HEAVY METALS ACCUMULATION IN SOME TREES GROWING AROUND HONGSHI SHIVAM CEMENT FACTORY, NAWALPARASI, NEPAL

Amrit Campus

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

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DECLARATION

The dissertation entitled **"Heavy metals accumulation in some trees growing around Hongshi Shivam Cement Factory, Nawalparasi, Nepal"** has been done by myself, under the supervision of Prof. Dr. Mukesh Kumar Chettri and co-supervision of Asst. Prof. Dr. Jaya Prakash Hamal. It has not been published or submitted elsewhere, in whole or in part, for any other degree or professional qualification. Any literature, data or works created by others and cited in this thesis have been properly acknowledged and listed in the reference section.

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RECOMMENDATION

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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
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
NPI	:	National Pollution Inventory
p	:	Level of significance
Pb	:	Lead
SD	:	Standard Deviation
SPSS	:	Statistical package for social science
WHO	:	World Health Organization
Zn	:	Zinc

सोधसार

यस अध्ययनले नेपालमा रहेको होङ्शी शिवम् सिमेन्ट कारखानाबाट विभिन्न दूरीमा रहेका पाँचवटा रुख प्रजाति *Shorea robusta* (साल), *Mangifera indica* (आँप), *Ficus semicordata* (खनियु), *Ficus religiosa* (पिपल) र *Mallotus philippensis* (सिन्धुरे) का पातहरू, बोक्रा र त्यस वरपरका माटोको धुलोमा भारी धातुहरूको संकलनलाई अनुसन्धान गरेको छ। नमुनाहरू कारखानाबाट पुर्बतर्फ़ २०० मिटर र १००० मिटर दूरीमा रहेका स्थानहरू साथै ४ देखि ५ किमि टाढा रहेको नियन्त्रण स्थानबाट संकलन गरिएका थिए। Lead (सीसा), Copper (तामा), र Zinc (जस्ता) को मात्रा AAS प्रयोग गरेर मापन गरिएको थियो। नतिजाले सबैभन्दा उच्च धातुहरूको संकलन माटोको धुलोमा, र त्यसपछि क्रमस: बोक्रा र पातहरुमा देखायो। धातुहरूको संकलन २०० मिटरमा १००० मिटर र नियन्त्रण स्थानहरू भन्दा उल्लेखनीय रूपमा बढि थियो, जसले प्रदूषण बढी मात्रामा देखाउँछ। सबै नमूनाहरूमा Zinc लगातार सबैभन्दा प्रचुर धातु पाइयो। *Mallotus philippensis* र *Mangifera indica* ले अन्य प्रजातिहरूको तुलनामा उच्च धातु संकलन देखाउँछन्। MAI दरहरु २०० मिटर वरपर *Mangifera indica* पातहरूको लागि ४२. २२ र *Mallotus philippensis* बोक्राको लागि ३५. ६० सहित सबैभन्दा उच्च थिए। सम्बन्ध विश्लेषणले प्रायः सबै प्रजातिहरूको लागि माटो र बोटबिरुवाको धातु संकलनको बीचमा केही अपवाद सहित बलियो सकारात्मक सम्बन्ध देखायो । *Ficus religiosa* र *Mallotus philippensis* ले पातहरूमा सबै धातुहरूको लागि बलियो सम्बन्ध देखाए भने, *Mangifera indica* र *Ficus semicordata* ले बोक्रामा Lead र Zinc को लागि बलियो सम्बन्ध देखाए। यस अध्ययनले प्रजातिको विशिष्ट धातु संकलनका ढाँचाहरूमा अन्तर्दृष्टि प्रदान गर्दछ र औद्योगिक प्रदूषणको जैवसंकेतकका रूपमा केही रुख प्रजातिहरूको सम्भावना उजागर गर्दछ।

प्रमुख शब्दहरु: पातहरु र बोक्रामा रहेका भारी धातुहरू, धातु संकलन दर, प्रदूषणको जैवसंकेतक

ABSTRACT

This study investigated heavy metal accumulation deposited on soil dust, leaves, and bark of five tree species (*Shorea robusta, Mangifera indica*, *Ficus semicordata, Ficus religiosa,* and *Mallotus philippensis*) at varying distances from Hongshi Shivam cement factory in Nepal. Samples were collected from sites 200 m and 1000 m from the factory, as well as a control site 4-5 km away. Concentrations of lead (Pb), copper (Cu), and zinc (Zn) were analyzed using atomic absorption spectrometry. Results showed highest metal concentrations in soil dust, followed by bark and leaves. Metal concentrations were significantly elevated at 200 m compared to 1000 m and control sites, indicating a pollution gradient. Zinc was consistently the most abundant metal across all sample types. *Mallotus philippensis* and *Mangifera indica* generally exhibited higher metal accumulation compared to other species. Metal Accumulation Index (MAI) values were highest for *Mangifera indica* leaves (42.22) and *Mallotus philippensis* bark (35.60) around 200 m. Correlation analysis revealed strong positive relationships between soil and plant metal concentrations for most species, with some exceptions. *Ficus religiosa* and *Mallotus philippensis* showed strong correlations for all metals in leaves, while *Mangifera indica* and *Ficus semicordata* demonstrated strong correlations for lead and zinc in bark. This study provides insights into species-specific metal accumulation patterns and highlights the potential of certain tree species as bioindicators of industrial pollution.

Keywords: Bark, Bio-indicators, Heavy metals, Leaves, MAI, Pollution gradient.

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CHAPTER 1: INTRODUCTION

1.1. Background

1.1.1. Heavy metals

Heavy metals are naturally occurring metals with an atomic number greater than 20 and an elemental density greater than 5gm/cm^3 (Ali *et al.,* 2017, Khanna *et al.,* 2018). Altogether, 53 heavy metals are present in nature, but only seven (Cu, Fe, Mn, Zn, Mo, Co and Ni) are essential to living cells in plants to run physiological processes (Weast, 1984).

Exposure to heavy metals such as cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb) can be toxic to organisms, even in small doses. Some metals, including copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn), are essential micronutrients required by organisms in small amounts (Freedman, 1995). However, high concentrations of these metals can also have toxic effects (Chettri *et al.,* 1998).

Heavy metals come from natural or human-made sources, such as effluents from oil and gas industries (Pichtel, 2016) and metal mining and smelting (Chen *et al.,* 2016). Biological or physical processes do not easily break down heavy metals and can persist in the soil for extended periods, posing a long-term environmental threat (Suman *et al*., 2018). Heavy metals can be categorized as essential or non-essential elements based on their role in biological systems. Essential heavy metals such as copper, iron, manganese, nickel, and zinc are necessary for physiological and biochemical processes during the plant life cycle (Cempel and Nikel, 2006). On the other hand, non-essential heavy metals like lead, cadmium, arsenic, and mercury are highly toxic, serve no known function in plants (Fasani *et al.,* 2018), and can cause environmental pollution while severely impacting various physiological and biochemical processes in plants (Clemens, 2006).

1.1.2. Heavy metals and human health

Continuous exposure to heavy metals can be hazardous to human health. Serious health problems such as mental retardation, birth defects, autism, allergies, hyperactivity, paralysis, muscle weakness, brain damage, and kidney damage can arise from prolonged exposure to lead (Morre and Goldberg, 1985; Apostoli *et al.,* 1998). It can be lethal in severe circumstances. Lead can also induce edema by breaking down the blood-brain barrier (Jaishankar *et al.*, 2014). Zinc affects survival and may be a critical regulator of apoptosis and neuronal death following brain injury, despite the fact that it is thought to be largely non-toxic to humans (Fosmire, 1990; Plum *et al.*, 2010). Similarly, mucous membrane damage, sexual immaturity, skin lesions, gray hair, electrolyte imbalance, dehydration, dizziness, and lethargy are signs of zinc toxicity (WHO, 1996). Abdominal discomfort, jaundice, anorexia, extreme thirst, diarrhea, and vomiting linked to erosive gastropathy are the most prevalent gastrointestinal side effects of copper overdoses, with signs of overt acute copper toxicity depending in part on the mechanism of copper overload. (Gamakaranage *et al.*, 2011).

1.1.3. Plants and heavy metals pollution

Higher plants, particularly trees, are the preferred choice for biomonitoring to determine short- and long-term toxicity levels. Biomonitoring is a valuable tool for assessing the quantity and quality of pollutants. It has a significant advantage over other environmental protection methods due to its reliability, easy application, and lower maintenance costs (Dogan *et al.*, 2010).

The correlation between heavy metal pollution and the level of industrialization and chemical usage has been well established (Akinola *et al.*, 2008). Forest ecosystems face significant challenges due to heavy metal pollution (Nuhoglu, 2005). Various researchers have documented air pollution issues near cement factories (Afolabi *et al.*, 2012; Emetere and Dania, 2019; Samad *et al.*, 2020). The unregulated industrial activities and rapid urban development have led to the release of various pollutants, particularly heavy metals like Ni and V from petrochemical and gas industries and Pb from commercial activities, including cement production, iron and steel production, leak out of tires due to wear and tear, battery corrosion, or fuel combustion contribute to environmental pollution (Delshab *et al.*, 2017). These emitted heavy metals adhere to particulate matter and dust, settling on the ground and trees (Ugolini *et al.*, 2013). The distribution of heavy metals is influenced by site topography, wind velocity, particulate matter diameter, and surface characteristics of the materials on which they are deposited (Norouzi *et al.*, 2016). Heavy metals deposited on land may be transported by wind or runoff, re-entering vegetal tissues through various pathways, including soil, water, and air (Bilo *et al.*, 2017).

Air pollutants, such as heavy metals, generated by crushing limestone, bagging, and transporting cement, are carried by wind and deposited on soil, plants and water bodies (Kabir, 2010). Among the metals especially recognized in environmental studies on emission from cement plants to have toxic effects on soil and surrounding vegetation are arsenic, cadmium, lead, mercury, aluminum, beryllium, chromium, copper, manganese, nickel and zinc (Addo, 2012). The top soils near a cement factory are enriched with Zn, Cr, Cd, Pb and Hg, and these metals are released into the air from the cement kilns (Arpita and Mitko, 2011).

1.1.4. Heavy metals accumulation in leaves and bark

Plants can absorb contaminants from the environment differently (uptake, stabilization and translocation of materials) (Ghosh and Singh 2005; Franca *et al.,* 2010). Trees are very efficient in trapping atmospheric particles, mostly on their foliage (Nowak *et al.,* 2006; Qiu *et al.,* 2009). Concentrations of major and trace metals in plants depend on root uptake or dry and wet deposition on outer plant organs such as foliage or bark (Lehndorff and Schwark, 2010). Therefore, elemental analysis of plant samples has been an alternative, easy, and effective way of conducting ecological investigations in urban areas for many years (Celik *et al.,* 2005; Baycu *et al.,* 2006). Since the introduction of biomonitoring in the last century as an alternative to direct detection of pollutants in air quality control programs, many studies conducted worldwide have reported the effectiveness of tree bark and leaves as environmental indicators and the use of plants as bioindicators (De Nicola *et al.,* 2008; Fujiwara *et al.,* 2011; Gueguen *et al.,* 2012; Hassan and Basahi, 2013).

