besides, the Cu is an essential elements and its content in *Cinnamomum camphora* is below the toxic level as suggested by Pahlsson, (1989).

Correlations between total chlorophyll and heavy metal content were negatively significantly in most cases. Heavy metals such as (Hg, Cu, Cr, Cd, and Zn) have been reported to decrease the chlorophyll content in various plants in most cases (Aggarwal *et al.,* 2012). This decline in photosynthetic pigments is most probably due to the inhibition of the reductive steps in the biosynthetic pathways of photosynthetic pigments due to the high redox potential of many heavy metals. Among the studied plants, *Grevillea, Camphor* and *Callistemon* showed insignificant correlation of total chlorophyll with all metals. In the case of *Grevillea robusta* and *Cinnamomum camphora* this might be due to less metal accumulation as indicated by mean metal accumulation index (MAI) in these species. In the case of *Callistemon lanceolatus* though the MAI value is 15.61, the accumulation of Zn and Cu is within the threshold limit and below the toxic level. Besides this Pb accumulation in leaves are up to 40 mg/kg but negative correlation with total chlorophyll was not observed, indicating no effect of Pb on chlorophyll content. The possible reasons for this may be their localization in the cell.

Ultrastructural studies have revealed that Pb are deposeited mainly in the intercellular space, cell wall and vacuoles (Wierzbicka and Antosiewicz, 1993; Chettri *et al.,* (2000). A small deposits of Pb have also been reported to be seen in the ER, dictyosomes and dictyosome derived vesicles (Wierzbicka and Antosiewicz, 1993). (*Callistemon lanceolatus, Ficus religiosa, Populus euroamericana, Cinnamomum camphora, Grevillea robusta*, *Jacaranda mimoisifolia)* .

Significant Correlation of APTI with all metal content are mostly negative in all cases except with Cu content in *Cinnamomum camphora*. APTI is predicted on the basis of four major biochemical properties of leaves that are ascorbic acid, relative water content, total chlorophyll and leaf pH (Sing and and Rao,1983). APT varies with these parameters because all these parameters are influenced by the increase or decrease in metal content. From MAI it is evident that *Populus euroamericana, Jacaranda mimosifolia* and *callistemonlanceolatus* scored high MAI value including that the heavy metals accumulation might have affected the physiological parameters and this might have resulted in to negative correlation. Though *Cinnamomu mcamphora Grevillea robusta* and *Ficus reliogisa* scored less MAI values, out of these avoidance mechanism in *Cinnamomum camphora* might be high and resulted in to positive response to APTI with Cu accumulation.

CONCLUSION

The APTI values of all studied tree species were within intermediate range. Because of high MAI value, *Populus euroamericana, Jacaranda mimosifolia and Callistemon lanceolatus* have been identified as the suitable species for the plantation near the road side. Though *Cinnamomum camphora, Grevillea robusta* and *Ficus religiosa,* growing in the same polluted environment with others scoredless MAI value, indicating the metal avoidance mechanism in them. From above findings it can be concluded that Air pollution tolerance index (APTI) value of plants could not only be sufficient for selection of plants to mitigate air pollution. For selection of more suitable plants, Metal Accumulation Index (MAI) value of plants should also be considered. Hence *Populus euroamericana, Jacaranda mimoisifolia and Callistemon lanceolatus* with high MAI and intermediate APTI range are recommended for plantation on road side of Kathmandu.

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Impact of heavy metals and biochemical parameters on specific leaf area of roadside trees in Kathmandu, Nepal

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ABSTRACT

Kathmandu is seriously facing the air pollution problem because of the rapid increase in vehicular emissions. Most commonly grown roadside trees like *Populuseuro americana, Ficusreligiosa, Jacaranda mimosifolia, Grevillearobusta, Callistemon lanceolatus* and *Cinnamomumcamphora* from different sites of Kathmandu were collectedduring the winter season to measure specific leaf area (SLA), biochemical parameters and heavy metals (Cu, Pb and Zn) content. The relations among them (SLA, biochemical parameters, heavy metals) were also studied. Specific leaf area (SLA) ranged from 24.48± 0.87cm2 g-1 to 188.08±35 cm2 g-1and the highest SLA was observed in Jacaranda mimosifolia (188.08±35cm2 g-1).The mean value of biochemical parameters such as total chlorophyll (TCh) ranged from 0.64mg/g in Cinnamomumcamphorato 1.243 mg/g in Ficusreliogiosa whereas pH ranged from 5.28 in Callistemon lanceolatus to 6.75 in Grevillearobusta. Similarly Ascorbic acid rangedfrom 4.54 mg/g in Cinnamomumcamphora to 8.61mg/g in *Populuseuro americana* and relative water content (RWC) from 40.02 % in Jacaranda mimosifolia to 86 % in Cinnamomumcamphora. Mean value of Zn accumulation ranged from 14.83 mg/kg in *Ficusreligiosa* to 71.92 mg/kg in *Populuseuro americana*, Cu accumulation from 4.14 mg/kg in *Populouseuro americana* to 14.56 mg/kg in *Jacaranda mimosifolia* and Pb accumulation from 0.05 mg/kg in *Populuseuro americana* to 22.47 mg/kg in Callistemon lanceolatus. Pb and Cu accumulation at phytotoxic concentrations in leaves showednegative impact on SLA.

