HEAVY METAL ACCUMULATION IN SOME PLANTS AT BUTWAL SUB-METROPOLITAN CITY, NEPAL

A Dissertation Submitted for Partial Fulfillment of the Requirement for the Master's Degree in Botany

Submitted By:

RAMCHANDRA ADHIKARI

Batch: 2076

Exam Roll No.: 834/076

T.U. Registration No.: 5-2-50-435-2014

Submitted To:

Department of Botany

Amrit Campus

Institute of Science and Technology

Tribhuvan University

Kathmandu, Nepal

July, 2024

DECLARATION

The dissertation entitled **"Heavy metal accumulation in some plants at Butwal Sub Metropolitan City, Nepal"** has been done by myself, under the supervision of Assistant Prof. Dr. Laxmi Joshi Shrestha and co-supervision of Prof. Dr. Mukesh Kumar Chettri. This work has not been submitted to any other institution for any academic degree. Any information or contributions from others that are cited in this thesis have been appropriately acknowledged and listed in the references.

.....

Ramchandra Adhikari
Department of Botany
Amrit Campus
July, 2024

TRIBHUVAN UNIVERSITY

AMRIT CAMPUS

THAMEL, KATHMANDU, NEPAL
P.O.Box. No. 10278

Institute of Science and Technology
Amrit Campus

Ref. No. .....

Date: 2081-04-16

RECOMMENDATION

This is to recommend that the dissertation entitled "**Heavy metal accumulation in some plants at Butwal Sub- Metropolitan City, Nepal**" has been carried out by Mr. Ramchandra Adhikari for the partial fulfillment of Master's Degree of Science in Botany with special paper Plant Ecology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institutions.

.....

Supervisor
Asst. Prof. Dr. Laxmi Joshi Shrestha
Amrit Campus
Tribhuvan University
Kathmandu, Nepal

.....

Co-Supervisor
Prof. Dr. Mukesh Kumar Chettri
Amrit Campus
Tribhuvan University
Kathmandu, Nepal
July, 2024

Phone No.: Campus Chief 4411637 , Admin Off. 4410408
Hostel 4414236 , Examination 4421476
E-mail: amritcampus@ntc.net.np
Website: amritcampus.edu.np

TRIBHUVAN UNIVERSITY

AMRIT CAMPUS

THAMEL, KATHMANDU, NEPAL
P.O.Box. No. 192

Ref. No. .....

Date: 2081-04-06

Amrit Campus

LETTER OF APPROVAL

On the recommendation of supervisor "Assistant Prof. Dr. Laxmi Joshi Shrestha" this dissertation submitted by Mr. Ramchandra Adhikari entitled **"Heavy metal accumulation in some plants at Butwal Sub- Metropolitan City, Nepal"** has been approved for the examination in partial fulfillment of the requirements of the Master's Degree of Science in Botany with a special paper Plant Ecology.

.....

Head of Department
Asst. Prof. Dr. Jaya Prakash Hamal
Amrit Campus
Tribhuvan University
Kathmandu, Nepal

Phone No.: Campus Chief 4411637 , Admin Off. 4410408
Hostel 4414236 , Examination 4421476
E-mail: amritcampus@ntc.net.np
contact@amritcampus.edu.
Website: amritcampus.edu.np

TRIBHUVAN UNIVERSITY

AMRIT CAMPUS

THAMEL, KATHMANDU, NEPAL
P.O.Box. No. 102

Ref. No. .....

CERTIFICATE OF ACCEPTANCE

Date...2081-04-06

This dissertation work submitted by Mr. Ramchandra Adhikari, entitled "**Heavy metal accumulation in different plants at Butwal Sub-Metropolitan City, Nepal**" has been accepted as partial fulfillment of the requirements of the master's degree of science in Botany with a special paper on Plant Ecology.

Evaluation committee

External Examiner
Asso. Prof. Dr. Anjana Devkota
Central Department of Botany
Tribhuvan University
Kathmandu, Nepal

Internal Examiner/ HOD
Asst. Prof. Dr. Jaya Prakash Hamal,
Amrit Campus
Tribhuvan University
Kathmandu, Nepal

Supervisor/ Coordinator
Asst. Prof. Dr. Laxmi Joshi Shrestha
Amrit Campus
Tribhuvan University
Kathmandu, Nepal

Co-Supervisor
Prof. Dr. Mukesh Kumar Chettri
Amrit Campus
Tribhuvan University
Kathmandu, Nepal

Date of Examination: 21 July, 2024

Phone No.: Campus Chief 4411637 , Admin Off. 4410408
Hostel 4414236 , Examination 4421476
E-mail: amritcampus@ntc.net.np
contact@amritcampus.edu.
Website: amritcampus.edu.np

ACKNOWLEDGEMENT

I would like to express my deep and sincere gratitude to my supervisor and coordinator Asst. Prof. Dr. Laxmi Joshi Shrestha and co-supervisor Prof. Dr. Mukesh Kumar Chettri for their valuable guidance, support, encouragement, continuous suggestions throughout this research work.

I am equally grateful to Asst. Prof. Dr. Jaya Prakash Hamal, Head of the Department and Associate Prof. Dr. Shila Singh, Former Head of Department, for their kind support and motivating guidance. I appreciate all the faculty members and staffs of Department of Botany, Amrit Campus, who assisted me directly or indirectly during my research.

I am thankful to authorities of Butwal Sub metropolitan city and Butwal Industrial Area, Butwal, Rupandehi for allowing me to conduct this research. I would also thankful to department of Hydrology and Metrology for providing necessary climatic data.

I also want to express heartfelt thanks to Mr. Saroj Acharya and Mr. Bishal Subedi for supporting me during field visit time. I am kindly thankful to Enviro Chemical Laboratories for laboratory analysis of the collected samples.

Lastly, I express my kind appreciation to my friends for their kind support. I am also obliged to my family for their continuous support and encouragement to carry out this research work.

Ram Chandra Adhikari
rcadhikari87@gmail.com

ABSTRACT

Plants have traditionally been viewed as pollutant sinks, and they can accumulate higher concentrations of heavy metals in the urban environment. This study aimed to evaluate the effectiveness of common plant species in monitoring and mitigating heavy metals in Butwal Sub Metropolitan City. Heavy metal concentrations (Pb, Cu, and Zn) in roadside, industrial site and at control were measured to evaluate the influence of emissions of metals at those areas in different samples viz. leaves, barks and soil dust around those sites. Perkins Elmer Atomic Absorption Spectrophotometer (AAS) was used to estimate heavy metals concentrations in plants. Concentrations of Pb, Cu, and Zn were significantly higher in plants growing at industrial sites when compared to their roadside and control points. Among three metals, zinc showed maximum accumulation and contamination at all sites while lead showed less accumulation and contamination. The accumulation of Pb, Cu, and Zn in leaves sample was ranged from 0.25-1.60 mg/kg, 1.87-10.17 mg/kg and 20.07- 80.25 mg/kg; in barks sample Pb ranged from 1.17-3.82 mg/kg, Cu ranged from 1.9- 13.08 mg/kg and Zn ranged from 11.8-181.43 mg/kg while in soil samples, Pb, Cu and Zn ranged from 5.17-64.17, 5.75-208.83 and 34.07-236.67 mg/kg respectively. Similarly, accumulation of Pb, Cu, and Zn was higher in barks of *Azadirachta indica* at industrial site, *Shorea robusta* at roadside and *Azadirachta indica* at industrial site respectively. The soil sample of industrial area showed maximum accumulation of all three metals i.e. 64.17, 208.83 and 236.67 mg/kg for Pb, Cu and Zn. The plants that showed highest MAI value like *Terminalia alata*, *Ficus religiosa, Mangifera indica*, *Azadirachta indica* can be used for phytoremediation around industrial and roadside areas.

Keywords: AAS, Accumulation, *Azadirachta indica*, Heavy metals, Monitoring, MAI.

शोधसार

बिरुवाहरूलाई परम्परागत रूपमा प्रदूषक सिंकको रूपमा हेरिएको छ, र तिनीहरूले शहरी वातावरणमा भारी धातुहरूको उच्च सांद्रता जम्मा गर्न सक्छन्। यो अध्ययन बुटवल उपमहानगरपालिकामा भारी धातुहरूको अनुगमन र न्यूनीकरणमा साझा बोट प्रजातिहरूको प्रभावकारिता मूल्याङ्कन गर्ने उद्देश्यले गरिएको थियो। सडक छेउमा, औद्योगिक साइट र नियन्त्रणमा भारी धातु सांद्रता (Pb, Cu, र Zn) विभिन्न नमूनाहरूमा ती क्षेत्रहरूमा धातुहरूको उत्सर्जनको प्रभाव मूल्याङ्कन गर्न मापन गरिएको थियो। ती साइटहरू वरपर पातहरू, बोक्राहरु र धुलोको नमुनामा धातुहरुको परिक्षण गरिएको थियो । पर्किन्स एल्मर परमाणु अवशोषण स्पेक्ट्रोफोटोमिटर (AAS) प्रयोगगरि बिरुवाहरूमा भारी धातुहरूको सांद्रता अनुमान गर्न प्रयोग गरिएको थियो । Pb, Cu, र Zn को सांद्रता औद्योगिक साइटहरूमा बढ्ने बिरुवाहरूमा उनीहरूको सडकको किनार र नियन्त्रण बिन्दुहरूको तुलनामा उल्लेखनीय रूपमा उच्च थियो। तीन धातुहरू मध्ये, Zn सबै साइटहरूमा अधिकतम संचय र प्रदूषण देखायो जबकि Pb कम संचय र प्रदूषण देखायो। पातको नमूनामा Pb, Cu, र Zn को संचय 0.25-1.60 mg/kg, 1.87- 10.17 mg/kg र 20.07-80.25 mg/kg; छालाको नमूनामा Pb 1.17-3.82 mg/kg, Cu 1.9- 13.08 mg/kg र Zn 11.8-181.43 mg/kg सम्म रहेको छ भने माटोको नमूनाहरूमा Pb (5.17-64), Cu (5.75-208.83) र Zn (34.07-236.67) mg/kg सम्म रहेको थियो । त्यसैगरी औद्योगिक क्षेत्रमा निम *(Azadirachta indica)*, सडक छेउमा साल *(Shorea robusta)* र औद्योगिक क्षेत्रमा निम *(Azadirachta indica)* को बोक्रामा क्रमशः Pb, Cu र Zn बढी रहेको छ । औद्योगिक क्षेत्रको माटोको नमूनाले Pb, Cu र Zn को लागि तीनवटै धातुहरू अर्थात् 64.17, 208.83 र 236.67 mg/kg अधिकतम जम्मा भएको देखाएको छ। साज (*Terminalia alata)*, पिपल *(Ficus religiosa)*, आँप *(Mangifera indica)*, निम *(Azadirachta indica)* जस्ता उच्च MAI मान देखाउने बिरुवाहरू औद्योगिक र सडक छेउका क्षेत्रहरूमा phytoremediation को लागि प्रयोग गर्न सकिन्छ।

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

Page No.

LIST OF ABBREVIATION AND ACRONYMS

°C	:	Degree Centigrade
AAS	:	Atomic Absorption Spectrometer
ANOVA	:	Analysis of Variance
CBCI	:	Compressive Bio Concentration Index
cm	:	Centimeter
Cu	:	Copper
DW	:	Dry weight
E, W, S, N	:	East, West, South, North
GIS	:	Geographic Information System
HIA	:	Hetauda Industrial Area
HMs	:	Heavy metals
MAI	:	Metal Accumulation Index
Masl	:	Meter above sea level
Mg/kg	:	Milligram per kg
Mm	:	Millimeter
MS	:	Microsoft
P	:	Level of significance
Pb	:	Lead
SD	:	Standard Deviation
SPSS	:	Statistical package for social science
WHO	:	World Health Organization
Zn	:	Zinc

CHAPTER 1
INTRODUCTION

1.1. Background

Pollution of the environment is one of the most serious issues confronting the globe today. Various efforts have been made globally to save the environment, but it still appears to be an uphill battle. Increased urbanization and the industrial revolution are having a major effect on environmental destabilization through the emergence of heavy metal contamination.

1.1.1 Heavy metal

Any metallic element that has a very high density and is dangerous or toxic even at low concentrations is referred to as a heavy metal (Lenntech, 2004). Rather from being eliminated or changed by chemical or physical means, heavy metals are changed and cycled through the ecosystem (Conner, 1994). The highest possible levels of heavy metals in agricultural soils have an impact on the biosphere (Ferguson, 1990) and agricultural productivity (Baath, 1989; Chaudri *et al.,* 1993). Biomolecules are harmed at the cellular level by elevated levels of heavy metals in the environment (Somashekaraiah *et al.,* 1992; Stoths and Bagchi, 1995; Schutzendubel and Polle, 2002), followed by some visible injuries (Wang and Schapp, 1988) and in extreme cases may change the vegetation (Ernst, 1998).

According to Garbarino *et al.*, (1995) and Hawkes (1997), the term heavy metal refers to a broad category of metals and metalloids having atomic densities greater than $4g/cm^3$ or five times that of water. According to Njoku and Ayoka (2007), heavy metals include Hg, Cd, As, Cr, Pb, and Zn. Just seventeen of the 53 heavy metals found in nature can be accessed by living cells (Weast 1984). Micronutrients Fe, Mo, and Mn are necessary; trace elements Zn, Ni, Cu, V, Co, W, and Cr are of modest importance but toxic in excess. Ag, Sb, U, Pb, Cd, As, and Hg are examples of heavy metals that have no biological role which are more or less toxic to living organisms (Godbold and Huttermann, 1985; Nies 1999).

Chemicals that are referred to as heavy metals, such as cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb), are harmful to organisms in even minor quantities. As critical micronutrients, some metals, such copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn), are needed by organisms in minute amounts (Freedman, 1972). For bio monitoring to offer data on levels of short- and long-term toxicity, higher plants, notably perennials, are preferred (Dogan *et al.*, 2014). The information gathered through bio monitoring is crucial for understanding the kind and concentration of contaminants. In addition to its lower application and maintenance costs, it also offers a significant benefit over other environmental protection techniques (Dogan *et al.*, 2010).