Plants can uptake and accumulate pollutants through their roots and leaf surfaces (Sawidis *et al.,* 2001). Particles can be dry-deposited on plant surfaces and accumulate through sedimentation under the influence of gravity through impaction resulting from wind (Nowak, 2004). They can also be retained on the leaf surface through internal leaf accumulation or retention (Gratani *et al.,* 2000). In particular, leaves can act as biological absorbers of pollutants (Gratani *et al.,* 2000; Pal *et al.,* 2002). Studies are in progress to search for suitable tree species and approve the validity of using their leaves as biomonitors. Thus, it is extremely important from an ecological point of view to use plant leaves as accumulative indicators of heavy metal contamination (Markert, 1993; WHO, 2000). Heavy metals on foliar surfaces have recently been confirmed to indicate environmental pollution using some key tree species in the Carpathian Mountains (Mankovska *et al.,* 2004).

Heavy metals can accumulate in the bark of trees, and their concentrations can be used to assess the effects of heavy metals pollution and monitor environmental air quality (Khatib *et al.*, 2020). The bark of trees accumulates various heavy metals, such as copper, iron, manganese, zinc, lead, nickel, cadmium, and chromium (Dogan *et al.*, 2014). The concentrations of these heavy metals in the bark can vary depending on factors such as the species of the tree, the location, and the pollution level in a particular area. Some studies have shown that the concentrations of heavy metals in tree bark can be higher than in leaves. For example, in a study conducted on *Juglans regia*, the heavy metal accumulation values were higher in leaf samples for Fe, Mn, Zn, Pb, and Cd. At the same time, Cu, Ni, and Cr showed higher accumulation values in bark samples (Dogan *et al.*,2014). Bark samples can be used for biomonitoring heavy metal pollution in forest areas. However, using bark in such studies can be challenging due to the lack of a standardized methodology for determining the exposure time of bark to pollutants. It has provided valuable insights into the impact of pollution on forest ecosystems (Świslowski *et al.*, 2020).

Trees are considered sensors that record environmental disturbances, as they live for a long time, stay in the same place all their lives, and are widespread geographically. These factors make them especially suitable biological archives (Gratani *et al.*, 2008). Conifers have much higher heavy metal content than deciduous species (Trüby, 2005). Among trees, evergreen species are better traps for particles than deciduous ones because of their longer leaf longevity, which can accumulate pollutants throughout the year (Gratani and Varone, 2007). Moreover, the observed effect of metal accumulation is a time-averaged result, which is more reliable than the direct determination of the pollutant concentrations in the air for a short period (Kord *et al.*, 2009).

1.1.5. Metal Accumulation Index (MAI)

Metal Accumulation Index (MAI) evaluates the ability of different species of trees to accumulate atmospheric heavy metals. Higher MAI-exhibiting plants can be employed as a barrier between contaminated and uncontaminated environments. Higher MAI values are associated with better accumulating capacity and are thought to be tolerant species. A plant grows more effectively in locations contaminated with heavy metals if its MAI is higher. High MAI native plants can be utilized as a natural remedy to reduce pollution in contaminated areas. Plants with higher MAI are thought to be resistant species and can function as sinks for heavy metal contamination (Jamil *et al.*, 2009; Nadgorska- Socha *et* *al.,* 2016).

1.2. Justification

It is evident that industrial emissions, such as from cement factories, cause an increase in the concentration of heavy metals in the air, giving rise to air pollution. The fall out of pollutants in the form of dust or particulate matter from the factory accumulates on the surface of the leaves and bark of trees. Hence, it was assumed that the measurements of heavy metals in the leaves and bark of trees around the cement factory would reflect the level of heavy metal pollution in the vicinity of the cement factory. The bark was tested to receive data on long-term contamination, while leaves were used to monitor short-term contamination. Heavy metal accumulation on leaves occurs through leaf absorption directly from surrounding polluted air and root uptake from contaminated soil (Souri *et al.,* 2019). The absorption of heavy metals by leaves depends on various factors, such as adsorption capacity and physical characteristics of leaves (Sulaiman *et al.,* 2018). Similarly, due to its structure, the bark retains the pollutants longer. The bark is exposed to air pollution directly from the atmosphere or stem flow. The reason for the efficient accumulation and retention of heavy metals in the tree bark is its structural porosity (Berlizov *et al.,* 2007). Though various heavy metals are prevalent in plants, Pb, Cu, and Zn were only tested during the analysis as it was found that those were common metals and played vital roles in the growth and development of plants according to previous research (Tisdale *et al.,* 1984; Marschner 1995; Raven *et al.,* 1999; Cakmak 2000; Islam *et al*., 2008).

Hongshi cement factory was established in 2015 at Sardi, Dumkibas, East Nawalparasi District. The main Cement plant of Hongshi cement factory is about 12 km southwest of the main East-West highway. After the establishment of this factory, air monitoring, especially for heavy metals, was not conducted. Besides this, the heavy metal contamination in the air around the factory is not known. As heavy metals have a direct impact on human health, it is essential to know the heavy metal pollution in the industrial impacted areas. Hence, the present study was initiated to monitor the heavy metals concentration in the soil dust, leaves and bark of trees surrounding Hongshi Cement factory.

1.3. Research question

i. What are the concentrations of different heavy metals in soil and plant parts (leaves and bark) around the factory?

1.4. Objectives

The main objective of this study is to determine the concentration of heavy metals in soil dust and selected tree species around the factory site.

The specific objectives are:

i. To determine the concentration of heavy metals (Pb, Cu, and Zn) in leaves and barks of selected tree species in East and South directions and distances from the factory.
ii. To measure the availability of those heavy metals in soil dust in East and South directions and distances from the factory.
iii. To investigate the relation between concentrations of various heavy metals in soil dust and plant parts of selected tree species.

1.5. Limitations of the study

i. Due to the limited budget, this study could not cover all plant species and focused on specific tree species within the study site.
ii. Heavy metal accumulation in trees is more evident during winter because of more dust pollution. So, this study was done only during the winter season. Fluctuations due to seasonal variations in metal accumulation was not observed.

CHAPTER 2: LITERATURE REVIEW

2.1. Roles of heavy metals in plants

Zinc (Zn) is essential to the physiology and metabolism of plants. Zinc's role in plant cytochrome synthesis, ribosomal fraction stabilization, and the activation of hydrogenase and carbonic anhydrase was emphasized by Tisdale *et al.,* (1984). Zn's role in protein synthesis, auxin control, pollen production, cellular membrane integrity, and carbohydrate metabolism was discovered by Marschner (1995). Additionally, he noticed that the zinc deficit causes smaller leaves, slowed development, chlorosis, and spikelet sterility, making plants more vulnerable to fungi and environmental challenges. Zn's function in controlling gene expression for environmental stress tolerance was also highlighted by Cakmak (2000), who connected Zn to plants capacity for adaptation.

Another crucial element with essential functions in plant physiology is copper (Cu). Cu has been identified as a structural component of regulatory proteins by Marschner (1995) and Raven *et al.,* (1999), who also highlighted Cu's role in critical activities such as electron transport during photosynthetic reactions, mitochondrial respiration, oxidative stress responses, cell wall metabolism, and hormone signaling. At high quantities, Cu can become poisonous due to its redox characteristics. According to Halliwell and Gutteridge (1984), the redox cycling of Cu2+ and Cu+ can lead to the generation of highly harmful hydroxyl radicals, which can harm proteins, lipids, DNA, and other biomolecules. Subsequently, it was discovered by Van Assche and Clijsters (1990) and Marschner (1995) that too much copper can damage plants and result in symptoms like chlorosis, necrosis, stunted development, leaf discoloration and inhibit root growth.

Continuing the examination of micronutrients and heavy metals in plants, lead (Pb) presents a significant challenge due to its toxic effects. Stefanov *et al.,* (1995) reported that lead exposure in plants results decline in photosynthetic rates. This decline is attributed to multiple factors: partial chloroplast ultrastructure damage, obstructed electron transport, inhibited activities of Calvin cycle enzymes, and CO2 deficiency due to stomatal closure. Building on earlier research on zinc and copper, which highlighted the importance of proper micronutrient balance, Islam *et al*., (2008) demonstrated that lead exposure severely limits seedling development and sprouting. They noted that even at low concentrations, lead inhibits the growth of both roots and aerial plant parts, emphasizing its potent phytotoxicity. Further expanding our understanding of lead's impact on plant physiology, Opeolu *et al.*, (2010) observed that high lead concentrations negatively affect flower production. These findings included reproductive impacts to the list of lead's detrimental effects on plants.

2.2. Heavy metal pollution in vegetation

According to Denneman and Robberse (1990), the target values for these elements in soil were 85 mg/kg for lead, 36 mg/kg for copper, and 50 mg/kg for zinc. Similarly, World Health Organization report (1996) established permissible values for these heavy metals in plants. The WHO guidelines set the permissible levels at 2 mg/kg for lead, 10 mg/kg for copper, and 0.60 mg/kg for zinc in plant parts.

Table 1: Permissible limit of heavy metals in soil dust and plant parts

Elements	Target value of soil*(mg/kg)	Permissible value of plant**(mg/kg)
Pb	85	2
Cu	36	10
Zn	50	0.60

Source: *****Denneman, C.A.J. and Robberse, J.G. (1990) and ******WHO (1996)

The use of plants as biomonitors has been extensively studied over the years to assess heavy metal contamination. Tomasevic *et al.*, (2004) evaluated horse chestnut and linden leaves as bioaccumulator in Belgrade city parks. They found the highest concentrations of heavy metals in horse chestnut leaves at Studentski Park site, indicating severe pollution levels. This early study laid the foundation for understanding how different plant species can be used to monitor environmental pollution. Further supporting these findings, Berlizov *et al.*, (2007) and Sawidis *et al.*, (2011) demonstrated that tree bark, due to its structural porosity, accumulates more heavy metals than leaves. Pine tree bark, in particular, was found to be more efficient bioindicator of urban pollution than plane tree bark. These studies reinforced the idea that certain parts of plants are more effective at indicating contamination levels.

Building on this foundation, Dogan *et al.*, (2010) highlighted the effectiveness of the Turkish red pine (*Pinus brutia*) as an indicator for detecting heavy metal pollution. Their research in Western Anatolia found high concentrations of metals like Pb, Cd, Cr, Fe, Ni, and Cu in the needles and bark of *P. brutia*, particularly in industrial areas. This study confirmed the superior efficiency of the bark over needles as a biosensor for heavy metal pollution, further emphasizing the role of tree bark in monitoring environmental health. In addition, Hoodaji *et al.*, (2012) argued that lichens and mosses are particularly effective, as they excel in accumulating and indicating elements without soil interference.

Further investigation by Pandey and Tripathi (2014) on *Shorea robusta* determining the impact of heavy metals like Cd, As, and Pb showed significant adverse effects on plant's morphological and biochemical parameters, with higher metal accumulation in roots compared to shoots. This research suggested the potential of *S. robusta* as a bioindicator for heavy metal pollution, adding another species to the list of effective phytomonitors. The impact of urbanization and industrialization on environmental pollution was evident in Kyrgyzstan, as shown by (Zeki *et al.*, 2015). Their study employed *Juniperus virginiana* to assess heavy metal concentrations and their effects on the plant's mineral nutrient status. The findings indicated that heavy metals significantly influenced the uptake of minerals like Mg, K, and Na, highlighting the contamination risk in the area.

Supporting these initial foundations, Safari *et al.,* (2018) surveyed the gas and petrochemical zone of Asaloyeh City, Iran, analyzing metal concentrations in various plant species. They found the highest concentrations of Ni, Pb, V, and Co in leaves and bark samples from industrial sites compared to urban and rural sites. This study emphasized the severe environmental impact of industrial activities on local vegetation, providing a stark reminder of the ongoing challenges in monitoring and mitigating heavy metal pollution in industrial regions.