***Key words :** Tree leaves, Total chlorophyll, Ascorbic acid, Relative water content, pH, Heavy metals*

Introduction

Carbon dioxide is the most important greenhouse gas which is now 50% higher than before the industrial revolution. Increase in CO_2 is mainly due to the burning of fossil fuels (WMO, 2021). Leaves play an important role to sequester carbon dioxide through photosynthesis from the atmosphere. Leaf surface area is one of the key factors which determines the potential of photosynthesis in leaves. Specific leaf area (SLA) is a ratio of leaf area per unit leaf dry mass which describes the distribution of leaf biomass in relation to leaf area (Pierce *et al.*, 1994). Specific leaf area is highly correlated with light level and decreases with increasing light intensity (Kellomäki and Oker-Blom, 1981; Evans and Poorter, 2001), hence SLA is related to a leaf's position in the crown. Trees generally have a lower SLA at the top of the canopy because they receive direct solar radiation whereas at the lower parts of the crown may be shaded and have a higher SLA. (Nagel and O'Hara, 2001; Goudie *et al.*, 2016). SLA

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has been considered as one of the most important characteristics of leaf which is used to measure plant growth (Wright *et al.*, 2004**;** Lambers and Poorter, 1992; Falster *et al.*, 2018). SLA is an important indicator of plant strategies for adaptation (Grime, 2006; Westoby *et al.*, 2002**)** and used widely in plant ecology, agronomy, and forestry (Poorter *et al.*, 2009). The level of influence on growth depends on plant species, pollutant type and concentration along with number of environmental factors (Wuytack *et al.*, 2011; Chaturvedi *et al.*, 2013). Kapoor *et al.*, (2013) investigated tree species *Dalbergiasissoo* and found reduction in photosynthetic pigments, pH and RWC in the polluted environment. Certain heavy metals like copper and zinc are essential micronutrients for plants because they are involved in numerous metabolic processes as constituents of enzymes and other proteins. However, toxic levels of heavy metals cause several toxic symptoms in plants such as the inhibition of photosynthesis and enzyme activity, growth retardation, disturbed mineral nutrition, water imbalance and the alteration of membrane permeability (Sharma and Dubey, 2005; Pandey *et al.*, 2009).

Kathmandu, the capital of Nepal is highly affected by air pollution due to the increase number of automobiles, densely populated and its unique bowl-shaped topography (Dhamala *et al.*, 2018) which prevents particulate matter freely escaping in to the atmosphere and it makes vulnerable situation in Kathmandu. Air quality of Nepal was ranked at 145th position out of 180 countries in Environmental Performance Index in 2020 (Shrestha *et al.*, 2021 and Wendling *et al.*, 2020), and Kathmandu is listed as one of the most polluted cities in the world (Subedi, 2021). Most of the previous studies related with plants and air pollution were mainly focused in Air Pollution tolerance Index (APTI) and heavy metal accumulation. But it is not cleared if the toxic effect of heavy metal are reflected on SLA or not. It is also not clear if SLA is dependent on different physiological parameters like RWC, pH, total chlorophyll and ascorbic acid. Therefore, the relation of heavy metal accumulation and the biochemical parameters (like total chlorophyll, ascorbic acid, pH and relative water content) with specific leaf area have been investigated in the present study. The result of this study will identify suitable roadside trees that can accumulate heavy metals and help to mitigate air pollution problems.

Materials and Methods

The study area

Kathmandu is a valley and located at 27°42′0′′ N and 85°18′0′′ E. It ranges from 1200 to 1400 m above sea level in subtropical zone. Bowl shaped topography makes it more prone to air pollution (Pradhan *et al*., 2012; Shrestha, 2001) and characterized by a typical monsoon climate with rainy summer and dry winter. An average summer temperature lies between 19 °C-35 °C and winter temperature between 2 °C-12 °C with an annual mean temperature 24 °C and an average rainfall of 1343 mm.

Study Method

Sample collection

Leaves of most commonly grown trees along the road side like *Populuseuroamericana, Ficusreligiosa, Jacaranda mimosifolia, Grevillearobusta, Callistemon lanceolatus* and *Cinnamomumcamphora* were collected from lower surface (above 3 m from the ground). The polluted sampling sites were Tinkune, Airport, Chabhil, Samakhusi, Basundhara, Dhumbarahi, Lainchaur, Swayambhu and Balaju, and a comparatively clean site Narayanthan was considered as a control site (Fig. 1). The leaf samples were collected in winter season (December-January) and used for the measurements of specific leaf area (SLA), heavy metals and biochemical parameters (total chlorophyll, pH, ascorbic acid relative water content).

Metal Analysis

For metal analysis, the leaves were washed with

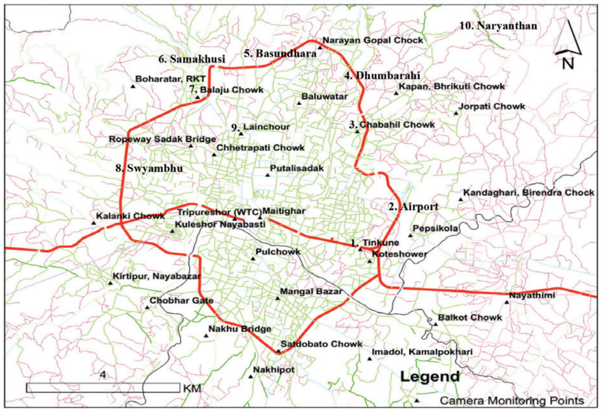

Fig. 1. Study site 1-Tinkune 2-Airport 3- Chabhil 4-Dhumbarahi 5-Basundhara 6-Samakhusi, 7-Balaju 8- Swayambhu 9-Lainchaur 10-Narayanthan