The total concentration of heavy metals normal range in plants is 3, 0.4-45.8 and 1-160 mg/kg for Pb, Cu and Zn respectively while 35, 30 and 90 mg/kg for Pb, Cu and Zn respectively for soils (Ross, 1994, Kabata-Pendias, 2000).

Table 1: Background, normal and critical concentrations of heavy metals in soil and plant parts

Metal	Background concentration (μkg^{-1})	Normal soil value (mg/kg)	Critical soil value (mg/kg)	Normal plant value µg/g DW	Critical plant concentration µg/g DW
Pb	$20 – 50^{N}$	35^{*}	400^{KP}	$0.1 – 100^{*}$ $0.1 – 100^{**}$	$20 – 30^{KP}$ 25 – 85#
Cu	$100^{@}$	30^{*}	125^{KP}	$4 – 15^{**}$	$20 – 100^{KP}$ $15 – 20^{KP}$
Zn	$300^{@}$	90^{*}	400^{KP}	$15 – 100^{*}$ $8 – 100^{**}$	200^{***} 60 – 900# $100 – 400^{KP}$

(Sources: ^{KP}K-Pendias and Pendias (1992); *Bowen (1979); **Alloway (1968); ***Davis and Beckett (1978); * Page et al. (1987); @French regulation of agricultural soils (^{N}Norrish, 1968 and Nriagu, 1978); # (Beckett and Davis, 1977; MacNicol and Beckett, 1985; Pahlsson, 1989).

Although heavy metals cannot be removed chemically or physically, they are recycled in ecosystems after they have accumulated as pollutants. According to Chamberlain (1960), Lagerwerff (1971), Bohn (1972), Buchaver (1973), Little (1973) and precipitation causes

heavy metals from the sky to eventually accumulate in the soil where they fall out. An average of 7,800 t of As, 1,000 t Cd, 19,000 t Cu, 516,000 t Mn, 19,000 t Pb, 66,000 t V, 46,000 t Zn, and 6,000 t Hg are released into the atmosphere annually (Niagu and Pacyna, 1988; Di Toppi *et al.,* 1999).

1.1.2 Source of heavy metals

The usage of inorganic fertilizers in agricultural techniques is growing daily. Numerous heavy elements, including Cd, are present in phosphorus fertilizers and are not removed during production (Sharply and Menzel, 1987). Furthermore, significant applications of poultry manure enhance the uptake of Cu and Zn in forages (Bomke and Lowel, 1991). This indicates that heavy metals in the environment typically come from two sources.

1.1.2.1. Natural sources

Soils and sediments are typically the last destination for trace metals that are generated by volcanic eruptions, earthquakes, and parent soil materials.

1.1.2.2. Human-caused sources

According to Ernst and Joosse (1983); Morselli *et al.,* (1999); it includes a wide range of industrial activities such as blast furnaces, metal smelters, electrolysis, cement production, iron and steel production, automobile traffic, household waste, wastewater sludge, agricultural debris (food additives, phosphate fertilizers, pesticides, and herbicides), energy supply (coal-fired power plants, petroleum combustion, nuclear power plants, high tension lines, heating systems), waste incinerations, etc.

According to the WHO (2006), traffic jams and vehicle emissions are the main causes of roadside soil pollution. Due to trace element emissions from motor cars, roadside plants have experienced both direct and indirect effects on their metabolism (Viskari *et al.,* 2000). The neurophysiological effects of these trace elements, such as lead (Pb), cadmium (Cd), arsenic (As), manganese (Mg), and nickel (Ni), have been shown to alter nervous system functioning and pose a health risk.

An intricate and interconnected biogeochemical system cycles harmful substances like heavy metals like cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) between the environment, the hydrosphere, the soil, and

even the biosphere of the earth (Castro-Gonzalez and Mendez-Armenta, 2008; Turkez *et al.,* 2012; Vila *et al.,* 2017).

The cycling process of heavy metals in the environment may be impacted by a number of natural and man-made factors, including ore deposits, weathering or erosion of bed rocks, volcanic activity, mining, smelting, industrial influx, and agricultural activities (Khan *et al.,* 2008; Krishna *et al.,* 2009; Ettler *et al.,* 2012). Numerous international studies (Al-Chalabi and Hawker, 2003) have identified the origins of trace elements in urban dusts as being vehicular traffic, industrial and commercial facilities, and the aging of buildings. Pb, Cd, As, Mg, and Ni are among the dangerous elements that can wind up on the side of the road due to metal corrosion, combustion, component wear, and fluid leakage. Most heavy metals leak out of tires due to wear and tear, battery corrosion, or fuel combustion.

1.1.3. Heavy metals and human health

1.1.3.1. Copper

Wilson's disease, lenticular and liver degeneration, cirrhosis, neurological symptoms, hemolytic anemia, Kayser-Fleisher ring, and bone damage can all be brought on by copper poisoning which results in mortality, seizures, nausea, vomiting, and diarrhea, lung and nasal septal perforations, lung and liver cancers may result from copper deposition (Scheinberg, 1979).

1.1.3.2. Lead

When children's blood contains 250–550 µg L-1 Pb, it is considered poisoning (WHO, 1996). Their growth, behavior, and IQ may be adversely affected by a lead content of at least 10 µg/dl (CDC, 1997). The levels of Pb in blood as high as 1.4 µg/l can have negative effects on the nervous system, reproduction, and even cause cancer (Apostoli *et al.,* 1998). Anemia, kidney damage, infertility, abnormal fetal development, and impaired brain function are all consequences of chronic lead poisoning (Moore and Goldberg, 1985).

1.1.3.3. Zinc

According to WHO (1996) and Dara (2004), symptoms of zinc toxicity include mucous membrane damage, sexual inadequacy lesions on the skin, grey hair, dehydration,

electrolyte imbalance, dizziness, and lethargy. As stated by Suranjana and Manas (2009), heavy metals bio-accumulate and impair the functions of important organs and glands in the human body, particularly the brain, kidney, and liver.

Heavy metal pollution brought forth by growing industrialization and urbanization has greatly increased environmental instability. Heavy metals are changed and cycled through different forms in the environment rather than being removed or changed chemically or physically (Conner, 1994). Metals and soil's organic and inorganic constituents interact in important ways (Adriano, 1986). All terrestrial plants need soil to absorb nutrients and minerals. Heavy metal limits in agricultural soils affect the biosphere and agricultural output by preventing the breakdown of organic matter and decreasing soil fertility (Baath, 1989; Ferguson, 1990; Chaudri *et al.*, 1993).

1.2. Rationale of the study

There are few researches on heavy metal accumulation in higher plants in Nepal that are related to the study's emphasis. Therefore, little is known about the causes of metal accumulation, the functions of geological processes, and the involvement of human activity. In order to ascertain the environmental concentrations of heavy metals in a few chosen higher plants near various urban places within the Butwal Sub metropolitan city, the current study was conducted. From the literature review, it is found that none of any research has been done regarding to heavy metal accumulation in higher plants in Butwal. As Butwal, the capital of Rupandehi is a sub metropolitan city and is expanding its development and urbanization for last more than 10 years. This city contains industrial areas, heavy traffic areas, and residential areas. The concentration of heavy metals in the dust and plant parts, which indicates the heavy metal contamination in air, is not known. Therefore, the present study intends to study the concentrations of heavy metals in the road dust and plant parts to monitor the air quality of Butwal.

1.3. Research questions

i. What concentrations of heavy metals (Pb, Cu and Zn) are present in different plants in different sites around Butwal Sub-metropolitan city?

1.4. Objectives

1.4.1 General objectives

i. To ascertain the levels of heavy metals, present in various tree species in the vicinity of various urban areas.

1.4.2 Specific objectives

i. To measure the concentration of heavy metals (Pb, Cu, and Zn) in bark, and leaves of some plants at different sites (along huge traffic area, industrial site, and relatively clean site).
ii. To measure the availability of those heavy metals in soil dusts around roadside, industrial site and relatively clean site (control).
iii. To analyze the correlation between metal concentrations in soil dust samples and plant parts.

1.5. Limitations of the study

i. Because of financial constraints, this study was unable to include all plant species in the sub metropolitan city.
ii. Only three metals were considered for the analysis because of financial constraints.

CHAPTER 2
LITERATURE REVIEW

2.1. Heavy metals in Leaves and barks

According to Dogan *et al.*, (2010), Turkish red pine (*Pinus brutia*) can be employed as excellent bio monitors for detecting heavy metal pollution. The research region has become one of Turkey's fastest growing areas due to increased industrial activity, human population, and urbanization. They also discovered that *Pinus brutia* needles and barks might be used as a bio-monitoring tool for trace element pollution in Western Anatolia. The concentration of metals such as Pb, Cd, Cr, Fe, Ni, and Cu in red pine needles and bark was determined using atomic absorption spectrometry. They gathered samples at 30 distinct stations, including industrial, suburban, roadside, rural, and control areas. The authors discovered that the greatest concentration of these components was detected.

Akan, (2013) gathered street dust and neem tree (*Azadirachta indica*) samples in Maiduguri Metropolis, Borno State, Nigeria. The researchers found that the stem bark of *Azadirachta indica* had the lowest levels of all metals in plant samples while the concentrations of metals in the leaves were significantly greater. Chromium (Cr), lead (Pb), nickel (Ni), cobalt (Co), cadmium (Cd), and arsenic (As) were found in higher concentrations in plant samples than the World Health Organization's recommended thresholds for medicinal plants.

The effects of lead (Pb), arsenic (As), and cadmium (Cd) on the morphological and biochemical characteristics of *Shorea robusta* were examined, along with the plant's ability to absorb certain heavy metals. According to the study, at all three of the heavy metal concentrations 1, 5, and 10 mg/l. The majority of the plant characteristics of *S. robusta* were significantly impacted negatively. The maximum accumulation of heavy metals was found to be 0.053 mg/g of Cd in the root, 0.17 mg/g of As in the leaf, and 0.201 mg/g of Pb in the soil. The accumulation of heavy metals was found to be higher in the root than the shoot of *S. robusta*. Most of the morphological and biochemical characteristics showed concentration-dependent alterations, which may help identify appropriate bio-indicators of heavy metal pollution (Pant and Tripathi, 2014).

According to Zeki *et al.*, (2015), undesired and uncontrolled urbanization and industrialization caused by excessive population growth and industry development has resulted in serious environmental contamination in Kyrgyzstan during the previous few decades. In their investigation, the authors used a dioecious plant, *Juniperus virginiana*, to examine the rate of heavy metal concentration in the area and its impact on the plant's mineral nutritional status. *Juniperus virginiana* was discovered to be capable of accumulating large amounts of metals. According to the author's research, the presence of heavy metals had a major influence on the uptake of mineral elements, particularly Mg, K, and Na, which were abundant in *J. virginiana* due to metal deposition. The mineral-nutrient uptake pattern was altered because of metal deposition in the plant, which showed a contamination risk in the area.

Janata, *et al.*, (2016) conducted a study in the city of Chiang Mai, Thailand, to determine the levels of heavy metals (such as Cu, Fe, and Zn) in tree bark layers and to test the factors that influence atmospheric heavy metal accumulation in the bark of *Cassia fistula*, a common tree species found along roadsides and planted for decoration. The scientists discovered that the highest concentrations of heavy metals were detected in cork layers (outermost layers), indicating that the metals in the outermost layer of bark were most likely caused by atmospheric pollution as a result of direct environmental exposure. Concentrations of heavy metals had no effect on specific characteristics such as the tree's exposed directions to the source or its trunk size (5-30 cm). So, the authors concluded that use of *Cassia fistula* tree as a heavy metal bio indicator should be considered appropriately.

The heavy metals Zn, Pb, Cu, Cr, and Cd associated with transportation were found to contaminate roadside soils in Hunan Province, China, and wild plants growing alongside roadways were found to absorb these metals. The authors looked at the amount of heavy metals (HMs) and their chemical fractionation in plants and soils. A significant amount of the mobile component demonstrates the labile nature of Cd, Pb, and Zn and their ability to be absorbed by wild plants. The ability of heavy metals (HMs) to accumulate in plants was evaluated using the metal accumulation index (MAI) and the biological accumulation factor (BAF). The results showed that while most HM plant tissues had larger accumulation capacities in their bark than in their leaves, all of those plant species exhibited good phytoextraction capabilities. With the highest MAI value of 5.99,

Cinnamomum camphora (L.) Presl is a great choice for bio-monitoring and ought to be planted close to highways where HM contamination is prevalent (Zhai *et al.,* 2016).

In terms of Cu, Ni, Mn, Fe, Zn, and Cd, Agnieszka et al. (2017) investigated the cumulative properties of conifer needles *Pinus armandii*, *P. banksiana*, *P. mugo*, *P. nigra*, *P. sylvestris*, and *P. wallichiana*. The author found that whereas *P. sylvestris* accumulates Ni and Cu through the bark of *P. mugo*, *P. armandii*'s bark gathers the maximum amounts of Mn, Fe, Cu, and Cd.

The metal uptake of plant *Ficus religiosa*'s leaves collected from nine experimental sites in Gorakhpur, Uttar Pradesh, was investigated. An atomic absorption spectrophotometer was used to determine the amount of lead present in the leaves. The World Health Organization (WHO) recommended limits for lead content were greatly exceeded by the lead content found in *Ficus religiosa* leaves. Lead bioaccumulation peaked at site 2 (Gayatri Nagar), in which dry weight of *F. religiosa* was 14.1 mg kg-1 (Agrahari *et al.,* 2018).

2.2. Heavy metal accumulation in soil dust samples

Aksoy and Ozturk (1997) proposed *Nerium oleander L.* as a bio monitor for heavy metals. They measured Pb, Cd, Zn, and Cu concentrations in both unwashed and washed leaves and soils. Heavy metal concentrations in surface soil correlated significantly with washed leaf samples.

A research was conducted on the heavy metal content of soil and rainfall in industrial, urban, and rural areas. There has been evidence of a positive association between soil contamination in the forest and rainfall and the heavy metals Zn, Pb, and Cu (Lee and Kim, 1998).