2.3. Heavy metals accumulation in vegetation in Nepal

Focusing on metals such as Cr, Ni, Pb, Zn, Ca, Mn, Fe, Si, and Al, Pandey *et al.*, (2000), analyzed lichen samples from in and around Hetauda Industrial Area (HIA) in Makwanpur district, Nepal. They found that samples collected within the industrial area had significantly higher metal concentrations than those from outside. Notably, the lichen species *Pyxine meissnerina* accumulated higher levels of all analyzed metals when growing inside the industrial area, illustrating the impact of industrial activities on environmental metal contamination.

Sharma and Chettri, (2005) conducted a comprehensive study evaluating heavy metal levels in vegetables and soil from agricultural fields in Kathmandu Valley. Their findings revealed significant contamination: copper (65.5 mg/kg) and lead (146.75 mg/kg) were highest in Shankhamul; cadmium (2 mg/kg) in Nakhu; nickel (29.25 mg/kg) in Balkhu; cobalt (15.25 mg/kg) and manganese (675 mg/kg) in Balkhu; chromium (73.75 mg/kg) in Banasthali; zinc (162 mg/kg) and iron (75,636 mg/kg) in Khusibun. Spinach and cress leaf showed high accumulations of Cu and Zn; red radish had elevated Pb levels, broad-leaf mustard accumulated Ni, Cr, and Fe, while cress leaf had high Mn levels.

Continuing their research on heavy metal accumulation in various vegetables across Kathmandu Valley, Sharma and Chettri, (2008) investigated *Brassica juncea* var. *cuneifolia* (broad leaf mustard), *Lepidotum sativum* L. (cress leaf), *Spinacia oleracea* L. (spinach), *Raphanus sativus* L. (radish), *Brassica rapa* L. (turnip), *Daucus carota* L. (carrot), and *Solanum tuberosum* L. (potato). Their study indicated that concentration of Cu, Pb, Cd, Zn, and Fe in some agricultural fields exceeded regular soil values, suggesting widespread soil pollution. Particularly concerning was the accumulation of Pb in leafy and underground vegetables, excluding potato tubers, highlighting the risk of this toxic metal entering the food chain.

Recently, Parajuli *et al.*, (2020) highlighted the consequences of excessively high iron concentrations (around 30,000 mg/kg) in soil on plant health. Their research emphasized that while iron is essential for plants, such high levels can deplete other essential elements, negatively impacting plant growth. They identified certain plant species exhibiting bronze coloration of shoots, indicative of iron accumulation, including *Cyanotis vaga*, *Hypericum japonicum*, *Begonia picta*, *Gonostegia hirta,* and *Isodon coetsa.* Additionally, they noted high bioaccumulation factors in *Anaphalis busua* and *Elsholtzia blanda,* proposing these plants as indicators for geo-botanical exploration of iron ore deposits.

2.4. Heavy metals content in soil and their uptake by plants

A study was conducted to determine the impact of heavy metals {cadmium (Cd), arsenic (As) and lead (Pb)} on the morphological and biochemical parameters of *Shorea robusta* and to investigate the uptake capacity of this plant for individual heavy metals. The study showed that all three heavy metals had a significant adverse impact on most of the plant parameters of *S. robusta* at all the given concentrations of 1, 5 and 10 mg/l. The accumulation of heavy metals was found to be higher in the root than the shoot of *S. robusta*, with maximum accumulation being 0.053 mg/gm in root for Cd, 0.17 mg/gm of

Zn in leaf and 0.21mg/gm of Pb in soil. Concentration-dependent changes were observed in most of the morphological and biochemical parameters, which may thus serve to determine suitable bio-indicators of heavy metal pollution (Pandey and Tripathi, 2014).

Furthermore, Jose (2021), investigated the accumulation of heavy metals like As, Pb, and Cd by plants belonging to the family Amaranthaceae and soil occurring around the polluted area of Thrissur District. Plants taken in this study were *Hygrophila angustifolia* and *Alternanthera tenella*. The concentration of metals in the root, shoot and leaf varied across the plant species and parts. The observed metallic levels in plants were not within the FAO/WHO limit guideline values for heavy metal levels in food. However, heavy metal content in soil was within the permissible limits the World Health Organization (WHO), 1996 recommended.

2.5. Metal Accumulation Index (MAI) of leaves and bark samples

The study by (Zhai *et al.*, 2016) analyzed the concentration and chemical fractionation of HMs in soils and plants in China. The research was performed to investigate pollution of traffic-related heavy metals (HMs—Zn, Pb, Cu, Cr, and Cd) in roadside soils and their uptake by wild plants growing along highways in Hunan Province, China. The results of HMs were in the order of Cd > Pb > Zn > Cu > Cr. The accumulation capacity for most HMs plant tissues was bark > leaf. The highest MAI value (5.99) in *Cinnamomum camphora* indicates the potential for bio-monitoring and a good choice for planting along highways where there is contamination with HMs. During the study by Karmakar and Padhy (2019), MAI values ranged from 3.06 to 7.68 and 9.48 to 9.82; 2.65 to 4.96 and 4.29 to 6.08; 3.35 to 8.14 and 5.04 to 11.65; 3.59 to 4.64 and 5.21 to 7.98 at Ballavpur Wildlife Sanctuary (BWLS) of West Bengal, India, and Barjora forest for *Shorea robusta*, *Acacia auriculiformis*, *Eucalyptus globulus* and *Azadirachta indica* respectively.

2.6. Previous study on dominant Vegetation Type around study area

The study area around Hongshi-Shivam Cement Factory (Sardi) comprised Tropical Sal and Mixed Broad-Leaved Forest. The area's vegetation was mainly dominated by Sal (*Shorea robusta*), a part of the Binayi Community Forest zone. Some other vegetation types included population of Saaj (*Terminalia paniculata*), Sisoo (*Dalbergia sissoo*), Asna (*Terminalia elliptica* Willd.), Barro (*Terminalia bellirica*), Kyamuno (*Syzygium nervosum*), and Khayar (*Senegalia catechu*), intermingled with the Sal Forest.

Additionally, a feeble population of the shrub Ader (*Ricinus communis*) found in the eastern part of the study area. In contrast, subshrubs Rudilo (*Pogostemon benghalensis*) and Thakar (*Senna tora*) occupied the western parts (Tamang, 2015).

Although much work has been done on monitoring heavy metal accumulation in different lower plants and vegetables in Kathmandu (Chettri *et al.*, 1997; Shakya *et al.*, 2004; Sharma and Chettri, 2005), very little research on heavy metal accumulation has been done conducted outside the Kathmandu valley. Besides this, in the context of Nepal, a few research studies have been conducted on higher plants using leaves or bark (Parajuli and Chettri, 2020; Hamal and Chettri, 2022). From these studies, it is evident that the biomonitoring of heavy metals around cement factories in Nepal is very scarce. Hence, the concentration of various heavy metals in different higher plants around the Hongshi Shivam Cement factory has been planned and studied using leaves samples, bark samples, and soil dust.

CHAPTER 3: MATERIALS AND METHODS

3.1. Study area

The study was conducted around Hongshi Shivam Cement Pvt. Ltd, which lies at Binayi Tribeni Rural Municipality, Sardi- Dumkibas, East Nawalparasi. It lies in Gandaki Province, Western Nepal. It is located to the North of Mahendra Highway at 27°36′15.62″N Latitude and 83°56′57.83″E Longitude, at an altitude of 164 m above sea level.

The study area was divided into three sites: a) site at about 200 m from the factory, b) site at about 1000 m away from the cement factory, and c) a control site about 4-5 km away from the factory. The site at about 200 m distance was located towards the east-south direction from the factory, and was an area with high vehicular movement and low residential density. The other site was located around 1000 m away from the factory in the same direction. It was little farther from the roads and had more residential density than that at 200 m site. The control site (Deurali) was situated about 4-5 km away from the factory in the west direction. It had low vehicular movement, low residential density, and less pollution.

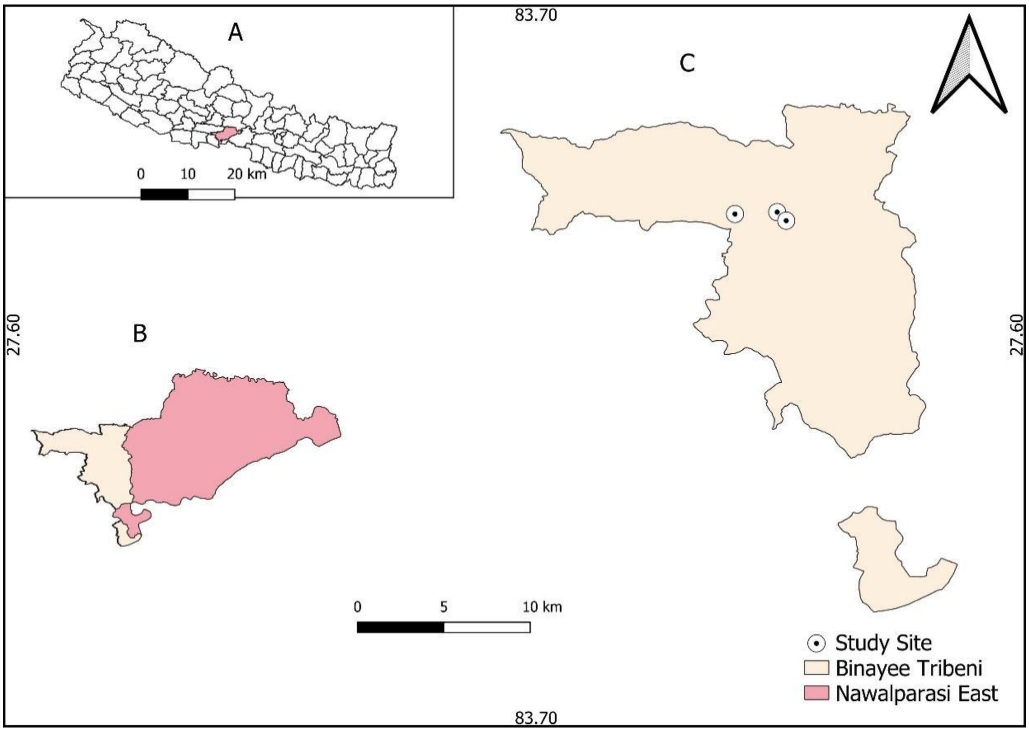

Figure 1: Map of study area(A), Map of Nawalparasi East District (B), Map of Binayi Tribeni Rural Municipality showing study Plots (C). Source: QGIS Version 2.3

Figure 2: Google map with plot locations for each site (Source: Google Earth Pro)

3.2. Studied tree species

Tree species growing within the periphery of the study area, i.e., Hongshi-Shivam Cement Factory, Sardi, were chosen randomly for the study (Table 2). The basis for selecting those tree species was according to their availability as they are common tree species found in the Terai region, as given by the previous literature by many researchers. The plant material (bark and leaves) and soil dust were collected from 200 m and 1000 m away from the factory, as well as from the control site, which had fewer disturbances. The tree species were identified according to Howland (1984) and websites (Plants of the World Online, 2023) and International Plant Names Index (2023) by comparing the local and Latin names and families of the studied plant species.