running tap water; rinsed with double deionized water and then dried at room temperature (20 °C) about 24-48 hours. Again dried at 60 °C for 48 hours. The dried leaf samples were grinded using mortar and pestle to prepare representative samples. 1g DW of the representative sample was dipped in 8 ml concentrated HNO_3 (Merck). These were left over night at room temperature. On the next day, the mixture was warmed for 2 h at 50 °C and subsequently heated at 160 °C for 4 h. The cooled extracts were filtered through Ash less filter paper (What man 589) and were diluted to prepare 25 ml with double deionized water (Sawidis *et al.*, 1995c). The filtrates were then analyzed for Cu, Pb and Zn by using Perkin Elmer (2380) Atomic Absorption Spectrometer (AAS) at wavelength 324.7 nm for Cu, 283.3nm for Pb and 213.9 nm for Zn (Welz, 1985). Two plant materials of National Bureau of Standard (USA) each with Nos. 1537 (Tomato leaves) and 1575 (Pine needles) were analyzed following the same procedure and the metal recoveries obtained were 95.5%, 94.2% and 97.5% for Cu, Pb and Zn respectively. The relative standard deviation of the measurements was 2.7% for Cu. 7.5% for Pb and 3.9%).

Biochemical parameters

Total Chlorophyll

Total Chlorophyll was obtained according to Barnes *et al*., 1992). 0.05 g of leaves were cut in to smaller pieces and placed in test tubes containing 5 ml DMSO. Then test tubes were incubated in a water bath at 60-65 °C for an hour. From preliminary studies this time was judged satisfactory for the full decolonization of tissues and kept for cooling at room temperature about 30 min. Further these were filtered and their absorptions were measured at 665 nm and 648 nm being the final stages. Measurment of absorption of blank DMSO was carried out with the help of Spectrophotometer. Finally total chlorophyll (TCh) concentration (a, b and total) was expressed as mg/g fresh weight and determined by the following formulae (Barnes *et al.*, 1992).

Chlorophyll a (mg/g F.W) = (14.85 A665 -5 .14 A648) (1)

Chlorophyll b (mg/g F.W) = (25.48 A665 – 7.36 A648) (2)

Total chlorophyll (mg/g F.W) = (7.49 A665 + 20.34 A648) (3)

Where: A665 = absorption value at 665 nm

A648= absorption value at 648 nm

Leaf extract pH

5 g of a leaf sample was crushed with the help of motor and pestle. 50 ml deionized water was added in to it and then obtained suspension was measured with a digital pH meter (Apriyantono *et al.,* 1989)

Ascorbic acid (AA)

Ascorbic acid content (expressed in mg/g) was measured according Bajaj and Kaur (1981) method.1g of the fresh leaf was taken and then 4 ml oxalic acid EDTA extracting solution was added to it. Again one ml of Orthophospheric acid and 1 ml 5% tetraoxosulphate acid was added to this mixture, and finally 2 ml of ammonium molybdate and 3 ml of water added. The solution thus obtained allowed to stand for 15 min after which the absorbance at 760 nm is measured with a spectrophotometer. The concentrations of ascorbic acid in the sample then extrapolated from a standard ascorbic acid curve.

Estimation of relative water content (RWC)

RWC was determined according to Barrs and Weatherly (1962). RWC is a ratio of the amount of water in the leaf tissue at sampling to that present when fully turgid. A composite sample of leaf discs was taken and the fresh weight was taken and then leaf was floated on water for up to 24 h. The turgid weight was then recorded, and the leaf tissue was subsequently oven-dried to a constant weight at about 85°C for 24 h and reweighted. RWC is calculated by using the following formula:

Relative water content (%) = {(F-D) / (T-D)} ×100
F = Fresh weight of leaves (g)
D = Dry weight of leaves (g)
T = Turgid weight of leaves (g)

Specific leaf area (SLA)

Seven matured leaves from lower surface of different directions around the canopy of each tree species were collected. Leaf samples were rinsed with distilled water and then photos were taken along with scales. The collected leaf samples were then dried in hot air oven at 60 °C for 24 hours. Weigh of the completely dried leaves were recorded using three digital electronic balance. The area of the photographed leaves were measured by using Image J program (Abràmoff *et al.,* 2004). Finally, SLA (cm^2 g^{-1}) of each leaf was calculated by dividing leaf area by corresponding leaf dry weight.

Statistical Analysis

All the data analysis was conducted using IBM SPSS statistics program version 25. To evaluate the significant differences in SLA between polluted and control sites one sample t-test was conducted. Pearson correlation was calculated between the % decreased in SLA and biochemical parameters, and also between the % decrease in SLA and metal content in leaves.

Results

Metal accumulation

Mean concentrations of Zinc (Zn) was found to be higher at polluted sites than at control sites in all studied plants. Highest Zn was recorded in *Populuseuro americana* (71.92 mg/kg) and lowest in *Ficusreligiosa* (14.83 mg/kg) at different polluted sites of Kathmandu (Fig. 2). Highest mean value for Zn accumulation (71.92 mg/kg) was recorded in *Populuseuro americana* and it ranged from 14.46 mg/kg to 130.57 mg/kg at different polluted sites. Lowest mean value for Zn accumulation was recorded in *Ficusreligiosa* (14.83 mg/kg) and it ranged from 8.13 to 20.87 mg/kg at different polluted sites in Kathmandu.