In Sudbury, Ontario, Canada, heavy metal buildup was noted in both soil and the needles of *Pinus banksiana*, or jack pine. The location was well-known for producing precious metals and rich sulfide ores containing Ni, Cu, and Fe. There were notable positive associations found between Ni and Co ($r = 0.74$, $P \leq 0.05$) and Cu and Co ($r = 0.77$, $P < 0.05$). Plant absorption patterns and homogeneous deposition were suggested by the significant correlation ($r = 0.82$, $P < 0.05$) found between Cu and Ni concentrations (Gratton *et al.,* 2000).

El-Hasan *et al.*, (2006) investigated heavy metal dispersion in urban street dust in Karak, Jordan. Heavy metals (Pb, Cu, Zn, Ni, Fe, Cr, Cd, and Mn) are more concentrated in urban areas, indicating that automobiles are the primary cause of pollution through emissions and wear and tear.

Using the atomic absorption spectrophotometric method, 96 roadside soils and 96 street dusts were collected from three distinct areas (peripheral, industrial, and urban) in Kavala, Greece. The samples were then examined for Pb, Cu, Zn, Ni, Cr, Cd, As, and Hg. The findings demonstrated that, in comparison to the values from the control location, dust and soil samples from the urban and industrial area included considerable quantities of the metals under investigation. In roadside soil, the mean values were 359.4, 42.7, 137.8, 58.2, 193.2, 0.2, 62.3, and 0.1 μg g−1, while in street dust; the mean values were 300.9, 123.9, 271.6, 57.5, 196.0, 0.2, 16.7, and 0.1 μg g−1. Patterns of street dust dispersal are essentially the same. The results show that samples of street dust from urban and industrial areas had elevated concentrations of Pb, Cu, Zn, and As, along with notable amounts of Cd and Hg. The main sources of these contaminants seem to be the surrounding phosphoric fertilizer and petrochemical industry plants, as well as local vehicular traffic (Christoforidis and Stamatis 2009).

2.3. Heavy metal monitoring in Nepal

The elements Cr, Ni, Pb, Zn, Ca, Mn, Fe, Si, and Al were examined in lichen samples that were gathered in and around the Hetauda Industrial area (HIA), Narayani zone, Makwanpur district, Nepal. There is more metal in the samples taken from inside the industrial area than there is outside. Higher concentrations of all the metals examined were found in *Pyxine meissnerina* growing inside the industrial region (Pandey *et al.*, 2000).

Vegetables and soil from agricultural lands in the Kathmandu valley were tested for heavy metals. Agricultural soil contained the following elements: manganese (675 mg kg-r), chromium (73.75 mg kg-r), zinc (162 mg kg-'), iron (75636 mg kg"l), copper (65.5 mg kg-r), lead (146.75 mg kg-r), cadmium (2 mg kg-r), nickel (29.25 mg kg-r), cobalt (15.25 mg kg-r), and nickel (29.25 mg kg-r). Spinach had the highest concentrations of Cu, Pb, and Zn, while red radish had the highest concentration of Pb. Broad-leaf mustard had the highest concentrations of Ni, Cr, and Fe, while cress leaf had the highest concentration of

Mn, based on the average of the vegetables analyzed. Non-essential but harmful metals, such as Pb, were found in higher concentrations than typical plant values (0.1-10 mg kg-t) in all vegetables gathered from various sampling sites except potatoes (Sharma and Chhetri 2005).

Parajuli, *et al*., (2020) concluded that though iron is essential elements for plants but very high concentrations of iron in soil (around 30,000 mg/kg) do not support trees, which may be due to depletion of other essential elements. The bronze coloration of shoots in plants like *Cyanotis vaga*, *Hypericum japonicum*, *Begonia picta*, *Gonostegia hirta*, *Trumphetta rhomboidea*, *Osbekia stellata* and *Isodon coesta* indicated large amount of iron accumulation in plants and this morphological coloration effects should be considered as indicator for exploration of iron ore deposits. Besides this the bioaccumulation factor in herb *Anaphalis busua* and shrub *Elsholtzia blanda* are high, hence these plants should also be included as tool of geo-botany for exploration of iron ore deposits.

Although many works have been done to analysis of heavy metal accumulation in different lower plants and vegetables in Nepal (Chettri *et al.*, 1997; Pandey and Pal 2002; Shakya, *et al.*, 2008; Devkota *et al.*, 2019; Hamal and Chettri, 2022). But till now no research on heavy metal accumulation using higher plants have been conducted outside the Kathmandu valley. Similarly, in context to Nepal, few researches were conducted in higher plants (using leaves and bark). The barks of several trees have already been used for heavy metal monitoring namely Oak, Elm, Willow Poplar, Pine, Olive, Cedar and eucalyptus (Poikolainen, 1997; Mandiwana *et al.*, 2006; Berlizov *et al.*, 2007).

2.4. Metal Accumulation Index (MAI)

A survey of the gas and petrochemical zones in the vicinity of Asaloyeh, Iran, was carried out by Safari, *et al.*, (2018); in two industrial, two urban, and two rural sites in the Asaloyeh industrial zone. Metal concentrations such as Ni, Pb, V, and Co were measured in leaves and bark samples of *Nerium oleander*, *Conocarpus erectus*, *Bougainvillea spectabilis wild*, and *Hibiscus rosa-sinensis*, and compared to the background site (BS). The authors found that the leaves of *C. erectus* (12.77 mg/kg), *N. oleander* (26.04 mg/kg), *N. oleander* (23.17 mg/kg), and *C. erectus* (23.17 mg/kg) had the highest concentrations of nickel, lead, vanadium, and cobalt, respectively. Researchers came to the conclusion that there was a noticeable variation in the amounts of heavy metals in trees between rural

and urban areas, as well as BS, which suggests that industrial activities were successful in contaminating the surrounding area with metals. *H. rosa-sinensis* and *C. erectus* had the highest MAI (Maximum Metal Accumulation Index) factor, indicating that these trees are effective in reducing air-born metals in polluted environments, and *N. oleander* and *C. erectus* had the highest AOM (Air Originated Metal) factor. Thus, it would be advantageous to grow these trees in industrial regions where there is significant air pollution.

From the literature review, it is found that few studies have been done regarding heavy metal accumulation in higher plants in the Kathmandu Valley. However, none of the research has been conducted in urban areas outside the Kathmandu Valley. So, the present study was carried out to assess the concentration of different heavy metals in the urban areas of the Butwal sub-metropolitan city using leaves and barks of different higher plants.

CHAPTER 3
MATERIALS AND METHODS

3.1 Study area

3.1.1 Physiographic location and Sampling sites

The study area was located at Butwal sub-metro-politan city in Rupandehi district, which has a dusty and smoky atmosphere due to pollution of heavy traffic and emissions from various industries. Geographically, the location of the study area ranges from 83° 21′ 49.15″E to 83° 30′21.40″E and 27° 36′54.33"N to 27° 44′ 56.85" N and at the altitude of 150 masl. The total geographic area covered is 101.58 km^2 (10158.24 ha). Butwal has a city population of 195,054 as per the 2021 AD Nepal census. The present study site is located in the confluence of the two prominent national highways viz. Mahendra highway and Siddhartha highway linking to the hills and mountains in the north.

Higher tree species like *Shorea robusta, Terminalia alata, Mangifera indica, Azadirachta indica*, *Ficus religiosa* are very common and were considered for the present study. Plant samples (barks and leaves) and soil dust samples were collected from three sampling sites (Table 2).

Table 2: Sites description

S.N.	Sites	Location
1	Roadside	Along Mahendra Highway from Tinau bridge, Fulbari chowk to Rajpur Khola
2	Industrial site	Butwal Industrial area, Ramgram
3	Control site	Shivanagar Community Forest, Charange, Ward no. 15, Butwal

The industrial area is in the vicinity of the Butwal industrial area at Base Camp, Butwal. Altogether, 82 various factories dealing with plastic, polyethylene pipe, aluminum products, metal factories, etc. are located at this site: the roadside (along Mahendra highway) and the control site, Shivanagar community forest, which is located about 5 km northeast of the industrial area and the National Highway.

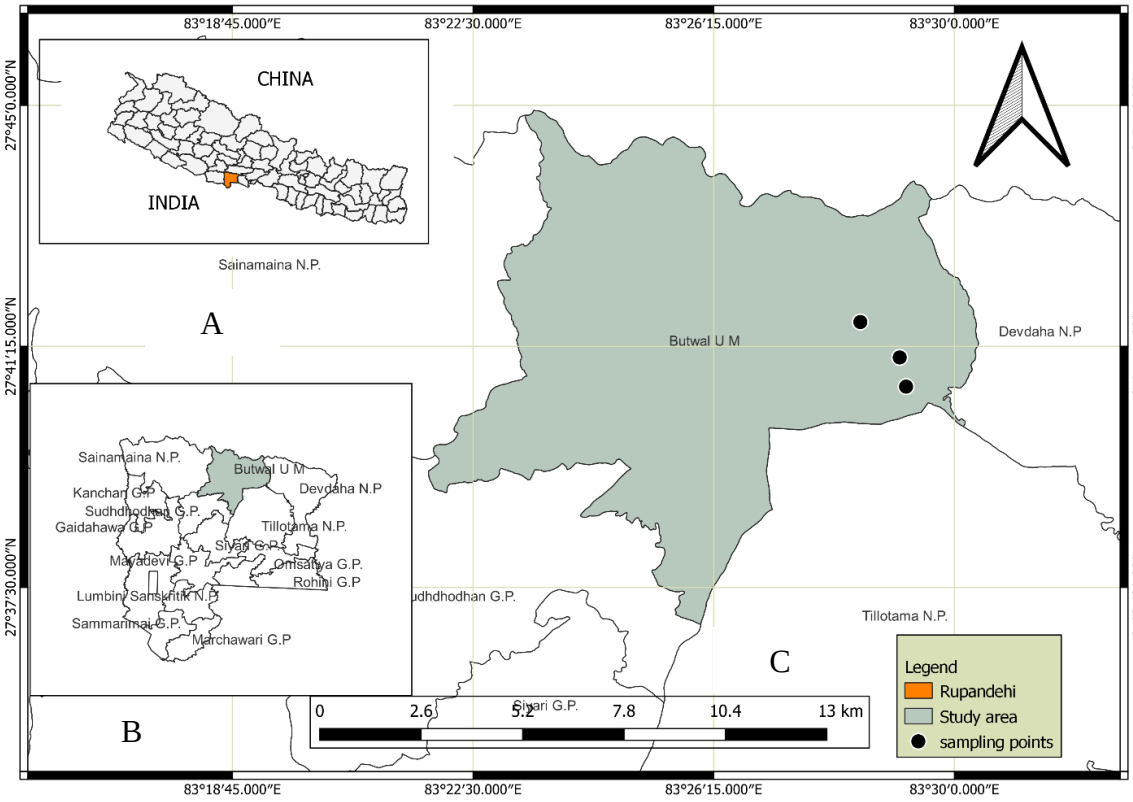


Figure 1: Map of study area; District Map of Nepal (A), Map of Rupandehi District showing Study area (B), Map of Butwal sub-metropolitan city showing study Plots (C). (Source: QGIS Version 23)

3.1.2. Climate

The study area has a subtropical monsoon type climate. The Department of Hydrology and Metrology in Katmandu, Nepal provided the metrological data for the Bhairahawa station, Rupandehi. The acquired data were used to compute the monthly average maximum temperature, minimum temperature, and precipitation. The Bhairahawa station (Lat. / Long. 27.506 N, 83.4204 E, Altitude: 150masl) provided the climatic data for the years 2012–2022. The mean maximum temperature ranged from 19.71°C to 36.5°C, with the highest temperature recorded in the months of April through July. The mean minimum temperature ranged from 9°C to 26.52° C, with the lowest temperature recorded in January and February. July had the highest monthly mean precipitation of 419.75 mm and less precipitation was recorded in November i.e. 0.045mm (DHM, 2022).

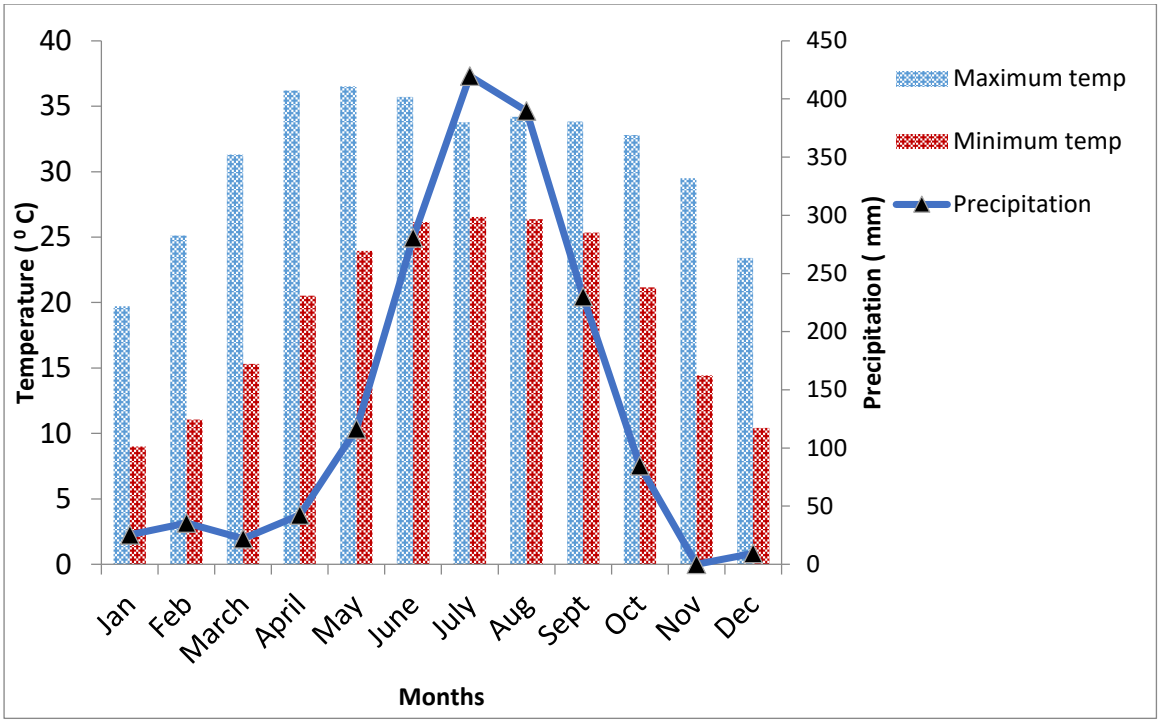


Figure 2: Ten years (2012-2022) average monthly maximum and minimum temperature and precipitation data of Bhairahawa station, Rupandehi (Source: DHM, 2022)

3.1.3. Vegetation

Tree species such as *Cassia fistula*, *Albizia chinensis*, *Dalbergia sissoo* were found frequently distributed alongside the river bank (Shakya *et al*., 2000). *Shorea robusta, Terminalia alata, Dalbergia sissoo*, and other plants dominate the vegetation of Butwal sub metropolitan cities. Other major tree species found in study sites include *Ficus religiosa, Ficus benghalensis, Azadirachta indica, Bombax ceiba, Mangifera indica, Aegle marmelos, Dalbergia sissoo, Saraca asoca, Neolamarckia cadamba, Eucalyptus camaldulensis, Melia azedarach, Ficus benjamina, Premna barbata, Tamarindusindica, Ficus racemose, Morus alba* etc.