Table 2. Name of tree species taken for leaves and bark samples collection for heavy metal analysis

Tree Species (Latin Name)	Family	Local Names
Ficus religiosa L.	Moraceae	Pipal
Ficus semicordata Buch.-Ham. ex Sm.	Moraceae	Khannim
Mallotus philippensis (Lam.) Müll.Arg.	Euphorbiaceae	Sindhure
Mangifera indica L.	Anacardiaceae	Aap
Shorea robusta C.F.Gaertn.	Dipterocarpaceae	Sal

3.3. Climate

The study area lies in the terai region. The terrain of the study area is flat, with an elevation of 150 masl. The climate is tropical type. Metrological data were collected from the Department of Hydrology and Metrology, Kathmandu, Nepal, for the Dumkauli Station, Nawalparasi. According to the climatic data from 2012-2022 of Dumkauli Station, maximum temperatures are highest from June to September, peaking around 35℃ in July or August. Minimum temperatures are lowest from December to February, with an average lowering of around 5°C. Precipitation was highest from June to September, reaching a peak of around 600 mm in July or August. Similarly, precipitation was lowest from November to February, with average levels below 100 mm (DHM, 2022).

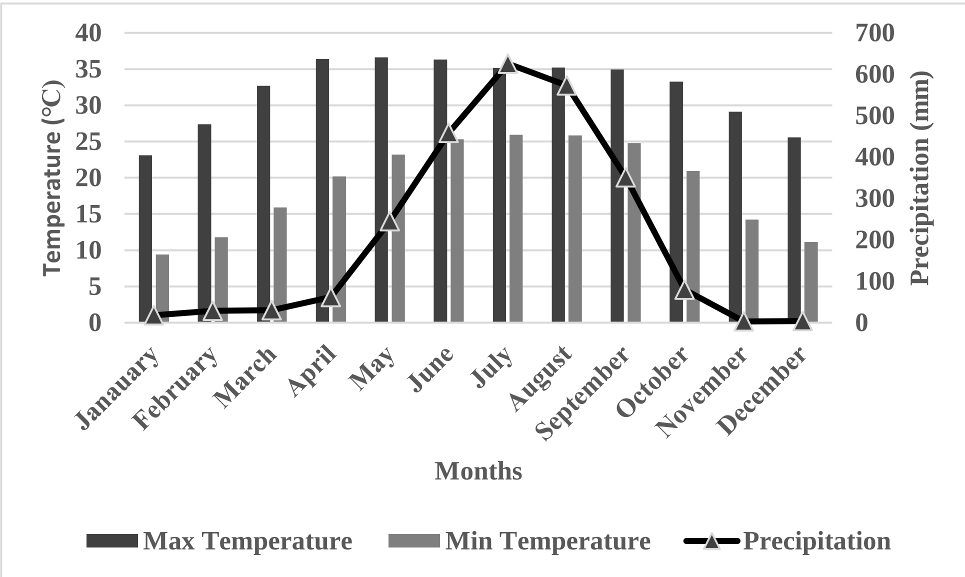

Figure 3: Ten years (2012-2022) average monthly maximum and minimum temperature and precipitation data of Dumkauli station, Nawalparasi East. (source: Department of Hydrology and Metrology, Government of Nepal, Kathmandu, 2022).

3.4. Study design

The study intended to monitor heavy metal accumulation in leaves, tree bark, and soil around Hongshi Shivam Cement factory at different distances and directions. The plant material and soil dust were collected from three distance ranges: 200 m, 1000 m and 4-5 km away from the factory (Figure 4). The samples from the clean area, assuming less pollution, exceeding 4-5 km from the factory, were considered as control. The samples were collected from two directions only, east and west, depending on the wind direction prevailing during winter months.

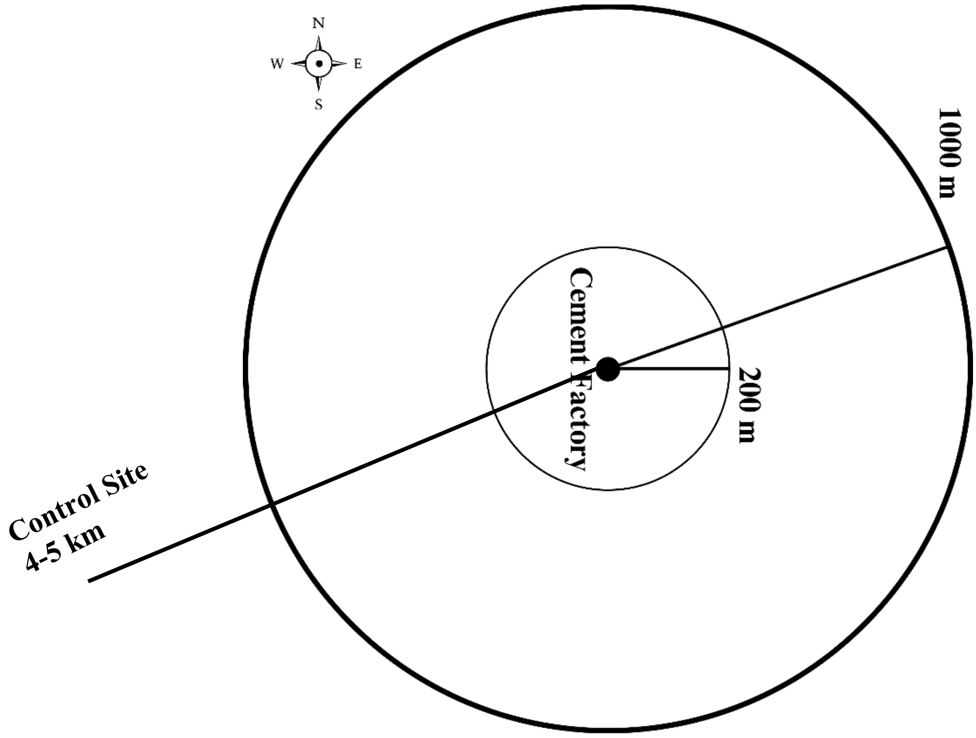

Figure 4: Diagrammatic representation of study area showing different distances from the factory.

3.5. Sample collection

The fully expanded mature leaves and bark from respective trees were collected in paper envelopes. To learn the atmospheric fall out of heavy metals, leaf and bark samples were not washed with water, but all the adhered dust on it was removed and cleaned with a fine brush. Leaves and bark samples of common 5 tree species and soil samples were collected during winter, i.e. January- February. The leaf and bark samples were collected from the trees having more or less similar DBHs (as far as possible) except *Mallotus philippensis* having less DBH than other four trees to avoid differences in metal accumulation in the bark due to age differences. The trees preferred were about the same age. Leaf samples were collected from all directions of the trees, especially the peripheral ones above 2 m, to avoid surface soil contamination. Similarly, bark samples were collected, using a stainless steel knife, from all directions of the trunk above 2 m from the ground. Leaf and bark samples were collected from 3 different individuals of each tree species at each site. For soil dust sample collection, 3 quadrats of 5x5 m^2 were laid at each site. Soil dust was collected using the brush from a 1x1 m^2 sub-quadrat surface of 4 corners and center and then homogenized to obtain a representative sample of each quadrat.

3.6. Sample preparation

The collected samples (leaves and bark) were air-dried for 2-3 days and then were brought to the laboratory. In the laboratory, the samples were oven-dried at a temperature of 70° C-100 °C for 72 hours till constant dry weight (Sawidis *et al.*, 1995). The dried bark and leaves were homogenized using a mortar and pestle to prepare representative samples. Later, they were sieved with 0.25 mm mesh to obtain uniform sample particles. Similarly, the soil dust samples collected from each site were oven-dried and homogenized following the same procedure.

3.7. Sample analysis

For sample analysis, the wet digestion method was adopted. One gram of sample (same for leaf, bark or soil) was wet digested in 5 ml conc. HNO3 was left overnight at room temperature in a conical flask (Chettri *et al., 1997*). On the next day, 5ml conc. HNO3 was added again, boiled slowly for 2 hours at 50◦C, then heated at 200◦C for about 4 hours until all the brown vapor stopped and then allowed to cool. The cooled extract was then filtered through ashless filter paper Whatman no. 40, and diluted with double de-ionized water to obtain 25 ml. The filtrates were then analyzed for Pb, Cu, and Zn using a Perkin Elmer Chemito-201 Atomic Absorption Spectrometer (Welz, 1985) with wavelengths of 283.3 nm, 324.7 nm, and 213.8 nm, respectively, and the values were recorded. Samples were screened in triplicate for every case. The metal analysis following this procedure was conducted at Enviro Chemical Laboratories, Old Baneshwor, Kathmandu.

3.8. Metal accumulation index (MAI)

Based on concentrations of heavy metals studied in each sample, MAI value of each sample was calculated using (Liu *et al.*, 2007).

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

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

3.9. Data Analysis

The quantitative data obtained from the experiments were initially tabulated in Microsoft Excel. The Statistical Package for Social Science (IBM SPSS version 20) was used for the statistical analysis. The data were checked for normality by the Shapiro–Wilk test. A parametric test (one–way ANOVA) was performed for normal data.

To determine whether there was a significant difference in the metal accumulation among plant samples and soil dust collected from different sites, one-way analysis of variance (ANOVA) was conducted, followed by Duncan's Multiple Range Test at $p=0.05$. Values were expressed in means ± S.D. (Bryman and Cramer, 2012). Correlation between the metal concentrations in soil dust and plant parts was analyzed by calculating Pearson's correlation coefficient.

CHAPTER 4: RESULTS

Heavy metals concentration in barks and leaves of trees *Shorea robusta, Mangifera indica, Ficus semicordata*, *Ficus religiosa* and *Mallotus philippensis*, and in soil dust at different sites were analyzed and their results are presented below:

4.1. Heavy metals in soil dust

The highest concentrations of lead, copper, and zinc was recorded at the site around 200 m from the factory. The mean concentrations of lead, copper and zinc in soil dust were 44.83, 40.33 and 65.23 mg/kg respectively at a distance about 200 m around the factory. The amounts of lead, copper and zinc detected in the soil dust decreased with increasing distance from the factory. The concentrations of lead and zinc was slightly higher than the control at sites which were located about 1000 m away. Among the heavy metals, zinc concentrations in soil remained relatively higher across the different distances (Figure 5).