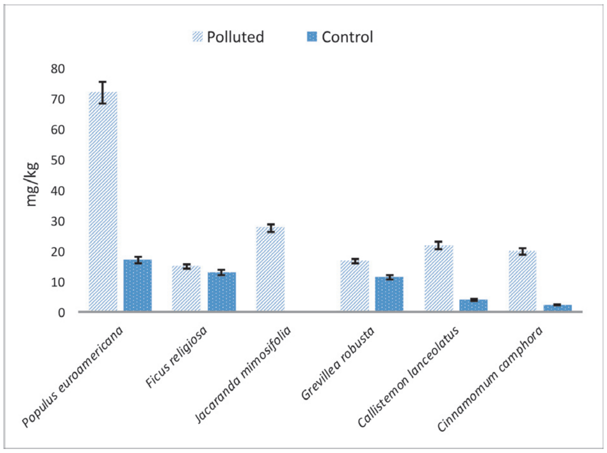

Fig. 2. Zinc concentrations (mean value) in the leaves of different tree species collected from various polluted and control sites

Mean concentrations of copper (Cu) was mostly higher at control sites than at polluted sites in all studied plants. Highest Cu was recorded in *Jacaranda mimosifolia* (14.56 mg/kg) and lowest in *Populuseuro americana* (4.14 mg/Kg)) at polluted sites (Fig.3). Highest mean value (14.56 mg/kg) for Cu accumulation was recorded in *Jacaranda mimosifolia* and it ranged from 5.72 mg/kg to 20.60 mg/kg at different polluted sites. Lowest mean value for Cu accumulation was recorded in *Populuseuro americana* (4.14 mg/kg) and it ranged from 0.05 mg/kg to 11.93 mg/kg at different polluted sites in Kathmandu.

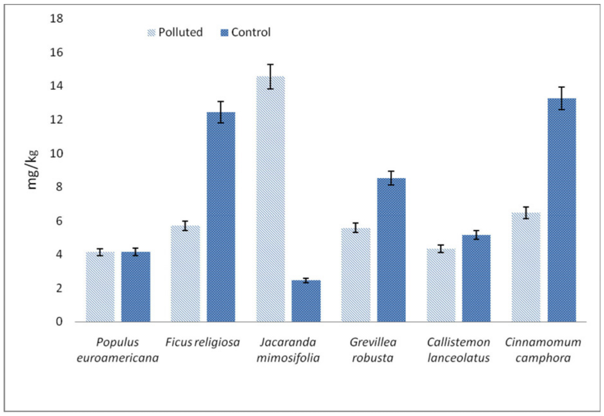

Fig. 3. Copper concentrations (mean value) in the leaves of different tree species collected from various polluted and control sites

Mean concentrations of lead (Pb) was mostly higher at polluted sites than at control sites in all studied plants. Highest Pb was recorded in *Callistemon lanceolatus* (22.47 mg/kg) and lowest in *Populuseuro americana* (0.05 mg/kg) at polluted sites (Fig.4). Highest mean value (22.47 mg/kg) for Pb was recorded in *Callistemon lanceolatus* and it ranged from 5.21 to 25.20 mg/kg at different polluted sites in Kathmandu.

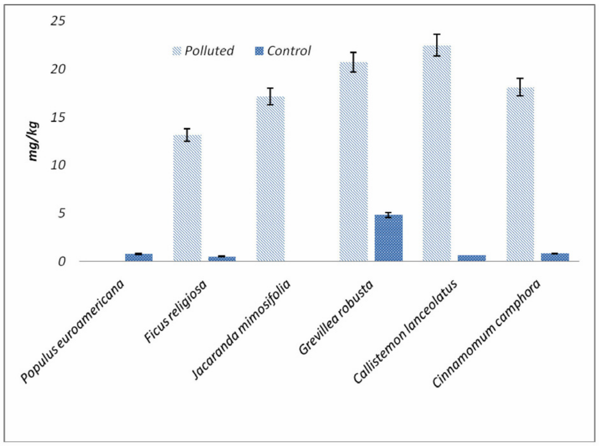

Fig. 4. Lead concentrations (mean value) in the leaves of different tree species collected from various polluted and control sites

Biochemical parameters

Mean value of total chlorophyll (TCh) was found to be higher at control sites than at polluted sites in all studied plants except in *Jacaranda mimosifolia*. Highest TCh mean value at control site was recorded in *Ficusreliogiosa* (1.243mg/g) and lowest recorded in *Cinnamomumcamphora* (0.64 mg/g). But at polluted sites highest TCh mean value was recorded in *Jacaranda mimosifolia* and lowest in *Cinnamoum camphora* (Fig. 5). Mean value of pH was mostly found to be higher at control sites than at polluted sites in all studied plants except in *Populuseuro americana* and *Jacaranda mimosifolia*. Highest pH mean value at control site was recorded in *Grevillearobusta* (6.75) and lowest recorded in *Jacaranda mimosifolia* (5.43). But at polluted sites highest pH mean value was recorded in *Ficusreligiosa* (6.33) and lowest in *Callistemon lanceolatus* (5.28)(Fig. 6). Mean value of Ascorbic acid(AA) was generally found to be higher at control sites than at polluted sites in all studied plants except in *Grevillearobusta and Cinnamomumcamphora*. Highest Ascorbic acid mean value at control site was recorded in *Populuseuroamericana* (8.61mg/g) and lowest recorded in *Cinnamomumcamphora* (4.54 mg/g). But at polluted sites highest Ascorbic acid mean value was recorded in *Populuseuroamericana* (8.10 mg/g) and lowest in *Ficusreligiosa* (4.80 mg/g) (Fig 7). Mean value of Relative water content (RWC) was generally found to be higher at control sites than at polluted sites in all studied plants except in *Ficusreligiosa* and *Jacaranda mimosifolia*. Highest Relative water content (RWC) mean value was recorded in *Cinnamomumcamphora* (86 %) and lowest recorded in *Jacaranda mimosifolia* (40.02 %) at control site. But at polluted sites highest Relative water content (RWC) mean value was recorded in *Ficusreligiosa* (80.78 %) and lowest in *Grevillearobusta* (60.51%) (Fig. 8).