3.2. Sample collection

The representative samples were collected in late winter, i.e., February – March 2023. To receive the short-term contamination and long-term contamination, leaves and bark samples were tested as bio monitors for pollution, respectively. The bark samples were collected from DBH height (1.35 m). Similarly, leaf samples were collected from all directions (E, W, S, N) at a height of 3m above. A list of trees used for the metal collection study is given in Table 3. Soil dust samples were collected using a brush from

the soil surface. Representative soil samples at each sampling site were collected by mixing soil dust samples collected from five different points, each from top soil surface of ground by using brush and hand driven stainless steel augers. Dried leaves, twigs or litter present in the soil dust were removed manually.

3.3. Sample preparation

The collected samples were placed in paper envelopes and carried to the laboratory for further cleaning and representative sample preparation. The collected leaves and barks were cleaned in dry condition by gentle shaking and were not washed, as the purpose of the study was to estimate the accumulation ability of plant parts to reflect the atmospheric pollution. Stainless steel knife was used to cut a sample of bark from the center of the tree trunk at a height of roughly 1.35 meters above ground. Before being collected, the tree's bark debris was meticulously removed using a synthetic hard brush. The sample did not contain the barks that were afflicted by fungus. Bark has the advantage of being widely available without compromising the health of the trees, and its structure helps it hold onto contaminants for longer (Berlizov *et al.*, 2007). Trees of the same age were selected for the samples.

Table 3: List of plant species used for the analysis of heavy metals

S. No.	English Name	Local Name	Botanical Name	Family Name	Collected sites
1	Sal tree	Sal	*Shorea robusta* Gaertn.	Dipterocarpaceae	• Roadside • Industrial site • Control
2	Indian laurel	Saaj or asna	*Terminalia alata* Heyne.ex Roth	Combretaceae	• Roadside • Industrial site • Control
3	Fig	Peepal	*Ficus religiosa* L.	Moraceae	• Roadside • Industrial site • Control
4	Mango	Aap	*Mangifera indica*	Anacardiaceae	• Roadside • Industrial site • Control
5	Margosa or indian lilac	Neem	*Azadirachta indica* A. Jees.	Meliaceae	• Roadside • Industrial site • Control

To obtain uniform soil particles, soil dust samples were oven dried for 72 hours at temperatures between 70 and 100 degrees Celsius until their dry weight (DW) remained consistent. After that, they were sieved through a 0.25 mm mesh filter. The same oven drying method that was used for the soil samples was also applied to the samples of leaves and bark. Following oven drying, the samples soil, leaves, and barks were homogenized to produce representative samples for every study site. To create representative samples, each sample was homogenized using a mortar and pestle.

3.4. Sample digestion and analysis of heavy metals

Subsequently, 1g DW of representative soil dust samples, bark and leaf samples were digested in concentrated HNO3 (Sawidis *et al.*, 1995; Chettri *et al.*, 1997) at 2000 C for 4-5 hours of continuous heating. The Whatman ashless filter paper was used to filter the digested samples, keeping a fixed final volume with double deionized water. Using the Chemito-201 Atomic Absorption Spectrophotometer (AAS) with the corresponding wavelengths of 283.3 nm, 324.7 nm, and 213.8 nm, respectively, the concentrations of heavy metals such as Pb, Cu, and Zn in the filtrate were determined (Welz, 1985). Samples were screened in triplicate for every case.

3.5. Metal Accumulation Index (MAI)

Using the data obtained in the present study, we developed an accumulation index to assess the overall performance of the trees in terms of metal accumulation. Since this index is for metals, it is, therefore, called the metal accumulation index (MAI) (equation 1). Based on the pioneer work on air quality indices developed by (Liu *et al.*, 2007), we developed the following calculation of MAI:

$$\mathrm{MAI} = \left(\frac{1}{\mathrm{N}}\right) \sum_{j=1}^{N} I_j \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where N = the total number of metals analyzed and Ij = x/dx is the sub-index for variable j, obtained by dividing the mean value (x) of each metal by its standard deviation (d).

3.6. Statistical analysis

Microsoft Excel was used for simple calculations and data management. The statistical analysis was conducted using IBM SPSS version 20, the Statistical Package for Social Science. The normality of the data was examined. The parametric test was used to know the variations among the different studied plants and sites.

With the help of one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test, the means of heavy metals in each of the plant parts; leaves, barks, and soil dust samples were compared across the sites. Correlation between the metal concentrations in soil dust and plant parts was analyzed by calculating Pearson’s coefficient of correlation

CHAPTER 4
RESULTS

Heavy metals concentration in barks and leaves of *Shorea robusta, Mangifera indica, Azadirachta indica, Terminlia alata* and *Ficus religiosa* tree and soil dust from different sites (roadside, industrial area and control site) were analyzed. And their results were presented in table below. Likewise, MAI value also calculated for every plant species.

4.1 Soil samples

The concentrations of three heavy metals (lead, copper, and zinc) in soil samples collected from three different sites: roadside, industrial area and relatively clean site (control site) is given in Table 2. The measurements are expressed in mg/kg and represent the average concentrations of each metal at the respective locations.

Table 4: Concentrations of heavy metals (Mean ± S.D.) in soil sample in three different urban sites (n=3).

Sites	Lead (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)
Roadside	15.25±1.64 B	15.08±2.88 B	55.92±3.71 B
Industrial area	64.17±1.9 C	208.83±4.37 C	236.67±5.3 C
Control site	5.17±2.01 A	5.75±1.52 A	34.07±4.50 A

Pb, Cu, and Zn contents in soil dust samples varied from 5.17 to 64.17 mg/kg, 5.75 to 208.83 mg/kg, and 34.07 to 236.67 mg/kg at various locations inside the Butwal sub metropolitan city. Industrial locations were found to have the greatest levels of heavy metal contamination in soil dust among the sites under study. Areas close to major roads and highways were used to collect soil dust samples for the roadside. Lead, copper, and zinc average values in the roadside soil dust were 15.25, 15.08, and 55.92 mg/kg, respectively.

Soil dust samples have been collected from sites around the Butwal industrial area at Base Camp, Butwal, for the Industrial Site. Altogether 82 various factories dealing plastic, polyethylene pipe, aluminum products, and iron industries are located at industrial site. Here, the concentrations of all heavy metals in the soil dust were significantly ($p=0.05$) higher in industrial areas as compared to the roadside and control sites (Table 4). The

average concentration of lead was 64.17 mg/kg, copper was 208.83 mg/kg, and zinc was 236.67 mg/kg.

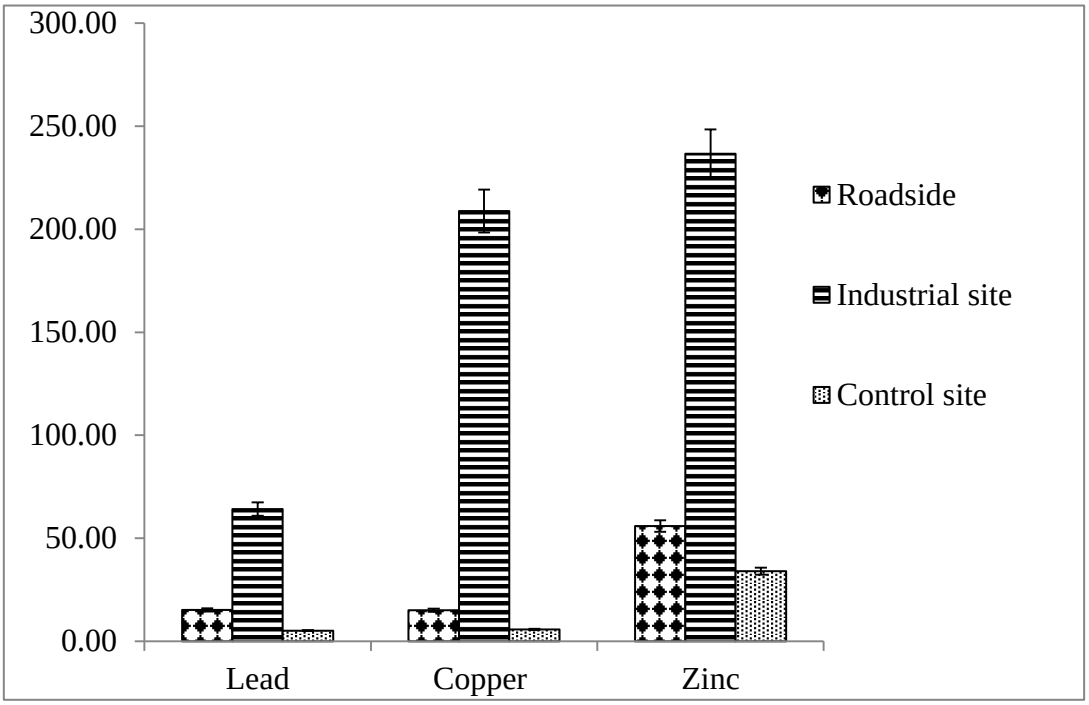


Figure 3: Average metal accumulation in soil dust samples

4.2 Barks samples

The concentration of lead, copper, and zinc in the bark of five distinct plant species growing at three different locations (roadside, industrial sites, and control site): *Shorea robusta, Mangifera indica, Azadirachta indica*, *Terminalia alata,* and *Ficus religiosa* (Table 5).

Table 5: Concentrations of heavy metals (Mean ± S.D.) in barks sample of different plant species at three different sites in Butwal (n=3).

Sites	Name of plant species	Lead (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)
Roadside	*Shorea robusta*	3.25±0.25C	13.08±0.52C	14.92±0.95A
	Mangifera indica	2.5±0.5BC	10.67±1.84B	53.5±1.25D
	Azadirachta indica	1.75±0.66AB	6.75±1.09A	40.0±0.5B
	Terminalia alata	1.33±0.29A	7.083±0.38A	43.0±1.0C
	Ficus religiosa	1.92±0.88AB	10.58±0.63B	132.5±2.50D
Industrial sites	*Shorea robusta*	2.67±0.76AB	6.9±0.79B	26.73±1,12A
	Mangifera indica	1.98±0.28A	4.52±0.63A	69.57±1.21D
	Azadirachta indica	3.82±0.39C	12.3±0.82C	181.43±1.43E
	Terminalia alata	3.32±0.35BC	5.27±0.74A	31.15±0.88B
	Ficus religiosa	1.92±0.63A	7.2±0.36B	47.03±1.46C
Control site	*Shorea robusta*	1.47±0.15A	5.03±0.15B	11.8±0.92A
	Mangifera indica	1.17±0.32A	2.1±0.27A	25.5±1.0B
	Azadirachta indica	1.47±0.15A	2.13±0.15A	26.57±1.50B
	Terminalia alata	1.53±0.15A	1.9±0.10A	13.33±1.04A
	Ficus religiosa	1.18±0.36A	5.17±0.45B	37.5±2.0C

Same capital letter after mean ± SD obtained from One-way ANOVA followed by Duncan Multiple Range Test, for different tree species within a site, do not differ significantly at p=0.05

Lead accumulation was significantly (*p=0.05*) high in the bark of *Shorea robusta* and *Mangifera indica* at road side (Table 5). But at industrial sites highest accumulation of Pb was recorded in *Azadirachta indica. A*t control sites Pb accumulation was insignificantly different among tree species.

Copper accumulation was significantly (*p=0.05*) high in the bark of *Shorea robusta* at roadside (Table 5). But at industrial areas highest accumulation of Cu was recorded in *Azadirachta indica*. At control site Cu accumulation was also significantly different among all plants and highest accumulation was observed in *Ficus religiosa*.

Zinc accumulation was significantly (*p=0.05*) high in the barks of *Ficus religiosa* at roadside (Table 5). But at industrial areas highest accumulation of Zn was recorded in

Azadirachta indica. But at control site Zn accumulation was higher in *Ficus religiosa*. The industrial areas showed the maximum accumulation of all three metals in comparison to roadside and relatively clean site (control).

4.2.1 Metal accumulation index (MAI) of barks samples for different tree species

The MAI value was calculated using the mean value of different metals present in bark and their standard deviation. Maximum MAI value was recorded in bark of *Ficus religiosa* (38.2) along roadside while least MAI value (6.36) was observed in bark of *Shorea robusta* around roadside. Similarly, *Mangifera indica* (22.23) around roadside, *Azadirachta indica* (21) around industrial areas, *Terminalia alata* around industrial areas (20.74) showed moderate MAI value.