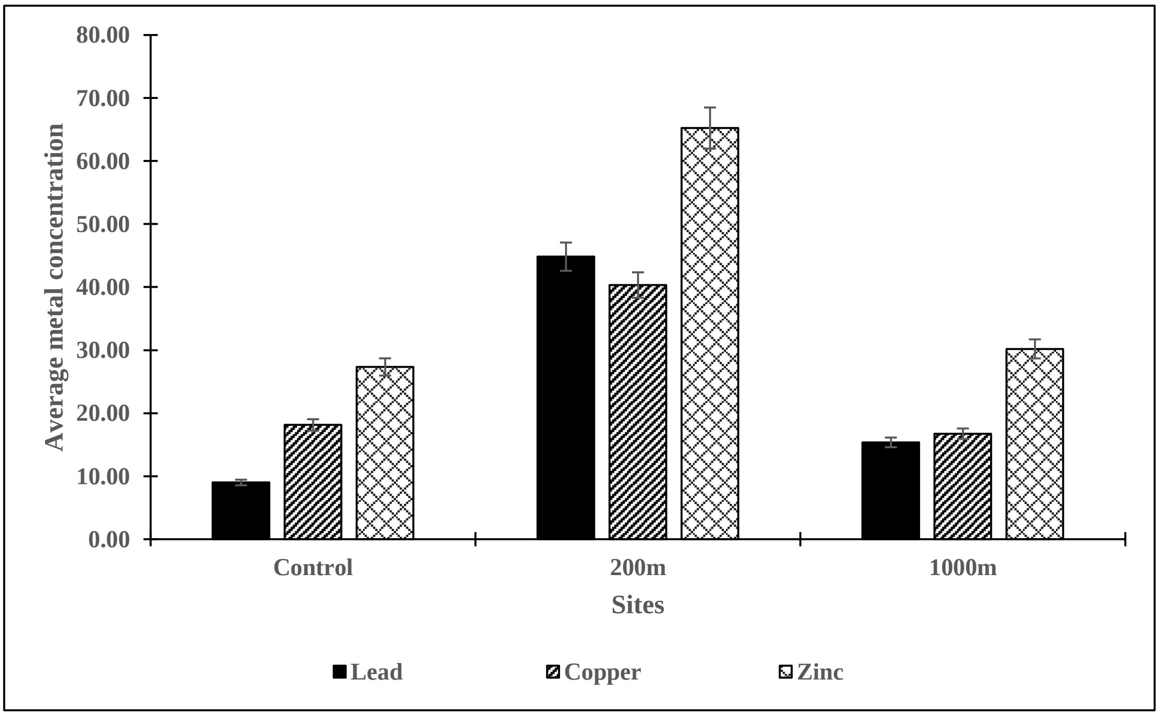

Figure 5: Concentrations of heavy metals (mg/kg) in soil dust at three different sites.

Concentration of heavy metals (mg/kg) in soil dust within a given site

Lead concentrations showed significant variation across all sites. The control site exhibited the lowest concentration (9 mg/kg), while at site 200 m around the factory demonstrated a marked increase to 44.83 mg/kg, indicating substantial lead pollution. At the site about 1000 m distance, Pb concentration was 15.37 mg/kg, significantly higher than that of control but lower than that at 200 m site (Table 3).

Copper concentrations also varied significantly, with the highest concentration observed at 200 m site (40.33 mg/kg). Interestingly, its concentration at the control site (18.13 mg/kg) and 1000 m site (16.73 mg/kg) did not differ significantly, suggesting a localized impact of copper pollution at vicinity of the factory only.

Zinc concentrations followed a similar pattern to copper, with the 200 m site showing significantly elevated levels (65.23 mg/kg) compared to both the control (27.33 mg/kg) and 1000 m site (30.20 mg/kg). The control and 1000 m sites did not differ significantly in zinc concentrations (Table 3).

Table 3: Concentration of heavy metals (mg/kg) in soil dust within a site. Values are (Mean ± SD) and different letters indicate significant variations (n=3). Same small letter after mean ± SD obtained from One-way ANOVA followed by Duncan's Multiple Range Test, for different tree species within a site, do not differ significantly at *p=0.05*.

Sites	Lead (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)
Control	9±1.50 a	18.13±2.60 a	27.33±1.96 a
200 m	44.83±2.25 c	40.33±1.92 b	65.23±2.06 b
1000 m	15.37±1.98 b	16.73±2.11 a	30.20±2.12 a

4.2. Heavy metals in leaves

The mean concentration of lead, copper, and zinc in the leaves of selected tree species (*Shorea robusta*, *Mangifera indica*, *Ficus semicordata*, *Ficus religiosa* and *Mallotus phillipensis*) growing at three sites (control, 200 m and 1000 m) was determined (Figure 6).

a. Lead: The concentration of lead in the leaves was relatively low at the control site (0.85

mg/kg). However, there was a significant increase in lead concentration in 200 m (2.11 mg/kg), indicating a higher exposure to lead pollution closer to the factory. The concentration was almost 2.5 times higher than the control site. However, at 1000 m site the lead concentration decreased (1.42 mg/kg) as compared to the 200 m site but was still higher than the control site.

b. Copper: The concentration of copper in the leaves was moderate at the control site (2.70 mg/kg). At 200 m site copper concentration was 5.99 mg/kg, which was a noticeable increase, indicating significant exposure of leaf to copper pollution at this distance. The concentration was more than double at 200 m site than that at control. At 1000 m site, the copper concentration decreased to 4.59 mg/kg than that at 200 m site, but remained higher than the control site. This suggests that copper pollution persists at a considerable distance from the factory.

c. Zinc: Among three metals, Zn concentration was highest at the control site (12.93 mg/kg). However, at 200 m site Zn concentration reached 29.15 mg/kg, which was significantly more than double that at the control site. This indicated substantial zinc pollution at 200 meters. At 1000 m, the zinc concentration decreased to 24.28 mg/kg from the 200 m site but remained higher than the control site. This indicated that zinc pollution was still impactful at 1000 m.

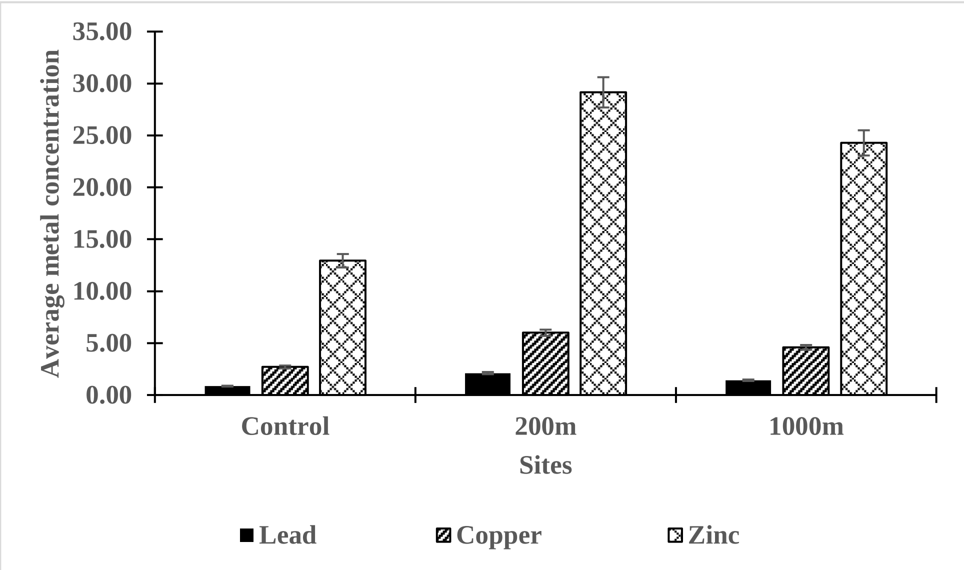

Figure 6: Average metal concentrations (mg/kg) in leaves at different sites from the factory.

Concentration of heavy metals(mg/kg) in leaves of different tree species within a site (Mean± SD)

Concentration of heavy metals (lead, copper, and zinc) in leaves of different plant species at three different sites (Control, 200 m from the factory, and 1000 m from the factory) was determined (Table 4).

At control site, lead concentrations ranged from 0.49 mg/kg in *Shorea robusta* to 1.26 mg/kg in *Mangifera indica*, with significant differences observed between these two species. Copper concentration showed no significant variation among species, ranging from 2.51 mg/kg to 3.20 mg/kg. Zinc concentration exhibited some significant differences among the species studied, with *Mallotus philippensis* showing the highest Zn concentration (15.05 mg/kg) while *Ficus semicordata* had the least (10.27 mg/kg).

At 200 m site, a general increase in metal concentrations was observed. Lead content in leaf ranged from 1.77 mg/kg to 2.54 mg/kg, with *Mallotus philippensis* significantly higher than *Shorea robusta* and *Ficus semicordata*. Copper concentrations showed marked variation, with significant differences among species, from 2.46 mg/kg in *Ficus semicordata* to 7.57 mg/kg in *Ficus religiosa*. Zinc content increased substantially, ranging from 18.23 mg/kg in *Mangifera indica* to 45.05 mg/kg in *Mallotus philippensis*, with all species showing significant differences.

At 1000 m site, metal concentrations generally decreased compared to 200 m site but remained elevated relative to the control. Significant differences were noted in lead concentrations from 1.09 mg/kg to 1.71 mg/kg. Copper levels varied significantly among species, from 2.33 mg/kg in *Shorea robusta* to 7.51 mg/kg in *Mangifera indica*. Zinc concentrations ranged from 17.0 mg/kg to 37.20 mg/kg, with *Mallotus philippensis* showing significantly higher accumulation than others.

Table 4: Concentration of heavy metals(mg/kg) in leaves of different tree species within a site. Values are (Mean± SD) and different letters indicate significant variations (n=3). Same small letter after mean ± SD obtained from One-way ANOVA followed by Duncan's Multiple Range Test, for different tree species within a site, do not differ significantly at *p=0.05.*

Sites	Name of species	Lead (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)
Control	*Shorea robusta*	0.49±0 .14 a	2.51±0.13 a	14.33±2.32 bc
	Mangifera indica	1.26±0.47 b	2.77±0.74 a	12.0±0.80 ab
	Ficus semicordata	0.53 ±0.10 a	2.51±0.15 a	10.27±1.33 a
	Ficus religiosa	1.06±0.12 ab	2.53±0.22 a	13.0±0.80 bc
	Mallotus philippensis	0.93±0.42 ab	3.20±0.44 a	15.05±0.47 c
200 m	*Shorea robusta*	1.83±0.47 a	4.98±0.59 b	22.49±1.11 b
	Mangifera indica	1.97±0.37 ab	7.47±0.15 c	18.23±0.25 a
	Ficus semicordata	1.77±0.41 a	2.46±0.14 a	34.73±1.22 d
	Ficus religiosa	2.44±0.20 ab	7.57±0.60 c	25.23±1.52 c
	Mallotus philippensis	2.54±0.14 b	7.50±0.70 c	45.05±1.62 e
1000 m	*Shorea robusta*	1.47±0.25 b	2.33±0.11 a	17.0±1.40 a
	Mangifera indica	1.41±0.18 ab	7.51±0.14 c	25.50±1.11 b
	Ficus semicordata	1.09±0.18 a	2.45±0.14 a	23.73±1.12 b
	Ficus religiosa	1.71±0.12 b	5.07±0.70 b	17.97±0.75 a
	Mallotus philippensis	1.43±0.20 ab	5.57±0.80 b	37.20±1.51 c

4.3. Heavy metals in bark

a. Lead: The mean lead concentration at the control site was 0.81mg/kg. This increased to 2.31 mg/kg at 200 m, indicating higher exposure to lead closer to the factory. At 1000 m, the concentration decreases to 1.66 mg/kg, suggesting a reduced lead deposition with increased distance from the factory (Figure 7).

b. Copper: As mentioned in the graph below, the mean copper concentration at the control site was 7.01 mg/kg. This concentration nearly doubled to 13.35 mg/kg at 200 m and further increased to 16.06 mg/kg at 1000 m. This trend indicated that copper concentration remained high even farther from the factory.

c. Zinc: The mean zinc concentration at the control site was 22.34 mg/kg. At 200 m, the concentration significantly increased to 55.91, demonstrating a substantial impact closer to the factory. At 1000 m, the concentration decreased to 33.39 mg/kg but remained higher than the control site.