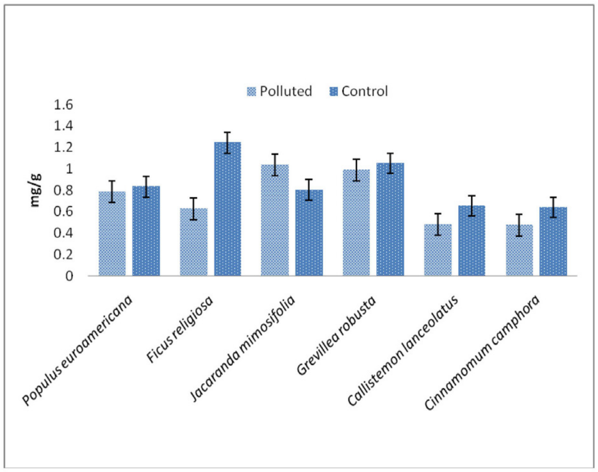

Fig. 5. Total chlorophyll content (mean value) in the leaves of different tree species collected from various polluted and control sites

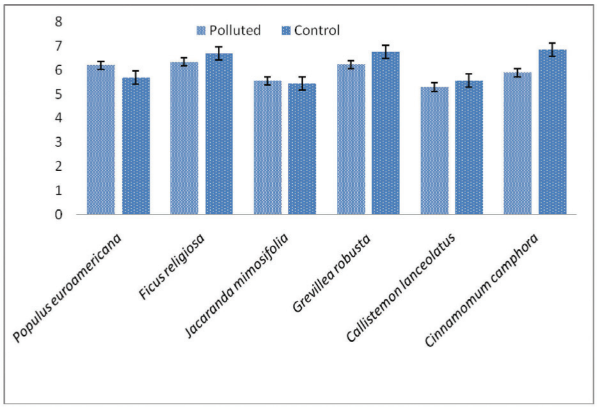

Fig. 6. pH (mean value) in the leaves of different tree species collected from various polluted and control sites

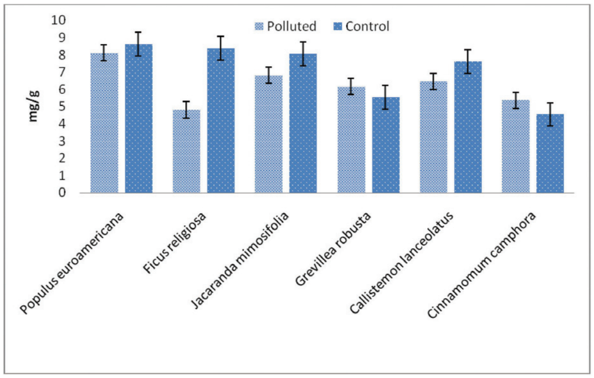

Fig. 7. Ascorbic acid (mean value) in the leaves of different tree species collected from various polluted and control sites

Specific leaf area (SLA)

Specific leaf area (SLA) in all the studied tree leaves decreased at polluted sites than at control sites. Specific leaf area (SLA) of different species and the SLA percentage decreased at polluted sites are given in Table 1. In this study SLA among the studied tree leaves ranged from 188.08±35.12 to 24.48± 0.87cm^2 g^{-1}. Decrease in SLA in polluted sites ranged from 78.18± 4.47 to 9.32±4.80 %. Though all the tree species showed decrease in SLA at polluted sites but was significant (at P=0.05) in *Populuseuroamericana* (at Tinkune, Airport, Dhumbarahi), *Ficusreligiosa* (at

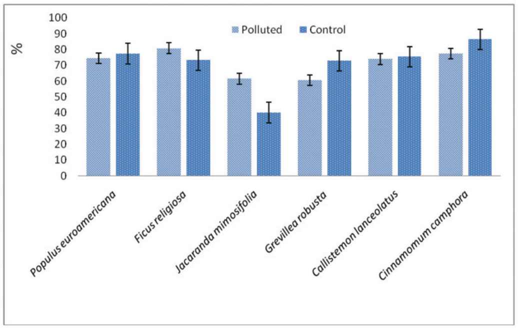

Fig. 8. Relative water content (meanvalue) in the leaves of different tree species collected from various polluted and control sites

Dhumbarahi, Basundhara, Balaju, Airport), *Jacaranda mimosifolia* (at Lainchaur, Basundhara, Swayambhu), *Grevillearobusta* (at Lainchaur, Basundhara) and in *Callistemon lanceolatus* (at Dhumbarahi, Balaju, Airport,Swayambhu).

Correlation between SLA and Bio chemicals

Results of Pearson correlations between SLA and biochemical parameters like total chlorophyll (TCh), pH, ascorbic acid (AA) and relative water content (RWC) is given in Table 2. Among all studied plants, % decrease in SLA of *Ficusreligiosa* showed positive

Table 1. SLA (cm^2 g^{-1}) and percentage decreased in SLA of different tree species growing along the road side in various polluted (P) and control sites (C) in Kathmandu.