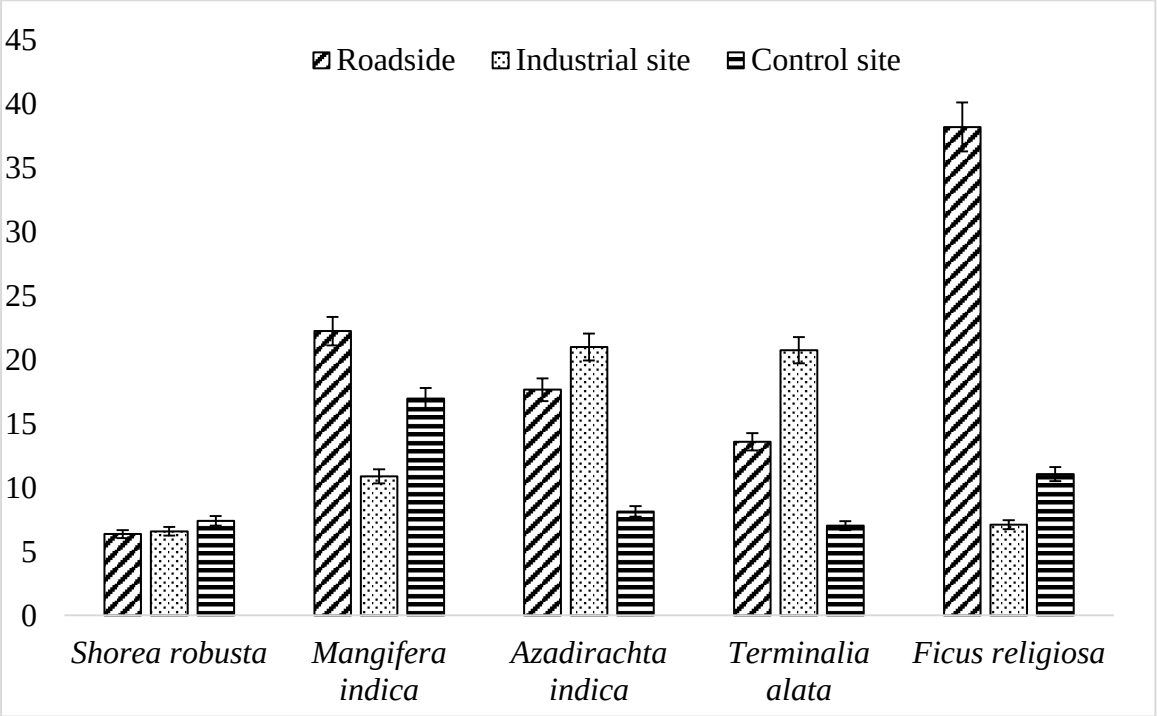


Figure 4: MAI at different sites in bark samples at Butwal sub-metropolitan city

4.3 Leaves

The concentration of lead, copper, and zinc in the leaves of the five different plant species (*Shorea robusta, Mangifera indica, Azadirachta indica, Terminalia alata,* and *Ficus religiosa*) growing at three locations (roadside, industrial sites, and control site) is given in Table 6.

Table 6: Concentrations of heavy metals in leaves sample of different plants (n=3)

Sites	Name of Plant species	Mean Lead (mg/kg)	Mean Copper (mg/kg)	Mean Zinc (mg/kg)
Roadside	*Shorea robusta*	1.26±0.20 AB	2.58±0.87A	23.83±1.38 A
	Mangifera indica	0.98±0.23 AB	10.0±2.50 C	37.33±3.25C
	Azadirachta indica	0.90±0.10 A	5.25±1.64 AB	42.42±2.38 C
	Terminalia alata	1.1±0.26 AB	7.83±1.53 BC	64.75±5.13 D
	Ficus religiosa	1.58±0.63 B	5.17±0.76 AB	30.83±1.38 B
Industrial sites	*Shorea robusta*	1.35±0.22 A	2.58±0.88A	55.083±3.63C
	Mangifera indica	1.6±0.36A	2.5±1.0A	41.23±0.751A
	Azadirachta indica	1.02±0.23A	5.25±0.66B	50.08±3.13B
	Terminalia alata	1.5±0.30A	9.75±0.25C	80.25±0.66D
	Ficus religiosa	1.03±0.42A	10.17±1.26C	47.33±2.25B
Control sites	*Shorea robusta*	0.48±0.23 AB	3±0.44B	20.07±0.51A
	Mangifera indica	0.78±0.30B	2.13±0.32A	24.26±1.50AB
	Azadirachta indica	0.58±0.14AB	3.07±0.38B	33.93±1.92C
	Terminalia alata	0.25±0.10A	1.93±0.25A	25.88±2.78B
	Ficus religiosa	0.33±0.14A	1.87±0.21A	29.08±4.64B

Same capital letter after mean ± SD obtained from One-way ANOVA followed by Duncan Multiple Range Test, for different tree species within a site, do not differ significantly at $p=0.05$.

Lead accumulation was significantly ($p=0.05$) high in the leaves of *Ficus religiosa* at road side (Table 6). But at industrial sites highest accumulation of Pb was insignificantly different among all tree species. *A*t control sites Pb accumulation was significantly highest in *Mangifera indica*.

Copper accumulation was significantly ($p=0.05$) high in the leaves of *Mangifera indica* at roadside (Table 6). But at industrial areas highest accumulation of Cu was recorded in *Terminalia alata* and *Ficus religiosa*. While Cu accumulation was also significantly different among all plants and highest accumulation was observed in *Azadirachta indica* at control site.

Zinc accumulation was significantly high (*p=0.05*) in the leaves of *Terminalia alata* at roadside (Table 6). Likewise, the highest accumulation of Zn was recorded in *Terminalia* too. But at control sites Zn accumulation was higher in *Azadirachta indica*.

4.3.1 Metal accumulation index (MAI) of leaves samples for different tree species

The MAI value was calculated using the mean value of different metals present in leaves and their respective standard deviation. Overall, the MAI value of all plants in industrial area was maximum except *Shorea robusta*. While, maximum MAI value (55.37) was observed in the leaves of *Terminalia alata* collected from industrial sites. Similarly, *Mangifera indica* (20.15), *Ficus religiosa* (13.35), *Azadirachta indica* (8.47) showed moderate MAI value, which indicates that these species of plant can be used as metal accumulator around industrial areas. But along roadside, all plants showed moderate MAI value (Fig. 5).

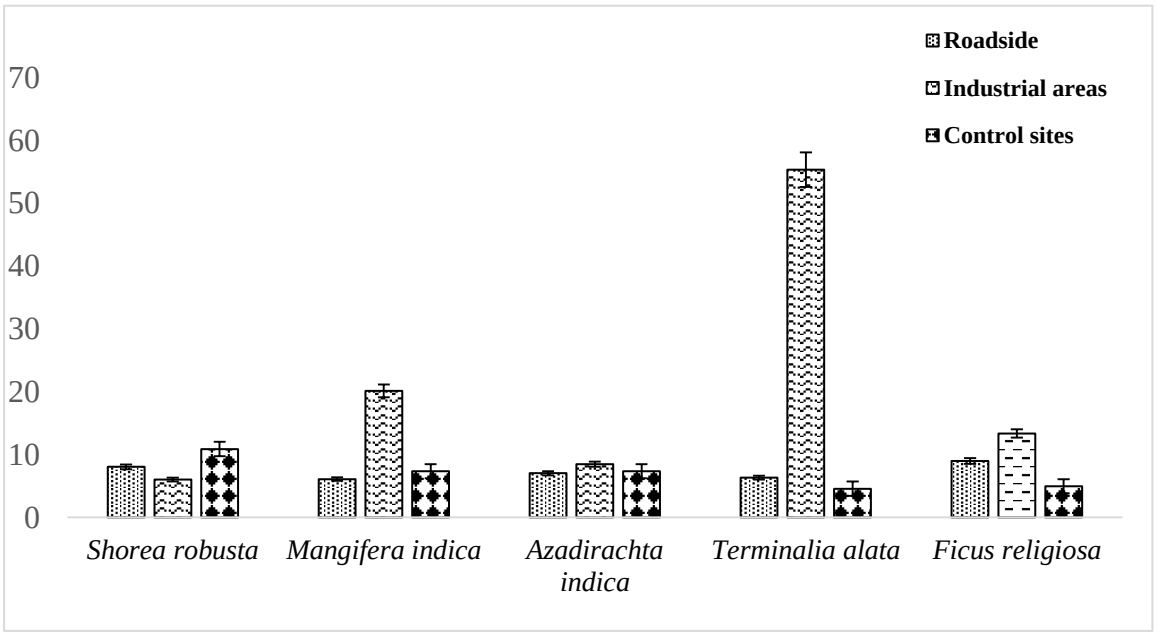


Figure 5: MAI at different sites in leaves samples at Butwal sub-metropolitan city

4.4 Average concentration of heavy metals (mg/kg) within leaves of same plant species in different sites

4.4.1 Lead

The present study showed that all five tree specie's leaves had higher lead concentrations around industrial area compared to roadside and control sites. *Terminalia alata* had the lowest lead concentrations at the control site (0.25 mg/kg). Similarly, *Mangifera indica* had the highest lead concentration at industrial area (1.60 mg/kg) (Table 7).

Table 7: Average concentration of lead (mg/kg) within leaves of same plant species in different sites. Values are Mean ± S.D., n=3

Name of plant species\sites	Roadside	Industrial area	Control site
Shorea robusta	1.26±0.20 N	1.35±0.22 O	0.48±0.23 M
Mangifera indica	0.98±0.23 N	1.60±0.36 O	0.78±0.30 M
Azadirachta indica	0.90±0.10 N	1.02±0.23 O	0.58±0.14 M
Terminalia alata	1.10±0.26 N	1.50±0.30 O	0.25±0.10 M
Ficus religiosa	1.58±0.62 O	1.03±0.42 N	0.33±0.14 M

Same capital letter after mean ± SD obtained from One-way ANOVA followed by Duncan Multiple Range Test, for different tree species within a site, do not differ significantly at p=0.05.

4.4.2 Copper

The copper concentrations (in mg/kg) in the leaves of five different species i.e. *Shorea robusta, Mangifera indica, Azadirachta indica, Terminalia alata* and *Ficus religiosa*. The table reflects that the copper concentration in all five plant species leaves is higher in the tree grown closer to the industrial area. The tree species with almost highest copper concentration in the leaves at roadside is *Mangifera indica* (10 mg/kg), *Ficus religiosa* (10.16 mg/kg) at industrial site and *Azadirachta indica* (3.07 mg/kg) at control site (Table 8).

Table 8: Average concentration of copper (mg/kg) within leaves of same plant species in different site. Values are Mean ± S.D., n=3

Name of plant species\sites	Roadside	Industrial area	Control site
Shorea robusta	2.58±0.87 N	2.58±0.88 O	3.00±0.44M
Mangifera indica	10.0±2.50 O	2.50±1.00 N	2.13±0.32 M
Azadirachta indica	5.25±1.63 N	5.25±0.66 O	3.07±0.38 M
Terminalia alata	7.83±1.53 N	9.75±0.25 O	1.93±0.25 M
Ficus religiosa	5.16±0.76 N	10.16±1.26 O	1.87±0.21 M

Same capital letter after mean ± SD obtained from One-way ANOVA followed by Duncan Multiple Range Test, for different tree species within a site, do not differ significantly at p=0.05.

4.4.3 Zinc

The concentrations of zinc (mg/kg) in the leaves of five different tree species; *Shorea robusta, Mangifera indica, Azadirachta indica*, *Terminalia alata* and *Ficus religiosa*. The concentration of zinc in leaves of all five plant species is higher at industrial site compared to roadside and control site. Among them, the concentration of zinc is higher in *Terminalia alata* (80.25 mg/kg) at industrial area while *Shorea robusta* has less accumulation of zinc (20.07 mg/kg) at control site (Table 9).

Table 9: Average concentration of lead (mg/kg) within leaves of same plant species in different sites. Values are Mean ± S.D., n=3

Name of plant species\sites	Roadside	Industrial area	Control site
Shorea robusta	23.83±1.377 N	55.08±3.63 O	20.07±0.51 M
Mangifera indica	37.33±3.25 N	41.23±0.75 O	24.27±1.50 M
Azadirachta indica	42.41±2.37 N	50.08±3.13 O	33.93±1.92 M
Terminalia alata	64.75±5.13 N	80.25±0.66 O	25.88±2.78 M
Ficus religiosa	30.83±1.37 N	47.33±2.25 O	29.08±4.64 M

Same capital letter after mean ± SD obtained from One-way ANOVA followed by Duncan Multiple Range Test, for different tree species within a site, do not differ significantly at p=0.05.

4.5 Average concentration of heavy metals (mg/kg) within bark of the same plant species in different sites

4.5.1 Lead

The lead concentration (in mg/kg) in the bark of same five tree species in different sites. The table shows that the concentration of lead in bark of *Azadirachta indica* (3.82 mg/kg), *T. alata* (3.32 mg/kg) and *F. religiosa* (1.92 mg/kg) is higher at industrial site whereas *Shorea robusta* and *Mangifera indica* has higher concentration of lead around roadside i.e. 3.25 mg/kg and 2.50 mg/kg. While the concentration of lead is less in all plant species (1.18-1.53 mg/kg) around control site except *Ficus religiosa* (1.92 mg/kg) of roadside (Table 10).

Table 10: Average concentration of lead (Pb) within bark of same plant species in different sites. Values are Mean ± S.D., n=3

Name of plant species\ sites	Roadside	Industrial area	Control site
Shorea robusta	3.25±0.25 O	2.67±0.76 N	1.47±0.15 M
Mangifera indica	2.50±0.50 O	1.98±0.28 N	1.17±0.32 M
Azadirachta indica	1.75±0.66 N	3.82±0.39 O	1.47±0.15 M
Terminalia alata	1.33±0.29 M	3.32±0.35 O	1.53±0.15 N
Ficus religiosa	1.92±0.88 O	1.92±0.63 N	1.18±0.36 M

Same capital letter after mean ± SD obtained from One-way ANOVA followed by Duncan Multiple Range Test, for different tree species within a site, do not differ significantly at p=0.05.