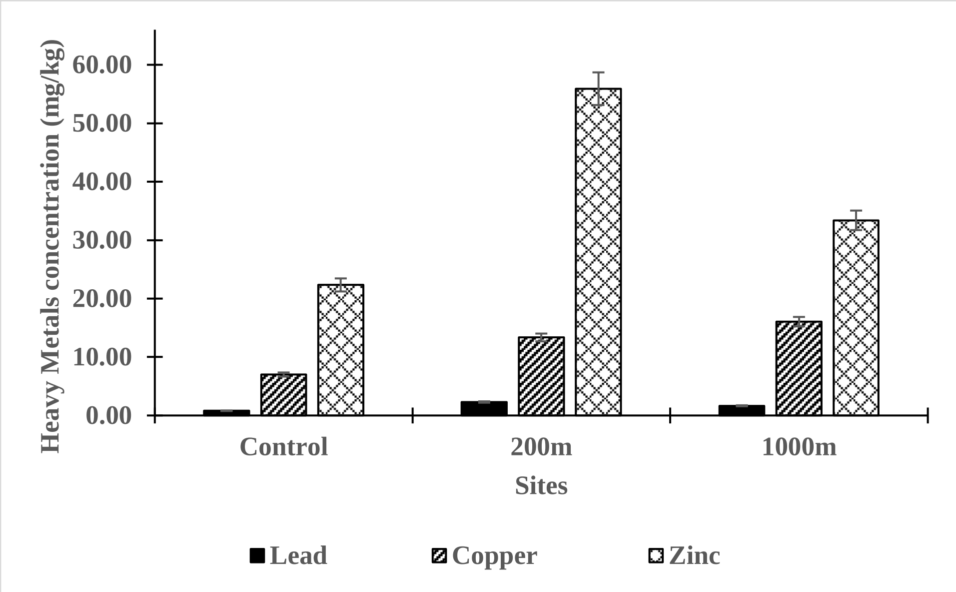

Figure 7: Average metal concentrations (mg/kg) of bark samples at different sites from the factory.

Concentration of heavy metals (mg/kg) in barks of different species within a site (Mean± SD)

At the control site, lead concentration ranged from 0.5 mg/kg in *Shorea robusta* to 1.48 mg/kg in *Ficus religiosa*, with significant interspecific variations. Copper concentration demonstrated marked differences, ranging from 2.53 mg/kg in *Mangifera indica* to 12.4 mg/kg in *Mallotus philippensis*. Zinc concentration also varied significantly, with the lowest in *Shorea robusta* (14.33 mg/kg) and the highest in *Mallotus philippensis* (35.33 mg/kg) (Table 5).

At 200 m site, a notable increase in metal concentrations was observed among all species. Lead concentration ranged from 1.20 mg/kg in *Shorea robusta* to 3.14 mg/kg in *Ficus religiosa*, showing significant interspecific differences. Copper concentrations varied substantially, from 6.47 mg/kg in *Ficus semicordata* to 25.10 mg/kg in *Mallotus philippensis*. Zinc accumulation increased from 17.13 mg/kg in *Ficus semicordata* to an exceptionally high 117.30 mg/kg in *Mallotus philippensis*, with all species exhibiting significant differences demonstrated by Table 5.

At 1000 m site, metal concentration generally decreased compared to 200 m site but remained elevated relative to the control. With significant interspecific variations, lead concentration varied from 1.01 mg/kg in *Shorea robusta* to 2.67 mg/kg in *Ficus religiosa*. Copper content ranged from 4.93 mg/kg in *Mangifera indica* to a notably high 44.83 mg/kg in *Mallotus philippensis*. Zinc concentrations varied significantly, ranging from 16.60 mg/kg in *Ficus semicordata* to 72.33 mg/kg in *Mallotus philippensis* (Table 5).

Table 5: Concentration of heavy metals (mg/kg) in bark of different tree species within a site. Values are (Mean± SD) and different letters indicate significant variations (n=3). Same small letter after Mean ± SD obtained from One-way ANOVA followed by Duncan's Multiple Range Test, for different tree species within a site, do not differ significantly at *p=0.05*.

Sites	**Name of species**	**Lead (mg/kg)**	**Copper (mg/kg)**	**Zinc (mg/kg)**
Control	***Shorea robusta***	0.5±0.08 a	10.23±0.87 c	14.33±0.96 a
	Mangifera indica	0.5±0.09 a	2.53±0.35 a	20.0±1.40 c
	Ficus semicordata	1.06±0.12 b	4.87±0.42 b	25.03±1.12 d
	Ficus religiosa	1.48±0.28 c	4.97±0.75 b	16.98±2.02 b
	Mallotus philippensis	0.51±0.10 a	12.43±0.80 d	35.33±1.06 e
200 m	***Shorea robusta***	1.20±0.36 a	12.70±0.95 c	27.53±0.85 b
	Mangifera indica	2.19±0.35 b	9.83±0.96 b	67.73±1.20 d
	Ficus semicordata	2.51±0.12 b	6.47±0.18 a	17.13±1.40 a
	Ficus religiosa	3.14±0.41 c	12.63±1.11 c	49.87±1.40 c
	Mallotus philippensis	2.49±0.11 b	25.10±0.66 d	117.30±2.61 e
1000 m	***Shorea robusta***	1.01±0.09 a	14.73±1.13 d	16.93±0.70 a
	Mangifera indica	1.04±0.08 a	4.93±0.70 a	30.60±1.15 b
	Ficus semicordata	1.54±0.15 b	7.50±0.13 b	16.60±3.12 a
	Ficus religiosa	2.67±0.51 d	8.30±0.62 b	30.47±1.15 b
	Mallotus philippensis	2.05±0.22 c	44.83±1.42 d	72.33±1.92 c

Concentration of heavy metals (mg/kg) in leaves and barks of different tree species between selected sites (Mean± SD)

Shorea robusta: The concentration in leaves significantly increased at 200 m (9.76 mg/kg) compared to the control (5.78 mg/kg), then decreased at 1000 m (6.93 mg/kg). In bark, the metal concentration at 200 m (13.81 mg/kg), with control (8.35 mg/kg) and around 1000 m (10.89 mg/kg) (Table 6).

Mangifera indica: Leaves showed a significant increase from control (5.34 mg/kg) to 1000 m (11.48 mg/kg), with a peak at 200 m (9.22 mg/kg). In bark, concentrations increased sharply at 200 m (26.59 mg/kg), much higher than control (7.68 mg/kg) and 1000 m (12.19

mg/kg) (Table 6).

Ficus semicordata: Leaves had a substantial rise at 200 m (12.99 mg/kg) compared to control (4.43 mg/kg), with a decrease at 1000 m (9.09 mg/kg). In bark, there is no significant differences in heavy metal accumulation between sites ($p > 0.05$) demonstrated by Table 6.

Ficus religiosa: Leaves showed significant differences, increasing from control (5.53 mg/kg) to 200 m (11.74 mg/kg) and 1000 m (8.25 mg/kg). Bark concentrations increased sharply at 200 m (21.88 mg/kg) from control (7.81 mg/kg), with a slight decrease at 1000 m (13.81 mg/kg) (Table 6).

Mallotus philippensis: Leaves demonstrated significant differences including rise at 200 m (18.36 mg/kg), from control (6.39 mg/kg) and decrease at 1000 m (14.73 mg/kg). Bark showed the highest increase at 200 m (48.30 mg/kg) compared to control (16.09 mg/kg) and 1000 m (39.74 mg/kg). Across species, 200 m generally shows the highest concentrations, particularly in bark of *Mangifera indica* and *Mallotus philippensis* (Table 6).

Table 6: Concentration of heavy metals (mg/kg) in leaves and bark of different tree species between selected sites. Values are (Mean± SD) and different letters indicate significant variations (n=3). The same small letter after Mean ± SD obtained from One-way ANOVA followed by Duncan's Multiple Range Test for different tree species between sites do not differ significantly at *p=0.05*.

Species	**Sites**	**Leaves (mg/kg)**	**Bark (mg/kg)**
Shorea robusta	**Control**	5.78±0.86 a	8.35±0.63 a
	200 m	9.76±0.72 b	13.81±0.69 c
	1000 m	6.93±0.59 a	10.89±0.64 b
Mangifera indica	**Control**	5.34±0.65 a	7.68±0.61 a
	200 m	9.22±0.26 b	26.59±0.82 c
	1000 m	11.48±0.48 c	12.19±0.64 b
Ficus semicordata	**Control**	4.43±0.52 a	10.32±0.54 a
	200 m	12.99±0.59 c	8.70±0.56 a
	1000 m	9.09±0.46 b	8.55±1.13 a
Ficus religiosa	**Control**	5.53±0.38 a	7.81±1.02 a
	200 m	11.74±0.77 c	21.88±0.97 c
	1000 m	8.25±0.53 b	13.81±0.76 b
Mallotus philippensis	**Control**	6.39±0.44 a	16.09±0.65 a
	200 m	18.36±0.81 c	48.30±1.12 c
	1000 m	14.73±0.83 b	39.74±1.14 b

4.4. Heavy metals concentration on leaves, bark and soil dust

At all sites average heavy metal concentrations in soil was higher than that in bark and leaves. At control site, soil exhibited the highest heavy metal concentration (18.16 mg/kg), followed by bark (10.05 mg/kg), and then leaves (5.50 mg/kg). At 200 m site; a marked increase in heavy metal concentrations was observed across all sample types. Soil showed the most substantial increase, reaching 50.13 mg/kg, while bark and leaves increased to 23.86 and 12.42 mg/kg respectively. At 1000 m site, heavy metal concentrations decreased compared to the 200 m but remained elevated relative to the control site. Soil concentrations dropped considerably to 20.77 mg/kg, while bark and leaves showed moderate decrease in average heavy metal concentrations to 17.04 and 10.10 mg/kg respectively. This trend indicated a gradual reduction in heavy metal contamination as distance from the factory increased.

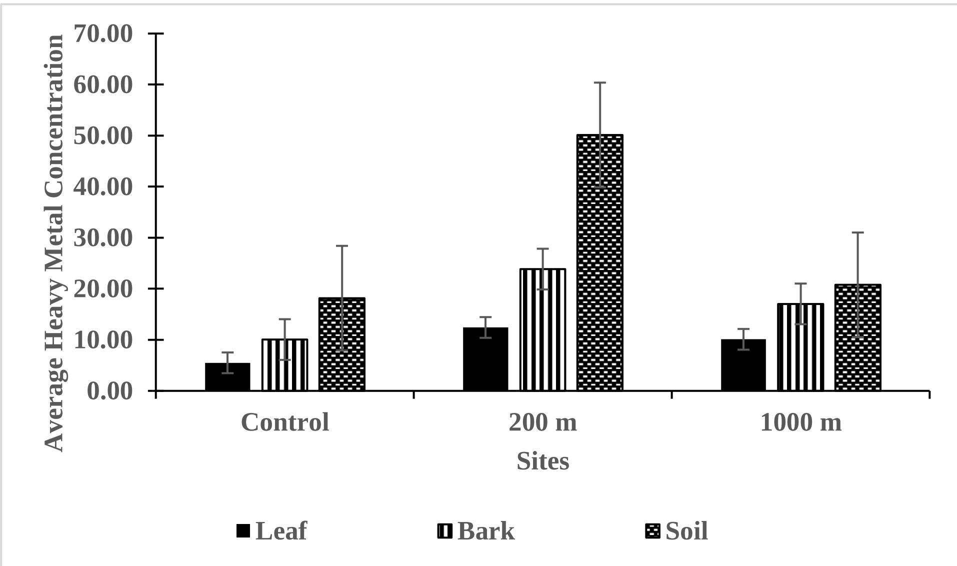

Figure 8: Average metal concentrations (mg/kg) in leaves, bark and soil samples at different sites.