Plants	Experimental (P) and control (C) site	SLA Mean ± Sd	%Decreased in SLA Mean ± Sd	t-value*
Populuseuroamericana	Tinkune (P)	8.48±19.66	38.99±13.47	5.015*
	Airport(P)	112.73±20.60	20.69±7.68	4.668*
	Chabhil(P)	93.50±31.32	27.93±16.78	2.883
	Samakhusi (P)	89.92±37.40	34.80±21.99	2.740
	Basundhara (P)	114.90±34.87	9.32±4.80	3.363
	Dhumbarahi (P)	61.03±12.33	50.67±10.11	8.685*
	Narayanthan (C)	131.07±32.21		
Ficusreligiosa	Dhumbarahi (P)	99.13±17.38	25.21±2.90	15.069**
	Basundhara (P)	81.85±6.35	34.02±14.07	4.189*
	Balaju (P)	69.31±9.00	47.31±1.79	45.790**
	Airport (P)	61.12±6.81	53.87±7.59	12.289**
	Narayanthan (C)	132.70±23.55		
Jacaranda mimosifolia	Lainchaur (P)	164.08±36.56	13.00±5.66	3.974*
	Dhumbarahi (P)	112.47±86.85	40.94±44.98	1.576
	Basundhara (P)	74.22±17.06	41.83±14.73	4.917*
	Airport (P)	128.52±4.74	23.77±14.07	2.925
	Swayambhu(P)	41.77±8.67	73.57±1.26	101.335**
	Narayanthan (C)	188.08±35.12		
Grevillearobusta	Lainchaur (P)	84.12±17.64	25.69±10.48	4.245*
	Dhumbarahi(P)	72.12±6.03	27.48±13.71	3.471
	Basundhara(P)	101.28±6.94	13.38±1.70	13.638**
	Airport(P)	80.08±25.83	28.98±19.05	2.634
	Balaju(P)	61.55±31.94	48.20±26.83	3.111
	Narayanthan(C)	114.58±29.75		
Callistemon lanceolatus	Dhumbarahi(P)	24.48± 0.87	78.18± 4.47	48.605**
	Balaju(P)	35.60±13.45	31.73±7.47	4.584*
	Airport(P)	61.79±22.33	40.84±30.55	4.793*
	Swayambhu(P)	69.31±5.66	21.05±9.11	21.211**
	Narayanthan(C)	116.15±28.74		
Cinnamomumcamphora	Dhumbarahi(P)	91.11±14.18	10.90±10.83	1.743
	Basundhara(P)	88.74±9.58	32.72±22.63	2.504
	Balaju(P)	99.74±0.08	16.90±7.52	3.891
	Airport(P)	104.54±25.05	32.94±22.68	2.514
	Narayanthan(C)	139.44±37.04		

*P=0.05 and ** P=0.001 obtained from one sample t-test of % decreased SLA for each tree species with N value ranging from 15 to 21.

significance correlation with total chlorophyll (TCh) and pH. In *Jacaranda mimosifolia* and *Callistemon lanceolatus* positive significance relation was observed between % decrease in SLA and Ascorbic acid.

Correlation between SLA and metal content

Results of Pearson correlations between % decrease in SLA and heavy metal contents (Zn, Cu, Pb) in leaves is given in Table 3. Percentage decrease in SLA of *Populuseuro americana* showed significant (P=0.05) negative correlation with Zn but significant (P=0.05) positive with Pb. In *Ficusreligiosa* % decrease in SLA showed positive significance correlation with Cu and Pb but in *Jacaranda mimosifolia*% decrease in SLA showed negative correlation with Zn and Cu. In *Grevillearobusta*% decrease in SLA showed negative significance correlation with Pb and in *Callistemon lanceolatus* it showed negative significance correlation with Zn. In *Cinnamomum camphora*, % decrease in SLA showed negative significane correlation with Zn but positive with Cu.

Discussion

Metal accumulation

Zn is one of the essential element in many biochemical process such as auxin, chlorophyll, carbohydrate and protein synthesis (Broadley, 2007). Onderand Dursun (2006) reported that the highest Zn concentrations were found in Cedarneedles at an industrial site. Zinc accumulation in *Populus* differed from 26.1 to 139 mg kg^{-1}, in unpolluted and polluted sites in Greece (Sawidis *et al.*, 1995). In this study it was found that plants grown in urban localities accumulated more Zn than other metals. The range of Zn concentration between 10 to 150 mg kg^{-1} (Padmavathiamma and Li, 2007; Hu *et al.*, 2014) is conventional for plants and toxic concentrations range from 300 to 400 mg kg^{-1} depending on plant species (Broadley *et al.*, 2007). In the present study the highest Zn accumulations was detected in *Populus*. The amount of Zn accumulation in leaves are not at a toxic level. Copper is an essential trace element for plants. Both of its deficiency and excess affect many enzymes which catalyze oxidation and reduction reactions (Ouzounidou, 1994; Celik *et al.*, 2005; Doganlar and Atmaca, 2011). The conventional range of Cu concentrations in the plants is 3 to 30 mg kg^{-1} (Kabata and Pendias, 2001) but its phytotoxic concentrations range is 20 to100 mg kg^{-1}(Padmavathiamma and Li, 2007). In the present study, the highest increase in Cu content was found in *Jacaranda mimosifolia.* Though the mean value (14.15 mg/kg) of Cu content in it is within the normal range but at some sites its concentrations were

Table 2. Correlations of percentage decreased in SLA with TCh, pH, AA and R WC of leaf at polluted sites

Plants	% decrease SLA-TCh	% decrease SLA-pH	% decrease SLA-AA	% decrease SLA-RWC
Populuseuroamericana	0.021	-0.107	-0.217	-0.101
Ficusreligiosa	0.807**	0.572*	0.356	0.0376
Jacaranda mimosifolia	0.381	0.312	0.801**	0.078
Grevillearobusta	0.169	0.227	0.212	0.266
Callistemon lanceolatus	0.175	-0.220	0.694**	-0.100
Cinnamomumcamphora	0.274	0.362	-0.134	0.154