4.5.2 Copper

The concentration of copper (in mg/kg) in barks of five tree species: *Shorea robusta, Mangifera indica, Azadirachta indica, Terminalia alata* and *Ficus religiosa.*

The concentration of copper is higher in all plant species (13.08 mg/kg in *Shorea robusta*) around roadside except *Azadirachta indica* (12.3 mg/kg). While the accumulation of copper is less in *Terminalia alata* (1.9 mg/kg). Overall, it can be said that roadside plants have higher copper concentrations than industrial and control sites, respectively (Table 11).

Table 11: Average concentration of Copper (mg/kg) within bark of same plant species in different sites. Values are Mean ± S.D., n=3

Name of plant species\sites	Roadside	Industrial area	Control site
Shorea robusta	13.08±0.52 O	6.90±0.79 N	5.03±0.15 M
Mangifera indica	10.67±1.84 O	4.52±0.63 N	2.10±0.27 M
Azadirachta indica	6.75±1.09 N	12.3±0.82 O	2.13±0.15 M
Terminalia alata	7.08±0.38 O	5.27±0.74 N	1.90±0.10 M
Ficus religiosa	10.58±0.63 O	7.20±0.36 N	5.17±0.45 M

Same capital letter after mean ± SD obtained from One-way ANOVA followed by Duncan Multiple Range Test, for different tree species within a site, do not differ significantly at p=0.05.

4.5.3 Zinc

The concentration of zinc within barks of same plants in different sites of *Shorea robusta, Mangifera indica, Azadirachta indica*, *Terminalia alata* and *Ficus religiosa*. It can be seen that the concentration of Zn in barks of selected plant species is higher in industrial area i.e. (181.43 mg/kg in *Azadirachta indica*) while the concentration is less in *Shorea robusta* (11.8 mg/kg) at control site. Overall, we can say that by analyzing the above mentioned table, the concentration of zinc is higher in plants around industrial site than roadside and control respectively (Table 12).

Table 12: Average concentration of Zinc (mg/kg) within leaves of same plant species in different sites. Values are Mean ± S.D., n=3

Name of plant species\sites	Roadside	Industrial area	Control site
Shorea robusta	14.92±0.95 N	26.73±1.12 O	11.8±0.92 M
Mangifera indica	53.5±1.25 N	69.57±1.21 O	25.50±1.0 M
Azadirachta indica	40.0±0.5 N	181.43±1.43 O	26.57±1.50 M
Terminalia alata	43.0±1.00 O	31.15±0.88 N	13.33±1.04 M
Ficus religiosa	132.5±2.50 O	47.03±1.46 N	37.5±2.00 M

Same capital letter after mean ± SD obtained from One-way ANOVA followed by Duncan Multiple Range Test, for different tree species within a site, do not differ significantly at $p = 0.05$.

4.6. Correlation coefficient between heavy metals in soil dust and barks of different plant species

Results of Pearson correlation between soil dust and bark of different tree species are given in Table 13. Significant ($p < 0.01$) positive correlation was observed between soil dust lead – bark lead among *Azadirachta indica* and *Terminalia alata*. Similarly, Table 12 showed the significant correlation between soil dust copper content and bark copper content of *Azadirachta indica* was observed. The positive significant ($p<0.01$) correlation of zinc content in soil dust and in bark of *Azadirachta indica, Shorea robusta and Mangifera indica* were recorded, which reveals that as the level of metal concentration in soil dust increases, there is increase in bark of those trees. However, there was a weak positive insignificant correlation between soil dust lead – bark lead ($r = 0.491$), soil dust copper – bark copper, ($r=0.04$) and soil dust zinc – bark zinc ($r=0.324$) for *Ficus religiosa*.

Table 13: Correlation coefficient between Soil Pb-Bark lead, soil Cu- bark Cu and soil Zn- bark Zn of different plants with level of significance

Name of Plant Species	Soil lead - Bark lead		Soil copper - Bark copper		Soil zinc - Bark zinc	
	r	Significant	r	Significant	r	Significant
Shorea robusta	0.227	ns	0.215	ns	0.964	***
Mangifera indica	0.09	ns	0.18	ns	0.846	**
Azadirachta indica	0.859	**	0.88	**	0.999	***
Terminalia alata	0.886	***	0.1	ns	0.281	ns
Ficus religiosa	0.491	ns	0.04	ns	0.324	ns

r = correlation coefficient, Level of significance: *p<0.05, **p<0.01, ***p<0.001, ns = not significant

4.7 Correlation coefficient between heavy metals in soil dust and leaves of different plant species with their respective level of significance

Results of Pearson correlation between soil dust and leaves of different tree species are given. Significant (p< 0.01) positive correlation was observed between soil dust lead – leaves lead among *Mangifera indica* and *Ficus religiosa.* Similarly, significant correlation between soil dust copper content and leaves copper content of *Ficus religiosa* and *Terminalia alata* was observed. The positive significant (p<0.01) correlation of zinc concentration in soil dust and in leaves of all plants was observed which means that as the level of metal concentration in soil dust increases, there is increase in leaves of those trees. However, there was a weak positive insignificant correlation between soil dust lead – leaves lead (r = 0.564 for *Shorea robusta*, 0.186 for *Azadirachta indica* and 0.544 for *Ficus religiosa*), soil dust copper – leaves copper, (r=0.179 for *Shorea robusta*, 0.435 for *Mangifera indica*, 0.448 for *Azadirachta indica*) and soil dust zinc – leaves zinc showed significantly positive correlation for all plant species (Table 14).

Table 14: Correlation coefficient between soil Pb-leaves lead, soil Cu- leaves Cu and soil Zn- leaves Zn of different plants with level of significance

Name of Plant Species	Soil dust lead - leaves lead		Soil dust copper - leaf copper		Soil dust zinc - leaves zinc	
	r	*p*- value	r	*p*- value	r	*p*- value
Shorea robusta	0.564	ns	0.179	ns	0.995	***
Mangifera indica	0.708	*	0.435	ns	0.737	*
Azadirachta indica	0.186	ns	0.448	ns	0.878	**
Terminalia alata	0.701	*	0.717	*	0.78	*
Ficus religiosa	0.544	ns	0.933	***	0.966	***

r = correlation coefficient

Level of significance: *p<0.05, **p<0.01, ***p<0.001, ns= not significant

CHAPTER 5
DISCUSSION

The increase in urban population, development, and activity by humans elevates vehicular and industrial exhaust, resulting in rising heavy metal contamination of soil and air. Heavy metal pollution is an issue of concern due to its persistent nature, potential threat to food safety, and negative effects on ecological systems (Zhiyuan *et al.,* 2011).

5.1. Heavy metal in soil samples

The average amounts of lead, copper, and zinc in soil from roadside and industrial sites were significantly greater than in the control site. This demonstrated that emissions from vehicles and industrial activity are significant contributors to heavy metal contamination. Similar findings were reported by Johansson *et al.,* (2009); Gunawardena *et al.,* (2012); Mahida *et al.,* (2023); and who found higher amounts of lead and copper in soil samples near roadways due to traffic pollution.

It has been noted that road dust re-suspension adds considerably to heavy metal concentration levels in urban areas (Karanasiou *et al.,* 2009; Athanasopoulou *et al.,* 2010). The presence of Zn, Pb, and Cu in all three sections is noteworthy. These metals are linked to traffic, road conditions, tire and brake wear, lubricants, paints, and fuels (García-Martínez and Poleto, 2014). The two most likely causes of street dust, and hence the trace elements detected in it, are deposition of previously suspended particles (atmosphere aerosol) and displaced urban soil (Ferreira-Baptista and De Miguel, 2005).

Soil samples from industrial locations were found to be significantly more enriched in all three heavy metals than those from roadside sites. This means that industrial activities are likely to emit a greater quantity and variety of heavy metals than traffic emissions. According to our findings, Butwal's industrial areas include a variety of factories such as a metal factory, an aluminum factory, a steel factory, a plastic factory, a textile fiber business, and others that may have contaminated the soil with heavy metals. In various regions of the world, heavy metal contamination in soil has been documented by Sharma *et al., (*2015); Ghosh *et al.,* (2020); showing higher heavy metal concentrations in soil near industrial facilities compared to roadside surroundings.

Due to its employment in the manufacturing of brass alloy it and their origin in brake linings, oil leak pumps, and cylinder head gaskets, automobiles may be the source of the highest concentration of zinc buildup in soil dusts in surrounding industrial sites. High concentrations of zinc have also been found in soil dust near roadway in earlier studies (Jiries *et al.*, 2003). On the other hand, there was also a high mean Zn concentration in the roadside dusts. Given that zinc is utilized in car tires as a vulcanization agent (Alloway, 1990), the increased wearing rate in Butwal's hot, tropical climate may be a factor in the high zinc concentration of the street dusts. Zinc can be found in lubricating oils, tires, and other vehicular activities and in carburetors.

Pb concentrations were 15.25 mg/kg along the roadside and 64.17 mg/kg in industrial zones. The average Pb concentrations found indicated that Pb deposition was caused by secondary industry combustion processes and vehicle emissions. Pb has been linked to fuel consumption, but with the progressive switch to unleaded fuels, it's unclear if there's a current source of the metal or if these are accumulations from a previous period. According to earlier studies (Man *et al.,* 2010), Pb is concentrated near industrial sites and along roadsides.

Due to its physical characteristics, copper is a heavy metal with many uses. It typically accumulates at the soil's surface as a result of human activity. Because zinc is needed to make brass alloy, brake linings, oil leak sump pumps, and cylinder head gaskets, it may be obtained by mechanical abrasion of automobile parts.

5.2. Heavy metals in leaves sample

The present study found that heavy metal concentrations (Pb, Cu and Zn) exceeded WHO guidelines i.e. 0.3, 73.3 and 99.4 mg/kg for Pb, Cu and Zn respectively. In the study areas, with the exception of Pb, which was just exceed the permitted mark at the control site (0.78mg/kg). In the present study the concentrations of heavy metals in leaves of all plants were mostly high at industrial sites. Previous research (Xiong, 1997; Roy *et al*., 2020;) has also reported high concentrations of heavy metals. The high concentrations of heavy metals at industrial sites of Butwal could be due to the presence of several industries, including textiles, plastics, rubber, tires, steel, and aluminum processing facilities.

Additionally, these businesses emit dangerous pollutants like SO2, CO2 etc. into the atmosphere, including heavy metals. Plants grown at roadside and industrial sites generally had higher concentrations of lead, copper, and zinc as compared to those at control sites. This suggests that traffic emissions and industrial activities can significantly contribute to heavy metal accumulation in plants. Our observation aligns with research of Hui *et al.,* (2017), who found that plants growing near roadways exhibited elevated levels of lead and copper due to traffic-related pollution. Pb contamination of roadside soil has been reported by various researchers (Abechi *et al.,* 2010; Yahaya *et al.,* 2010). Furthermore, Pb has been observed to have a lengthy period of residence in the soil (Shaw, 1989). Besides this, high traffic emissions on roadsides are a significant source of heavy metals in leaves. Similar high accumulation of heavy metals along road side leaves was also recorded by Wilson *et al.,* (2005); Enete *et al.,* (2012); Ogbonna et al., (2013).

Leaves of *Ficus religiosa* shows the highest overall accumulation of lead, copper, and zinc across most sites. Conversely, leaves of *Terminalia alata* tends to accumulate the least amount of heavy metals at sites. This indicates that plant species have varying capacities for heavy metal uptake and tolerance. Similar observations were reported by a Filipović-Trajković, *et al.,* 2012**,** where different plant species displayed significant differences in their ability to accumulate heavy metals from contaminated soil.

Shorea robusta is an exception, showing lead and copper concentrations at the roadside site similar to the control site. This suggests that this particular species may have mechanisms to resist or limit the uptake of these metals from polluted environments. Further research is needed to explore the physiological adaptations that might explain this observation.

5.3. Heavy metals in bark sample

It was found that the amounts of heavy metals in bark were greater than those in leaves in every research site. Solid particles that leached from stem fall find a huge surface area in bark. Dead tissue called bark always covers a tree's trunk; the majority of pollution accumulates in the layers of bark that are visible on the outside, down to a depth of around 8 mm (Rosca *et al.,* 2021). Bark has the advantage of being widely available without compromising the health of the trees and retaining pollution for a longer period of

time due to its structural makeup. Tree bark's structural porosity is the cause of its ability to collect and retain analyses well (Berlizov *et al.,* 2007).

The concentration of Zn in bark of *Ficus religiosa* was found to be highest at all sites. It is because of the large canopy cover, stem flow also high due to large girth of the *Ficus. Ficus religiosa* might have a specific affinity for zinc accumulation. Compared to the roadside, plants at industrial sites generally show a greater increase in metal concentration, particularly for *Azadirachta indica* (lead and copper) and *Mangifera indica* (zinc). This suggests that the specific industrial activities at these sites might contribute significantly to heavy metal pollution in plants (Ebong *et al.,* 2016). *Shorea robusta* shows a mixed response. Its lead concentration remains elevated at the roadside but decreases in the industrial site compared to the control. Copper concentration follows a similar pattern. Zinc concentration, however, is highest at the industrial site. This data reinforces the previous observation that *Shorea robusta* might possess mechanisms to limit lead and copper uptake in polluted environments, but its response to zinc appears to be more variable.

The contrasting trends seen here demonstrate the complex interactions between plants and heavy metals. According to Liu *et al.,* (2007) and Roy *et al.,* (2020), it illustrates the overall effectiveness of a given plant species to accumulate and metal tolerance capacities for different heavy metals. These capacities can be impacted by physiological adaptations and root shape. The increased uptake of metals at industrial sites is consistent with studies conducted by Başlar *et al.,* (2005); Zhuang *et al.,* (2007); Wang *et al.,* (2019); Roy *et al.,* (2020); Patel *et al.,* (2022); which found that plants growing close to industrial facilities had higher concentrations of heavy metals than those growing along roadsides.