4.5. Metal Accumulation Index (MAI)

4.5.1. Metal Accumulation Index (MAI) of leaves samples for different tree species

For *Shorea robusta*, MAI values were 9.75 in the control site, increasing to 10.86 at 200 m and further to 12.83 at 1000 m. *Mangifera indica* displayed a MAI value of 7.15 in the control site, which increased to 42.22 at 200 m, and decreased to 28.04 at 1000 m. In the case of *Ficus semicordata*, MAI values were 9.85 at the control site, increasing to 16.60 at 200 m and then slightly decreasing to 15.04 at 1000 m. *Ficus religiosa* had an MAI value of 12.13 at the control site, which increased to 13.86 at 200 m and further to 15.12 at 1000 m. Lastly, *Mallotus philippensis* showed an MAI value of 13.83 in the control site, increasing to 18.70 at 200 m, but then decreasing to 12.88 at 1000 m. MAI values at 200 m site suggested a peak in metal accumulation near the factory, followed by a reduction to a level slightly below the control site at a greater distance.

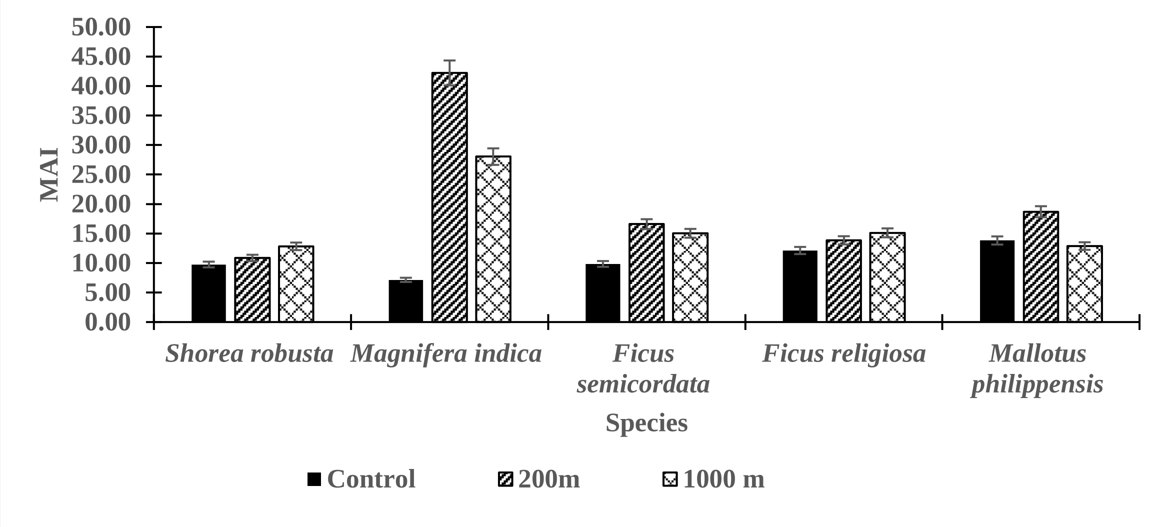

Figure 9: MAI of leaves samples at different sites from the factory.

4.5.2. Metal Accumulation Index (MAI) of bark samples for different tree species

For *Shorea robusta*, the MAI values observed were 10.96 in the control site, increasing significantly to 16.40 at 200 m and maintaining a slightly lower value of 16.10 at 1000 m. *Mangifera indica* exhibited an MAI value of 9.11 in the control site. At 200 m, the MAI value rose markedly to 24.28, but then decreased to 15.52 at 1000 m. Despite the reduction, the value at 1000 m remained higher than at the control site, suggesting residual effects of the factory's emissions at this distance. In the case of *Ficus semicordata,* MAI values were 14.22 in the control site, increasing to 23 at 200 m and further to 24.44 at 1000 m. *Ficus religiosa* had an MAI value of 6.77 in the control site, which increased substantially to 18.22 at 200 m and remained elevated at 14.99 at 1000 m. *Mallotus philippensis* showed the lowest MAI value at control site (18.06). At 200 m, the MAI value was almost doubled to 35.60 and similarly, remained significantly high at 26.11 around 1000 m site.

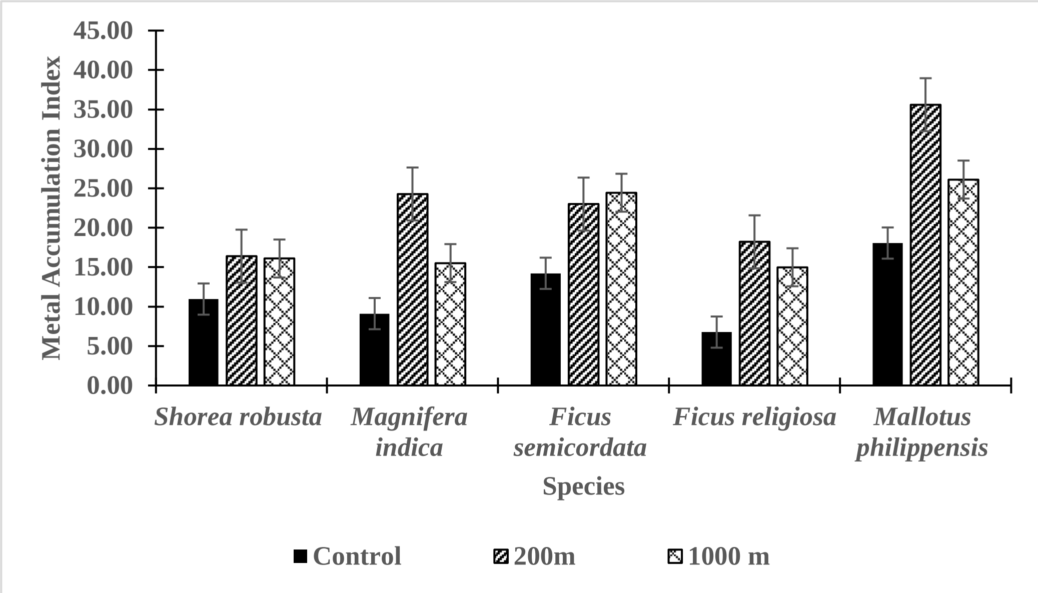

Figure 10: MAI of bark samples at different sites from the factory.

4.5. Correlation between soil dust and plant samples

4.5.1. Correlation between soil dust and leaves

a. Correlation of lead concentration between underlying soil dust and leaves of tree species:

All five species demonstrated strong positive correlations, with r values ranging from 0.77 to 0.96. Notably, *Mallotus philippensis* exhibited the strongest correlation (r = 0.96), followed closely by *Ficus religiosa* (r = 0.94). All correlations were statistically significant, with *Shorea robusta* and *Mangifera indica* showing significance at $p<0.05$, while the remaining three species display higher significance at $p<0.01$ demonstrated by Table 6.

b. Correlation of copper concentration between underlying soil dust and leaves of tree species:

The results showed more variability. *Shorea robusta* demonstrated an exceptionally strong correlation (r = 0.98, p<0.01), while *Ficus religiosa* and *Mallotus philippensis* also showed strong, significant correlations (r = 0.84 and r = 0.80 respectively, both at $p<0.01$). Conversely, *Mangifera indica* and *Ficus semicordata* exhibited weak, non-significant correlations (r = 0.45 and r = 0.08 respectively) (Table 6).

c. Correlation of zinc concentration between underlying soil dust and leaves of tree species:

Strong significant correlations were observed in *Shorea robusta* (r = 0.93, $p<0.01$), *Ficus religiosa* (r = 0.93, $p<0.01$), and *Ficus semicordata* (r = 0.87, $p<0.01$). *Mallotus philippensis* showed a moderately strong significant correlation (r = 0.75, $p<0.05$), while *Mangifera indica* displayed a weak, non-significant correlation (r = 0.03).

Table 7: Correlation between soil Pb-leaves Pb, soil Cu- leaves Cu and soil Zn- leaves Zn of different plants with level of significance

Name of Plant Species	Soil Pb-Leaf Pb		Soil Cu-Leaf Cu		Soil Zn-Leaf Zn	
	r	**Level of significance**	**r**	**Level of significance**	**r**	**Level of significance**
Shorea robusta	0.80	*	0.98	**	0.93	**
Mangifera indica	0.77	*	0.45	ns	0.03	ns
Ficus semicordata	0.91	**	0.08	ns	0.87	**
Ficus religiosa	0.94	**	0.84	**	0.93	**
Mallotus philippensis	0.96	**	0.80	**	0.75	*

r = correlation coefficient (2-tailed), Level of significance: *$p<0.05$, **$p<0.01$, ns= not significant.

4.5.2. Correlation between soil dust and bark

a. Correlation of lead concentration between underlying soil dust and bark of tree species

All five species exhibited strong positive correlations, with r values ranging from 0.73 to 0.98. *Ficus semicordata* showed the strongest correlation (r = 0.98, $p<0.01$), closely followed by *Mangifera indica* (r = 0.97, $p<0.01$). *Shorea robusta*, *Ficus religiosa*, and *Mallotus philippensis* demonstrated moderately strong correlations (r = 0.73, 0.78 and 0.79 respectively), all significant at $p<0.05$ demonstrated by Table 7.

b. Correlation of copper concentration between underlying soil dust and bark of tree species

Mangifera indica and *Ficus religiosa* demonstrated strong, highly significant positive correlations (r = 0.93 and r = 0.88 respectively, both at *$p<0.01$*). In contrast, *Shorea robusta*, *Ficus semicordata*, and *Mallotus philippensis* exhibited weak, non-significant negative correlations (r = 0.07, 0.09, and -0.17 respectively) (Table 7).

c. Correlation of zinc concentration between underlying soil dust and bark of tree species

Strong significant positive correlations were observed in four out of five species: *Shorea robusta* (r = 0.99, $p<0.01$), *Mangifera indica* (r = 0.98, *$p<0.01$*), *Ficus religiosa* (r = 0.94, *$p<0.01$*), and *Mallotus philippensis* (r = 0.92, *$p<0.01$*). Interestingly, *Ficus semicordata* showed a moderate negative correlation (r = -0.43), which is not statistically significant.

Table 8: Correlation between Soil Pb-Bark Pb, soil Cu- bark Cu and soil Zn- bark Zn of different plants with level of significance.

Name of Plant Species	**Soil Pb-Bark Pb**		**Soil Cu-Bark Cu**		**Soil Zn-Bark Zn**	
	r	**Level of significance**	**r**	**Level of significance**	**r**	**Level of significance**
Shorea robusta	0.73	*	0.07	ns	0.99	**
Mangifera indica	0.97	**	0.93	**	0.98	**
Ficus semicordata	0.98	**	0.09	ns	-0.43	ns
Ficus religiosa	0.78	*	0.88	**	0.94	**
Mallotus philippensis	0.79	*	-0.17	ns	0.92	**

r = correlation coefficient (2-tailed), Level of significance: *$p<0.05$, **$p<0.01$, ns= not significant

CHAPTER 5: DISCUSSION

The past few decades have shown a significant increase in the concentration of heavy metals in the environment due to industrialization, causing worldwide concern (Ashraf *et al.*, 2019). Plants can serve as bio-indicators, and air pollutants tolerant plants are a sink for controlling air pollution (Manjunath and Reddy, 2017). Trees and shrubs are incredibly efficient for filtering dust pollutants in the atmosphere (Farmer, 1993).