* Significant at P= 0.05 level and ** at P = 0.01 level

Table 3. Correlations of percentage decreased SLA with leaf metal content (Zn, Cu, Pb)

Plants	% decrease SLA-Zn	% decrease SLA-Cu	% decrease SLA-Pb
Populuseuroamericana	-0.460*	0.091	0.737**
Ficusreligiosa	-0.316	0.535*	0.599*
Jacaranda mimosifolia	-0.656**	-0.767**	0.117234
Grevillearobusta	-0.38	0.198	0.669**
Callistemon lanceolatus	-0.721**	0.285	-0.507
Cinnamomumcamphora	-0.769**	0.618*	-0.35314

*Correlation is significant at P= 0.05 and **Correlation is significant at P = 0.01 level.

at phytotoxic range i.e. above 20 mg kg^{-1}. The sufficient or normal Pb concentration in the plants can be in the range of 5 to10 mg kg^{-1} (Kabata-Pendias and Pendias, 2001) and its toxic concentrations is from 30 to 300 mg kg^{-1}. While Markert (1994) reported the toxic Pb range for plants to be between 3 and 20 mg kg^{-1}. In this study, Pb amounts in all plants and sampling points were within normal limits and were found higher than the control site.

Biochemical parameters

In all studied tree species, biochemical parameters showed different variations in polluted and control sites. Total chlorophyll were reduced in polluted sites which were in agreement with the observations of Prusty *et al*., 2005), and Sharma and Tripathi, 2009). The reduction in pigment concentration might be due to deposition of dust particles on leaf surfaces which reduced the light available for photosynthesis and resulted in the inhibition of chlorophyll biosynthesis (Prusty *et al.,* 2005), and the other one was due to dissolution of metals and polycyclic hydrocarbons cell sap as a result blocked the stomatal pores from exchange of air, thus developing the stress on plant metabolism and resulting in chlorophyll degradation (Kapoor *et al.,* 2013). pH is a biochemical parameter that serves as a sensitivity indicator of air pollution (Joshi *et al.,* 2011), and plants with a pH of around 7 are more pollution-tolerant. The leaf extract pH of plant species collected from polluted sites was lower than that of control sites which agreed with Rai and Panda, 2013). The decrease in leaf extract pH could be due to the effect of atmospheric SO_2 and NO_X. Ascorbic acid in plant leaves plays multiple functions to perform through cell wall synthesis, cell division, photosynthetic carbon fixation and also acts as a strong reducer protecting the plants against reactive oxygen species (ROS), thereby improving the tolerance ability of the trees against air pollution. With the increase in ascorbic acid content (Lima *et al.,* 2000), the tolerance level in plants also increases The present study revealed that most of tree species at control sites had higher ascorbic acid content than at polluted site but *Cinnamomum* and *Grevillea* had more average Ascorbic acid content at polluted sites. This indicates that the trees like *Cinnamomum* and *Grevillea* have developed better tolerance ability than others. The average value of RWC at polluted sites were slightly decreased in *Grevillea* and *Cinnanomum,* but was slightly increased in *Ficus*and *Jacaranda*. Chaturvedi *et al.,* 2013 reported that RWC was considerably reduced in polluted sites, and significantly negatively correlated with dust (Rai and Panda, 2013). Higher RWC in plants will help maintain their physiological balance when exposed to air pollution and favors drought resistance. In the present study, *Ficusreligiosa* and *Jacaranda mimosifolia* showed increase in mean RWC value at polluted sites, indicating their better tolerance than others.

Specific leaf area (SLA)

In all studied tree species specific leaf area (SLA) decreased at polluted sites than control sites and similar results were also reported by Abbasi *et al.*, 2018) in Tehran Metropolitan City, Iran. Reduction in SLA reduces water loss and transpiration. This is possibly a strategy of plants to improve stress tolerance (Xu *et al.*, 2009). Plants that are resistant to tension have higher relative water content (Mahecha *et al.*, 2013), which is evident in *Ficusreligiosa* and *Jacaranda mimosifolia* in the present study. Insignificant relation was mostly observed between the % decrease in SLA and biochemical parameters, which might be due to various strategies and physiological adjustments in plants to survive in adverse conditions.

Significant negative correlation was observed between % decreased in SLA and Zn accumulation in *Populus, Jacaranda, Callistemone* and *Cinnamomum* growing at polluted sites. In the present study the Zn accumulation in these plants are below the phytotoxic level as suggested by Chaney, (1993) but even then reduction % in SLA ranged from 9 to 50% in *Populus*, 13 to 73% in *Jacaranda*, 21 to 78% in *Callistemon* and 10 to 32% in *Cinnamomum*. This reduction in SLA might be due to deficiency of some other essential elements when there is stress in plant due to increased concentrations of one toxic metal (Sharma and Chettri, 2005). Significant positive relation was observed between the % decreased in SLA and Cu accumulation in *Ficus* and *Cinnamomum* and found in low concentrations of Cu in both these species. Cu is essential for various physiological processes such as a structural element in regulatory proteins, electron transport, mitochondrial respiration and oxidative stress responses (Marschner, 1999; Raven *et al.*, 1999) in low concentrations. The % decrease in SLA ranged from 25-53% in *Ficus* and 10-32% in *Cinnamomum.* The positive relation between them might be due to some other factors, which regulates SLA, but not the toxicity of Cu. Significant negative correlation was observed between Cu accumulation and % reduction of SLA in *Jacaranda*. In the present study, the highest accumulation of Cu was found in *Jacaranda mimosifolia* and similar results were also reported by Zeynep *et al*., (2012). The negative relation between them might be due to the concentration of Cu which reached up to 20.60 mg/kg at polluted sites and are at the phytotoxic range (Padmavathiamma and Li, 2007). The significant positive relation between % reduction of SLA and Pb content might be due to Pb toxicity. Markert (1994) reported the phytotoxic range of Pb for plants to be between 3 and 20 mg/ kg. As the Pb concentrations at the polluted sites reached up to 27.51 mg/kg in *Populus*, 39.61 mg/kg in *Ficus*, 37.8 mg/kg in *Grevillea* in the present study, and these concentrations are at phytotoxic range in plants as described by Markert (1994).