5.4. Correlation analysis

Pearson correlation analysis (Table 11 and Table 12) indicates that most of the metals are highly correlated. Significant positive correlation was observed between soil dust lead – leaves lead among *Mangifera indica* and *Ficus religiosa.* Similarly, significant correlation between soil dust copper content and leaves copper content of *Ficus religiosa* and *Terminalia alata* was observed. The bark of *Azadirachta indica* showed strongly positive relationship with soil dust particles for different metals (Pb, Cu and Zn). There was a weak positive insignificant correlation between soil dust lead – leaves lead ($r = 0.564$ for

Shorea robusta, 0.186 for *Azadirachta indica* and 0.544 for *Ficus religiosa*), soil dust copper – leaves copper, (r=0.179 for *Shorea robusta*, 0.435 for *Mangifera indica*, 0.448 for *Azadirachta indica*) and soil dust zinc – leaves zinc showed significantly positive correlation for all plant species. Similarly, there was a weak positive insignificant correlation between soil dust lead – bark lead (r = 0.491), soil dust copper – bark copper, (r=0.04) and soil dust zinc – bark zinc (r=0.324) for *Ficus religiosa.* As demonstrated by the correlation analysis of the current study, a number of studies (Berthelsen *et al.,* 1995; Ugolini *et al.,* 2013; Ogundele *et al.,* 2015; Suryawanshi *et al.,* 2016; Roy *et al.,* 2020; Unterbrunner *et al.,* 2007) reported a significant positive correlation between soil, bark, and leaf trace metals concentration.

5.5. MAI value

The present study found that the leaves of *Terminalia alata* in an industrial area had the highest average MAI value (55.37), while the bark of *Ficus religiosa* by a roadside had the next highest value (38.2). The findings show that different plant species have varying capacities for removing pollutants, and that these capacities depend on a number of different conditions. Similar species can have different MAI values at different place like, the MAI value of *Ficus religiosa* was 38.2 in bark around roadside while leaves of *Ficus religiosa* had moderate MAI value (13.35) around roadside.

Nadgorska Socha *et al.,* (2017) also showed site wise variations in MAI values for different plant species. When it comes to eliminating air pollutants, trees are the most effective plant group (Yang *et al.,* 2005; Hu *et al.,* 2014). Different plants species leaves and barks simultaneously collect different elements (Liu *et al.,* 2007). According to Hu *et al.,* (2014), the index is dependent on a number of variables, including local air chemistry, meteorological, sample height (tree), time of sampling, and plant features. Liu *et al.,* (2007) showed the highest MAI values (53.8) in *Catalpa speciose* at Beijing. Similar findings were reported where highest MAI values (3.89) have been identified in *Sabina chinensis* at Yan'an (Hu *et al.,* 2014).

CHAPTER 6
CONCLUSIONS

It can be concluded that, emissions from industrial area has greatly influenced the level of toxic metals in plants by the roadside since lower levels of these metals were recorded in the control samples. This data emphasizes the need for a micro understanding of plant-heavy metal interactions in polluted environments. While some species like *Ficus religiosa* show a highest metal accumulation, others like *Shorea robusta* exhibit low metal accumulation. Further research is needed to explore the underlying mechanisms and inform strategies for mitigating heavy metal pollution and developing effective phytoremediation approaches. This study has shown that, *Mangifera indica*, *Ficus religiosa*, and *Azadirachta indica* has the potential of accumulating toxic metals within its vicinity thus could be applied for phytoremediation studies and bio monitoring. Similarly, *Shorea robusta* and *Terminalia alata* exhibit complex responses.

Lastly, this present study concluded that the barks of *F. religiosa* showed highest MAI value around roadside plant species. Similarly, *M. indica, A.indica, T. alata* showed moderate MAI value at roadside area. So that these plants can be used for phytoremediation around roadside area. Likewise, the leaves of *T. alata* around industrial area showed highest MAI. Species like *M. indica*, *A. indica* and *F.religiosa* showed moderate accumulation in their leaves. So, these plants can be used as phytoremediation around industrial areas.

CHAPTER 7
RECOMMENDATIONS

The following recommendations have been made in the context of the investigation's findings:

i. From above mentioned result, it only represents a single study, and broader geographical comparisons might be necessary.
ii. Certain plant species, like those showing high metal tolerance, might be useful for phytoremediation. This technique utilizes plants to remove contaminants from the soil. Research into identifying and utilizing suitable plant species for phytoremediation in polluted areas is essential.
iii. Investigate the physiological mechanisms *Ficus religiosa* employs for efficient heavy metal accumulation.
iv. Plants that showed the highest MAI value, such as *Terminalia alata*, *Ficus religiosa*, *Mangifera indica*, and *Azadirachta indica*, can be used for phytoremediation around industrial and roadside areas.
v. Studies should be conducted to understand the underlying reasons for avoidance of heavy metal accumulation in *Shorea robusta.*

8. REFERENCES

Abechi, E. S., Okunola, O. J., Zubairu, S. M. J., Usman, A. A., and Apene, E. (2010). Evaluation of heavy metals in roadside soils of major streets in Jos metropolis, Nigeria. *Journal of Environmental Chemistry and Ecotoxicology*, **2**(6), 98-102.

Adriano, D. C. 1986. Trace elements in the terrestrial environments. *Springer*, New York, 533 pp.

Agrahari, P., Richa, R., Swati, K., Rai, S., Singh, V. K., and Singh, D. K. (2018). Ficus religiosa tree leaves as bio indicators of heavy metals in Gorakhpur city, Uttar Pradesh, India. *Pharmacognosy Journal*, **10**(3).

Akan, J. C., Inuwa, L. B., Chellube, Z. M., and Lawan, B. (2013). Heavy metals in leaf, stem bark of neem tree (*Azadirachta indica*) and roadside dust in Maiduguri Metropolis, Borno State, Nigeria. *Environment and Pollution***, 2**(1), 88.

Al-Chalabi, A. S., and Hawker, D. (1997). Response of vehicular lead to the presence of street dust in the atmospheric environment of major roads. *Science of the Total Environment*, **206**(2-3), 195-202.

Alloway, B. J., Jackson, A. P., and Morgan, H. (1990). The accumulation of cadmium by vegetables grown on soils contaminated from a variety of sources. *Science of the total Environment*, **91**, 223-236.

Apostoli, P., Kiss, P., Porru, S., Bonde, J. P. and Vanhoorne, M. 1998. Male reproductive toxicity of lead in animals and humans. Occup. *Environment Medicine* **53**: 364-374.

Aradhi K. Krishna, M. Satyanarayanan, Pradip K. Govil. Assessment of heavy metal pollution in water using multivariate statistical techniques in an industrial area (2009). A case study from Patancheru, Medak District, Andhra Pradesh, India. *Journal of Hazardous Materials*. **167** (1–3), 366-373. ISSN 0304-3894. https://doi.org/10.1016/j.jhazmat.2008.12.131.

Athanasopoulou, E., Tombrou, M., Russell, A. G., Karanasiou, A., Eleftheriadis, K., and Dandou, A. (2010). Implementation of road and soil dust emission

parameterizations in the aerosol model CAMx: Applications over the greater Athens urban area affected by natural sources. *Journal of Geophysical Research: Atmospheres*, **115**(D17).

Baath, E. 1989. Effects of heavy metals in soil on microbial processes and populations (a review). *Water, Air, Soil Poll*ution **47**: 335-379.

Baslar, S., Dogan, Y., Yenil, N., Karagoz, S., and Bag, H. (2005). Trace element biomonitoring by leaves of *Populus nigra L.* from Western Anatolia, Turkey. *Journal of Environmental Biology*, **26**(4), 665-668.

Berlizov, A. N., Blum, O. B., Filby, R. H., Malyuk, I. A., and Tryshyn, V. V. (2007). Testing applicability of black poplar (*Populus nigra L.*) bark to heavy metal air pollution monitoring in urban and industrial regions. *Science of the Total Environment*, **372**(2-3), 693-706.

Berthelsen, B. O., Steinnes, E., Solberg, W., and Jingsen, L. (1995). Heavy metal concentrations in plants in relation to atmospheric heavy metal deposition (Vol. 24, No. 5, pp. 1018-1026). *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.*

Bohn, H. L. 1972. Soil adsorptions of air pollutants. *Journal of Environmental Quality* **1**: 372-377.

Bomke, A. A. and Lowel, L. E. 1991. Trace element uptake by two British Columbia forages as affected by poultry manure application. Can. *Journal of Soil Sci*ence **71**: 305-312.

Buchauer, M. J. 1973. Contamination of soil and vegetation near a zinc-smelter by zinc, cadmium, copper and lead. *Environment Science and Technol*ogy **7**: 131-135.

Burton, E. D., Hawker, D. W., and Redding, M. R. (2003). Sludge-Derived Cu and Zn in a Humic-Gley Soil: Effect of dissolved metal-organic matter complexes on Sorption and Partitioning. *Soil and Sediment Contamination: An International Journal*, **12**(1), 23–46. https://doi.org/10.1080/713610959.

Castro-González, M. I., and Méndez-Armenta, M. (2008). Heavy metals: Implications associated to fish consumption. *Environmental toxicology and pharmacology*, **26**(3), 263-271.

CDC 1997. Screening young children for lead poisoning: Centers for Disease Control and Prevention, Guidance for state and local public health officials. Atlanta, GA.

Chamberlain, A. C. 1960. Aspects of the deposition of radioactive and other gases and particles. *International Journal of Air Pollution* **3**: 63-88.

Chaudri, A. M., McGrath, S. P., Giller, K. E., Rietze, E. and Sauerbeck, D. R. 1993. Enumeration of indigenous Rhizobium Leguminosarum biovar Trifoli in soil previously treated with metal contaminated sewage sludge. *Soil Biology and Biochemistry* **25**:301-309.

Chettri, M. K., Sawidis, T. and Karataglis, S. 1997. Lichens as a tool for biogeochemical prospecting. *Ecotoxicology and Environmental Safety*, **38** (1): 322-335.

Dara, S. S. 2004. *A text book of Environmental Chemistry and Pollution control. Chand S and Company Ltd. Ram Nagar, New Delhi*. 354 p

Dogan, Y., Ugulu, I., and S. Baslar: Turkish Red Pine as a Bio monitor (2010). A comparative study of the Accumulation of Trace Elements in Needles and Barks. *Ekoloji*, **19** :75-88.

Dogan, Y., Baslar, S., and Ugulu, I. (2014). A study on detecting heavy metal accumulation through biomonitoring: content of trace elements in plants at Mount Kazdagi in Turkey. *Applied Ecology and Environmental Research*, **12**(3), 627-636.

Ebong, G., and Etuk, H. (2016). Fractionation status and uptake potentials of trace metals in water bodies within Niger Delta Region, Nigeria. *International Research Journal of Pure and Applied Chemistry*, **13**(2), 1-18.

Enete I, Ogbonna C, Officha M (2012) Using trees as urban heat island reduction tool in Enugu city Nigeria based on their air pollution tolerance index. Ethiopian *Journal of Environmental Studies and Management* **5:** 482–486.

Ernst, W. H. O. 1998. Evolution and ecophysiology of metallophytes in Africa and Europe. In: (Eds) Breckle S W, Schweizer B and Arndt U. Results of worldwide ecological studies.

Filipović-Trajković, R., Ilić, Z. S., Šunić, L., and Andjelković, S. (2012). The potential of different plant species for heavy metals accumulation and distribution. *Journal of Food Agriculture and Environ*ment, **10**(1), 959-964.

Friedman, M. and Waiss, A. C. 1972. Mercury uptake by selected agricultural products and byproducts. *Environment Science Technology*.

Garbarino JR, Hayes H, Roth D, Antweider R, Brinton TI, Taylor H (1995). Contaminants in the Mississippi River, U. S. *Geological Survey Circular* 1133, Virginia, U.S.A. (www.pubs.usgs.gov/circ/circ1133/).

Ghosh, S. P., Raj, D., and Maiti, S. K. (2020). Risks assessment of heavy metal pollution in roadside soil and vegetation of national highway crossing through industrial area. *Environmental Processes*, **7**(4), 1197-1220.

Godbold, D. L. and Huttermann, A. 1985. Effect of zinc, cadmium and mercury on root elongation of *Picea abies* (Karst) seedlings, and the significance of these metals to forest die back. *Environmental Science and Pollution Research* **38**: 375-381.

Gunawardena, J., Egodawatta, P., Ayoko, G. A., and Goonetilleke, A. (2012). Role of traffic in atmospheric accumulation of heavy metals and polycyclic aromatic hydrocarbons. *Atmospheric environment*, **54**, 502-510.

Hu, Y., Wang, D., Wei, L., Zhang, X., Song, B., 2014. Bioaccumulation of heavy metals in plant leaves from Yan′ a city of the Loess Plateau, China. *Ecotoxicol. Environmental Safety* **110**, (82) 93-101, ISSN 0940-2993. https://doi.org/10.1016/j.etp.2010.06.011.

Hui, Z., Caiqiu, W., Jiping, G., Xuyin, Y., Qiao, W., Wenming, P., ... and Hanpei, Z. (2017). Assessment of heavy metal contamination in roadside soils along the Shenyang-Dalian highway in liaoning province, China. *Polish Journal of Environmental Studi*es **26**(4), 1539-1549.

Janta, R., Chantara, S., Inta, A., Kawashima, M., and Satake, K. (2016). Levels of road traffic heavy metals in tree bark layers of Cassia fistula tree. *International Journal of Environmental Science and Development*, **7**(5), 385.

Jaradat, Q. M., and Momani, K. A. (1999). Contamination of roadside soil, plants, and air with heavy metals in Jordan, a comparative study. *Turkish Journal of Chemistry*, **23**(2), 209-220.

Jiries, A. (2003). Vehicular contamination of dust in Amman, Jordan. *Environmentalist*, **23**, 205-210.

Johansson, C., Norman, M., and Burman, L. (2009). Road traffic emission factors for heavy metals. *Atmospheric Environment*, **43**(31), 4681-4688.

Karanasiou, A. A., Siskos, P. A., and Eleftheriadis, K. (2009). Assessment of source apportionment by Positive Matrix Factorization analysis on fine and coarse urban aerosol size fractions. *Atmospheric Environment*, **43**(21), 3385-3395.

Lagerwerff, J. V. 1971. Uptake of cadmium, lead and zinc by radish from soil and air. *Soil Sci*ence **3**:129-133.

Lago-Vila, M., Rodríguez-Seijo, A., Arenas-Lago, D. *et al.* Heavy metal content and toxicity of mine and quarry soils (2017). *Journal of Soils Sediments* **17**, 1331–1348. https://doi.org/10.1007/s11368-016-1354-0

Lenntech, Water Treatment and Air Purification (2004). Water Treatment, Published by Lenntech, Rotter dam sewage, Netherlands (www.excelwater.com/thp/filters/Water-Purification.htm).

Little, P. 1973. A study of heavy metal contamination of lead surfaces. *Environtal Pollution* **5**: 159- 172.

Liu, Y.J., Zhu, Y.G., Ding, H., 2007. Lead and cadmium in leaves of deciduous trees in Beijing, China: development of a metal accumulation index (MAI). *Environmental Pollution* **145** (2), 387-390.

Mahida, D. K., Makwana, V. M., Sankhla, M. S., Patel, A., and Dodia, P. (2023). Accumulation of Heavy Metals in Roadside Plants and Their Role in

Phytoremediation. In *Anthropogenic Environmental Hazards: Compensation and Mitigation* (pp. 119-141). Cham: Springer Nature Switzerland.

Man, Y. B., Sun, X. L., Zhao, Y. G., Lopez, B. N., Chung, S. S., Wu, S. C., ... and Wong, M. H. (2010). Health risk assessment of abandoned agricultural soils based on heavy metal contents in Hong Kong, the world's most populated city. *Environment international*, **36**(6), 570-576.

Morselli, L., Barilli, L., Olivieri, P., Cechini, M., Aromolo, R., Dicarlo, V., Rosa, F. and Gata, L. L. 1999. Heavy metal determination in dry surrogate depositions, characterization of an urban and a natural site. *Annali di Chimica*, **89**:739-755.

Nadgorska Socha, A., Kandziora-Ciupa, M., Trzę sicki, M., Barczyk, G., 2017. Air pollution tolerance index and heavy metal bioaccumulation in selected plant species from urban biotopes. *Chemosphere* **183**, 471-482.

Nies, D. H. 1999. Microbial heavy metal resistance. Applied microbial. *Biotechnol*ogy Journal International **51**: 730-750.

Njoku, P. C., and Ayoka, A. O. (2007). Evaluation of heavy metal pollutants from soils at municipal solid waste deposit in Owerri, Imo State, Nigeria. *Journal of Chemical Society of Nigeria*, **32**(1), 57-60.

Ogbonna C, Enete C, Egedeuzu C, Ogbochi F (2013) Heavy metal concentration in leaves of roadside trees in Umuahia Urban, South East Nigeria. *Resources and Environment* **3**: 141–144

Ogundele, D. T., Adio, A. A., and Oludele, O. E. (2015). Heavy metal concentrations in plants and soil along heavy traffic roads in North Central Nigeria. *Journal of Environmental and Analytical Toxicology*, **5**(6), 1.

Pandey, V., Upreti, D.K., Pathak, R. *et al.* Heavy Metal Accumulation in Lichens from the Hetauda Industrial Area Narayani Zone Makwanpur District, Nepal. *Environmental Monitoring and Assessment* **73**, 221–228 (2002). https://doi.org/10.1023 /A:1013173104533

Pant, P. P., and Tripathi, A. K. (2014). Impact of heavy metals on morphological and biochemical parameters of Shorea robusta plant. *Ekológia (Bratislava)*, **33**(2), 116-126.

Parajuli, I. and Chettri, M. K., (2020). Bioaccumulation of Iron in Plants and Their Possibilities as a Tool for Exploration of Hematite Ores. *Amrit Research Journal*, **1**(1), 1-12.

Parzych, A., Mochnacký, S., Sobisz, Z., Kurhaluk, N., and Polláková, N. (2017). Accumulation of heavy metals in needles and bark of Pinus species. *Folia Forestalia Polonica*, **59**(1), 34-44.

Patel, K., Chaurasia, M., and Rao, K. S. (2023). Heavy metal accumulation in leaves of selected plant species in urban areas of Delhi. *Environmental Science and Pollution Research*, **30**(10), 27622-27635.

Poikolainen, J. (1997). Sulphur and heavy metal concentrations in Scots pine bark in northern Finland and the Kola Peninsula. *Water, air, and soil pollution*, **93**, 395-408.

Rosca, M., Cozma, P., Minut, M., Hlihor, R. M., Bețianu, C., Diaconu, M., and Gavrilescu, M. (2021). New evidence of model crop Brassica napus L. in soil clean-up: comparison of tolerance and accumulation of lead and cadmium. *Plants*, **10**(10), 2051.

Ross, S. M. (1994). Sources and forms of potentially toxic metals in soil-plant systems.

Roy, S., Gupta, S. K., Prakash, J., Habib, G., and Kumar, P. (2022). A global perspective of the current state of heavy metal contamination in road dust. *Environmental Science and Pollution Research*, 1-22.

Safari, M., Ramavandi, B., Sanati, A. M., Sorial, G. A., Hashemi, S., and Tahmasebi, S. (2018). Potential of trees leaf/bark to control atmospheric metals in a gas and petrochemical zone. *Journal of environmental management*, *222*, 12-20.

Salim Akhter, M., and Madany, I. M. (1993). Heavy metals in street and house dust in Bahrain. *Water, Air, and Soil Pollution*, **66,** 111-119.

Sawidis, T., M.K. Chettri, G.A. Zachariadis, J.A. Stratis and M.R.D. Seaward, 1995. Heavy metal bioaccumulation in lichens from Macedonia in North Greece. *Toxicological and Environmental Chemistry* **50**, 157-166.

Scheinberg, H. 1979. Occurence, analysis and biological relevance. In: Metals and their compounds in the environment, (Ed) Merian E, New York: VCH, pp. 17-39.

Schutzendubel, A. and Polle, A. 2002. Plant responses to abiotic stresses: heavy metal–induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany* **53** (372): 1351-1365.

Severoglu, Z., Ozyigit, I. I., Dogan, I., Kurmanbekova, G., Demir, G., Yalcin, I. E., and Kari, G. K. (2015). The usability of Juniperus virginiana L. as a biomonitor of heavy metal pollution in Bishkek City, Kyrgyzstan. *Biotechnology and Biotechnological Equipment*, **29**(6), 1104-1112.

Shakya, J. D. (2000). Floristic conposition of Butwal area and its surrounding hills. *Banko Janakari*, **10**(1), 42-46.

Shakya, K., Chettri, M. K., and Sawidis, T. (2008). Impact of heavy metals (copper, zinc, and lead) on the chlorophyll content of some mosses. *Archives of Environmental Contamination and Toxicology*, **54**, 412-421.

Sharma, B., and Chhetri, M. K. (2005). Monitoring of heavy metals in vegetables and soil of agricultural fields of Kathmandu Valley. *Ecoprint: An International Journal of Ecology*, **12**, 1-9.

Sharma, K., Basta, N. T., and Grewal, P. S. (2015). Soil heavy metal contamination in residential neighborhoods in post-industrial cities and its potential human exposure risk. *Urban ecosystems*, **18**, 115-132.

Sharply, A. N. and Menzel, R. G. 1987. The impact of soil and fertilizers phosphorus on the environment. *Advance in Agronomy* **41**: 297-324.

S. Khan, Q. Cao, Y.M. Zheng, Y.Z. Huang, Y.G. Zhu (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing,

China. *Environmental Pollution*. **152** (3), 686-692. ISSN 0269-7491. https://doi.org/10.1016/j.envpol.2007.06.056.

Somashekaraiah, B. V., Padmaja, K. and Prasad, A. R. K. 1992. Phytotoxicity of cadmium ions on germinating seedlings of mung bean (Phaseolus vulgaris): Involvement of lipid peroxides in chlorophyll degradation. *Physiol Planta*, **85**: 85-89.

Stoths, S. J. and Bagchi, D. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Med*icine **18**:321-336.

Suranjana, R., and Manas, K. R. (2009). Bioremediation of heavy metals toxicity with special Reference to Chromium, special: 57-63. *ISSN 0974-1143*.

Suryawanshi, P. V., Rajaram, B. S., Bhanarkar, A. D., and Chalapati Rao, C. V. (2016). Determining heavy metal contamination of road dust in Delhi, India. *Atmósfera*, **29**(3), 221-234.

Thapa M. S., Poudel G. (2018): Assessing the Coverage of Urban Green Space in Butwal Sub-Metropolitan City, Nepal: A GIS based approach. Forestry: *Journal of Institute of Forestry, Nepal*. No. **15**: page 77 to 86.

Tjell, J. C., Christensen, T. H., and Bro-Rasmussen, F. (1983). Cadmium in soil and terrestrial biota, with emphasis on the Danish situation. *Ecotoxicology and Environmental Safety*, **7**(1), 122-140.

Ugolini, F., Tognetti, R., Raschi, A., and Bacci, L. (2013). Quercus ilex L. as bioaccumulator for heavy metals in urban areas: Effectiveness of leaf washing with distilled water and considerations on the trees distance from traffic. *Urban forestry and urban greening*, **12**(4), 576-584.

Unterbrunner, R., Puschenreiter, M., Sommer, P., Wieshammer, G., Tlustoš, P., Zupan, M., and Wenzel, W. W. (2007). Heavy metal accumulation in trees growing on contaminated sites in Central Europe. *Environmental pollution*, **148**(1), 107-114.

Viskari, E. L., Surakka, J., Pasanen, P., Mirme, A., Kossi, S., Ruuskanen, J., and Holopainen, J. K. (2000). Responses of spruce seedlings (*Picea abies*) to exhaust

gas under laboratory conditions. I Plant-insect interactions. *Environmental Pollut*ion, **107**, 89-98. http://dx.doi.org/10.1016/S0269-7491(99)00128-1.

Vivek Pandey, V. P., Upreti, D. K., Ramayan Pathak, R. P., and Amit Pal, A. P. (2002). Heavy metal accumulation in lichens from the Hetauda Industrial area Narayani zone Makwanpur district, Nepal.

Vojtěch Ettler, Bohdan Kříbek, Vladimír Majer, Ilja Knésl, Martin Mihaljevič (2012). Differences in the bio accessibility of metals/metalloids in soils from mining and smelting areas (Copper belt, Zambia). *Journal of Geochemical Exploration*, **113**: 68-75, ISSN 0375-6742. https://doi.org/10.1016/j.gexplo.2011.08.001.

Wang, D. and Schapp, W. 1988. Air pollution impacts on plants: current research challenges. I. S. I. Atlas. *Sci, Anim. Plant Sci.* 1(1): 33-39.

Weast, R. C. 1984. *Handbook of Chemistry and Physics* (64th edition), Boca Raton, CRC Press.

Welz, B. 1985. *Atomic Absorption Spectrometry*. VCH, Weinheim, Germany.

WHO 1996. Trace elements in Human nutrition and Health. World Health Organization, Geneva.

Wilson, B., Lang, B., and Pyatt, F. B. (2005). The dispersion of heavy metals in the vicinity of Britannia Mine, British Columbia, Canada. *Ecotoxicology and Environmental Safety*, **60**(3), 269-276.

Xiong, Z. T. (1997). Bioaccumulation and physiological effects of excess lead in a roadside pioneer species Sonchus oleraceus L. *Environmental pollution*, **97**(3), 275-279.

Yahaya, M. I., Ezeh, G. C., Musa, Y. F., and Mohammad, S. Y. (2010). Analysis of heavy metals concentration in road side's soil in Yauri, Nigeria. *African Journal of Pure and Applied Chemistry*, **4**(3), 022-030.

Yang, J., McBride, J., Zhou, X., Sun, Z.Y., 2005. The urban forest in Beijing and its role in air pollution reduction. *Urban for. Urban Green*, **3**, 65-78.

Yousaf, M., Mandiwana, K.L., Baig, K.S. *et al.* Evaluation of *Acer rubrum* Tree Bark as a Bioindicator of Atmospheric Heavy Metal Pollution in Toronto, Canada. *Water Air Soil Pollut* **231**, 382 (2020). https://doi.org/10.1007/s11270-020-04758-w

Zhai, Y., Dai, Q., Jiang, K., Zhu, Y., Xu, B., Peng, C. and Zeng, G. (2016). Traffic-related heavy metals uptake by wild plants grow along two main highways in Hunan Province, China: effects of soil factors, accumulation ability, and biological indication potential. *Environmental Science and Pollution Research*, **23**, 13368-13377.

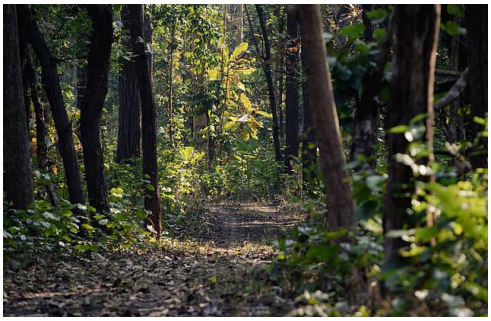
Zhiyuan, W., Dengfeng, W., Huiping, Z., and Zhiping, Q. I. (2011). Assessment of soil heavy metal pollution with principal component analysis and geoaccumulation index. *Procedia Environmental Sciences*, **10**, 1946-1952.

Zhuang, P., Yang, Q. W., Wang, H. B., and Shu, W. S. (2007). Phytoextraction of heavy metals by eight plant species in the field. *Water, Air, and Soil Pollution*, **184**, 235-242.

Photo plates

a) Butwal industrial area

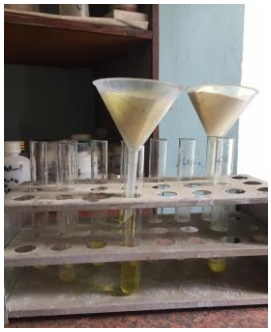
b) Mahendra highway, Butwal



C) Control site (Shivanagar CF)

d) Sample preparation

e) Digested sample



f) Sample filtration

g) Chemito-201 AAS

h) Analysis of heavy metals