Conclusion

From the above it can be concluded that though the studied biochemical parameters have their physiological role in leaves but their relation with SLA is mostly insignificant which might be due to various strategies for physiological adjustment in stressed conditions in plants. The impact of heavy metals in small concentrations on SLA are not evident, but when present in high concentrations i.e at phytotoxic range, their impacts on SLA is conspicuous. Based on heavy metal accumulation and SLA, all the studied roadside trees (like *Populuseuroamericana, Ficusreligiosa, Jacaranda mimosifolia, Grevillearobusta, Callistemon lanceolatus* and *Cinnamomumcamphora*) which could accumulate Pb and/or Zn at high concentrations at polluted sites and could withstand adverse environment by reducing their SLA are recommended for future plantation to mitigate roadside air pollution problem.

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Appendix 3: Certificate of international and national conference attended and paper presented

1. INTERNATIONAL CONFERENCE BIODIVERSITY,LIVELIHOOD AND CLIMATE CHANGE IN THE HIMALAYAS (December 12-14, 2010), Kathmandu, Nepal.

2. 7th NATIONAL CONFERENCE ON SCIENCE AND TECHNOLOGY (March 29-31, 2016, Kathmandu, Nepal) and Paper presented at conference.

3. INTERNATIONAL CONFERENCE ON BIODIVERSITY, CLIMATE CHANGE ASSESSMENT AND IMPACTS ON LIVELIHOOD (January 10-12, 2017) and paper presented at conference.

4. INTERNATIONAL CONFERENCE ON INTEGRATING BIOLOGICAL RESOURCE FOR PROSPERITY (February 6-7, 2020) Biratnagar Nepal.
5. Poster presented- Ph. D. FESTIVAL 2023, 9-10 October, University Campus. Kirtipur, Kathmandu, Nepal.

International Conference

Biodiversity, Livelihood and Climate Change in the Himalayas

12-14 December 2010; Kathmandu, Nepal

Certificate of Participation

Mr. Jaya Prakash Hamal, Nepal

Prof. Dr. Ram P Chaudhary
Member Secretary
ICBLCC 2010

Prof. Dr. Krishna K Shrestha
Chairman
ICBLCC 2010

Save the Himalayan Biodiversity for Future Generations

Nepal Academy of Science and Technology

CERTIFICATE

Awarded to

Jaya Prakash Hamal

for active participation/[✓ paper presentation]/poster presentation

in

THE 7th NATIONAL CONFERENCE ON SCIENCE AND TECHNOLOGY

SCIENCE, TECHNOLOGY AND INNOVATION FOR NEPAL'S GRADUATION TO DEVELOPING COUNTRY STATUS

March 29-31, 2016

Kathmandu, Nepal

Ramila Raut

Mrs. Ramila Shrestha Raut
Chief, Promotion Division

Buddhi

Dr. Buddhi Ratna Khadge
Secretary

Prof. Dr. Jiba Raj Pokharel
Vice-Chancellor

Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms.

Jay Prakash Hamal

has presented [✓ paper]/poster/attended

the International Conference on Biodiversity, Climate Change Assessment and Impacts on Livelihood

at Kathmandu on January 10-12, 2017.

Dr. Bishwa Nath Oli
Secretary, Ministry of Population and Environment

Nir Krakauer

Dr. Nir Krakauer
Co-Chair, Conference Organizing Committee
PI: USAID-IPM-IL-Biodiversity Project

Prof. Naba R Devkota
Director of Research
Agriculture and Forestry University

Prof. Mohan Siwakoti
Secretary, Conference Organizing Committee
Head, Central Dept. of Botany, TU

Prof. Pramod K Jha
Chair, Conference Organizing Committee
Nepal Coordinator & Co-PI
USAID-IPM-IL-Biodiversity Project

National Conference on Integrating Biological Resources for Prosperity

Certificate of Participation

This is to certify that

Mr. Jaya Prakash Hamal

has presented Paper / Poster / Attended the

National Conference on Integrating Biological Resources for Prosperity

held at Biratnagar on February 6-7, 2020.

Prof. Dr. Mohan Siwakoti
Chairman
Conference Organizing Committee
President, Botanical Society of Nepal

Prof. Dr. Sasinath Jha
Chairman
Nepal Biological Society

Dr. Bishwa Nath Oli
Secretary
Ministry of Forests and Environment

Hon. Jagadish Prasad Kusiyait
Minister
Ministry of Industry,
Tourism, Forests and Environment
Province No. 1

TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE & TECHNOLOGY
Kirtipur, Kathmandu

PARTICIPATION CERTIFICATE

AWARDED TO

JAY PRAKASH HAMAL

With appreciation and good wishes for participation with a poster presentation in the

Ph.D. Festival 2023

Held on 9-10 October, 2023 (22-23 Asoj 2080) in University Campus, Kirtipur, Nepal.

Prof. Dr. Binil Aryal
Dean, IoST, TU, Nepal

Organized by
Institute of Science & Technology, TU

Prof. Dr. Shiva Lal Bhusal
Rector, TU, Nepal