5.1. Heavy metals content in soil dust

The study revealed a clear pattern in heavy metal concentrations in soil dust, with the highest levels of lead, copper, and zinc found at the site closest to the factory, specifically at 200 m: 55 mg/kg, 40 mg/kg and 65 mg/kg respectively. Subsequently, the metal content in soil dust decreased with increasing distance from the pollution source, with 1000 m site showing lower levels, followed by the control site. This observed trend showed that closeness to the pollution source results in higher concentrations due to direct emissions and deposition. The decreasing concentrations with distance reflect contaminants gradual dispersion and dilution as they travel from the factory (Monfared *et al.*, 2013).

The concentration of Zn in soil dust samples collected from three selected sites was higher and varied widely between 27.33-65.23 mg/kg and the recommended limit by Dutch standard 1990 was 50mg/kg. It showed that Zn concentration in soil dust around 200 m from the factory exceeded the permissible limit of Dutch standard 1990. This finding is consistent with previous studies highlighting zinc's persistence in soil and its ability to exhibit elevated concentrations near industrial activities (Li *et al.*, 2015; Desaulty *et al.*, 2020). Zn is used in brake linings and it is released during mechanical abrasion of vehicles and from engine oil combustion and tires of vehicles (El-Gamal, 2000; Hjortenkrans, 2003; Dolan *et al.*, 2006). The concentration might be attributed to industrial production, the number of trucks carrying raw materials into the factory, the distribution of products out from the factory, and their emissions that pass through the roads around the study area continuously.

The concentration of lead and copper is seen comparatively lesser than the concentration of zinc in soil dust, as mentioned above. Similar to this study, under investigation by Al-Khashman (2004), the ranges of heavy metal concentrations on street dust in Karak

Industrial Estate were found to be 1.8–84.9, and 2.1–314.1 mg kg^{-1} dry soil for Cu and Pb respectively which exceeded the values than in our study. The researchers discussed two possible sources of heavy metals accumulation was due to anthropogenic and industrial activities.

Similar to our findings, Gabarrón *et al.*, (2017) discussed that each industrial activity contributes differently to the concentration of metals in soil dust, and the highest concentrations of Pb and Zn were found from refinery and fertilizer industries. It showed that the industrial activity had influence on the physicochemical properties of soil and road dust and on the bioavailability of all metals. The distribution of heavy metals in the soil around cement factory indicated that there were several locations with high concentrations of Pb, Cu, and Zn which were severely contaminated. The high contents of Pb, Cu, and Zn were consistent with the results of Bi *et al.*, (2006) and Wu *et al.*, (2010). The contributing factor to this high soil contamination can be related to the combustion of leaded fuel by vehicles involved in different activities around the factory (Banat *et al.*, 2005) coupled with the cement production activities that release dust containing Pb, Cu, and Cd (NPI, 1991).

5.2. Heavy metal accumulation in leaves

Zinc was consistently the most prevalent heavy metal in leaves of all tree species and distances, which was a startling finding from the research. This result is consistent with research showing that various industrial processes have led to the widespread presence of zinc in environmental matrices (Farooq *et al.*, 2008; Doğanlar *et al.*, 2011; Krupnova *et al.*, 2021). Higher zinc concentrations found in all tree species under investigation highlight how common zinc is in the area under study. In contrast, the study of these three metals (Pb, Cu and Zn), the result obtained indicated that Pb was the most abundant heavy metal in mango leaves (67.11 mg/kg) during the study by Asuquo *et al.*, (2020).

In the present study, among the tree species, leaves of *Mallotus philippensis* showed a considerably more significant amount of Zn (45.05 mg/kg), Cu (7.50 mg/kg), and Pb (2.54 mg/kg) around 200 m from the factory in comparison to 1000 m and control site in which Zn and Pb concentration exceeded the permissible value of plant as given by WHO report 1996. This finding suggested that *Mallotus philippensis* is more vulnerable to pollution and might have particular traits that increase absorption of these heavy metals.

The study on the same plant by Dalvi *et al.*, (2007) nearby roadsides in Mumbai, recorded zinc (2.50 ppm), copper (5.20 ppm) and lead (0.15 ppm) and discussed about the possibility for the absorption of these heavy metals. Furthermore, the study showed significant differences in the amounts of heavy metals in the leaves of selected tree species at various distances from the cement factory. The present study resembled with the findings reported by Princewill *et al.,* (2011); Adejoh, (2016); Egbe *et al.,* (2019); Bayouli *et al.,* (2020); Javanmardi *et al.,* (2022), who studied the accumulation of heavy metals, including Pb, Cu and Zn in leaves of plants nearby cement industries.

Agrahari *et al.,* (2018) investigated the metal concentration of the plant *Ficus religiosa* leaves collected from nine experimental sites in Gorakhpur, Uttar Pradesh. The lead concentration in leaves was determined with the help of an atomic absorption spectrophotometer. The bioaccumulation of lead was highest in site 2 (Gayatri nagar), i.e. 14.1 mg kg-1 dry weight of *F. religiosa*. In contrast the present study recorded higher bioaccumulation of lead in leaves of *Mallotus philippensis* i.e. 2.54 mg/kg around 200 m from the factory that was slightly higher than the limit recommended by WHO 1996.

5.3. Heavy metal accumulation in bark samples

The present study indicated a higher concentration of heavy metals in the bark of tree species closer to the factory site (200 m) exceeding the limit as per WHO (1996) and decreasing concentration as the distance from the factory increases. This pattern aligns with established knowledge of the dispersion of pollutants with the prevailing wind-carrying contaminants (Norouzi *et al.,* 2016). The wind direction in our study area was from west to east.

Regarding individual metals, the results consistently showed that zinc concentrations are generally the highest across all tree species and distances, followed by copper and lead. This finding aligned with previous studies highlighting the prevalence of zinc as a common environmental contaminant (Akan *et al.,* 2013; Chrabąszcz *et al.,* 2017; Xu *et al.,* 2017; Birke *et al.,* 2018). The elevated concentrations of zinc may be attributed to various industrial activities, as zinc is commonly released into the environment through emissions from cement industries.

Interestingly, the study identified variations among the tree species regarding heavy metal concentrations accumulated on the bark of collected trees. Bark of *Mallotus philippensis* and *Mangifera indica* tend to exhibit higher concentrations of heavy metals than other species. This differential response among tree species could be attributed to variations in physiological characteristics, such as the capacity for metal uptake and accumulation. The higher concentration in *Mallotus philippensis* and *Mangifera indica* may suggest a greater susceptibility of these species to heavy metal pollution or a potential higher affinity for metal uptake. A similar finding was obtained by Khan *et al.,* (2015). Their study implied that the bark of *Mangifera indica* is a hyperaccumulator and could be used as a bioindicator for pollution studies.

The results by Alatou *et al.,* (2019) showed that the highest concentrations were recorded in barks for copper (21.86 µg/g, 23.33 µg/g) and lead (14.53 µg/g, 63.33 µg/g), and in unwashed leaves for cadmium (0.57 µg/g, 1.19 µg/g) and zinc (48.94 µg/g, 47.6 µg/g) for *E. cladocalyx* F. Muell, and *C. sempervirens* var. *fastigiata,* respectively. Lead and zinc were the most accumulated metals in all samples compared to cadmium and copper. The studied metal content in urban areas was significantly higher than that of the control site, as in our study. This represents that cement plant had influenced the metal contamination of the surrounding area.

5.4. MAI value for leaves and bark samples

The present study found that *Mangifera indica* leaves around 200 m from the factory had the highest MAI value (42.22). Despite the reduction at the greater distance, the values remained significantly higher than the control for *Mangifera indica* leaves, indicating a strong influence of the factory's emissions on metal accumulation in this species. The bark of *Mallotus philippensis* around the same distance away from the factory had the next highest value (35.60). This demonstrated that bark samples of *Mallotus philippensis* accumulated metals more efficiently than the other species studied, and the effects of the factory's emissions were evident even at a greater distance. A previous study reported that as the surface of the plant part gets rougher, heavy metal accumulation increases due to their greater ability to trap the particles (Sawidis *et al.,* 2011).

By applying MAI index to individual species similar to the present study, the researcher found that the average MAI values for the investigated heavy metals in the following decreasing order: *Eucalyptus cladocalyx* F. Muell (4.5) > *Cupressus sempervirens var. fastigiata* (3.8) in tree leaves, and *C. sempervirens* (3.4) > *E. cladocalyx* (2.7) for barks. For *E. cladocalyx* leaves, the highest metal accumulation sub-index (6.51) was recorded for Pb, followed by Cu (6.04) and Zn (4.12) while the highest value for *C. sempervirens* was recorded for Cu (4.71) followed by Zn (4.3) and Pb (2.11). For barks of *E. cladocalyx*, the highest value was recorded for Cu (3.4), followed by Zn (3.3) and Pb (2.7) and for those of *C. sempervirens*, the researcher recorded the decreasing order of Cu (3.8) > Zn (2.09) > Pb (1.7) (Alatou *et al.*, 2019).

Nadgorska Socha *et al.*, (2017) also showed site wise variations in MAI values for different plant species. 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 several 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 speciosa* at Beijing, China.

5.5. Correlation between soil dust and plant samples

a. Correlation between soil dust and Leaves

Ficus religiosa ($r = 0.94$, $p < 0.01$) and *Mallotus philippensis* ($r = 0.96$, $p < 0.01$) demonstrated very strong positive correlations, suggesting these species have a high capacity for lead uptake. This may be attributed to their root morphology and biochemical processes facilitating Pb absorption. *Shorea robusta* ($r = 0.98$, $p < 0.01$), *Ficus religiosa* ($r = 0.84$, $p < 0.01$), and *Mallotus philippensis* ($r = 0.80$, $p < 0.01$) exhibit strong positive correlations, indicating efficient copper uptake. Conversely, *Mangifera indica* ($r = 0.45$, $p > 0.05$) and *Ficus semicordata* ($r = 0.08$, $p > 0.05$) did not show significant correlations, suggesting these species may have mechanisms that limit copper uptake. *Shorea robusta* and *Ficus religiosa* both showed strong correlations ($r = 0.93$, $p < 0.01$). This indicates a consistent pattern of Zn uptake in these species, which might be due to their efficient Zn transport systems. However, *Mangifera indica* ($r = 0.03$, $p > 0.05$) exhibited no significant correlation, possibly due to the plant's physiological mechanisms restricting Zn uptake to the leaves. This resembled with the previous studies by Berthelsen *et al.*, (1995); Ugolini *et al.*, (2013) which showed significant correlation between leaf trace metals and soil dust.

b. Correlation between soil dust and bark

All studied plant species exhibited positive and statistically significant correlations between soil Pb and bark Pb concentrations. *Mangifera indica* and *Ficus semicordata* demonstrated particularly strong correlations (r = 0.97 and 0.98, respectively; $p<0.01$), suggesting these species may be effective biomonitors for soil Pb contamination. The correlations between soil Cu and bark Cu concentrations showed marked variability among species. *Mangifera indica* and *Ficus religiosa* displayed strong positive correlations (r = 0.93 and 0.88, respectively; $p<0.01$), whereas other species show weak or non-significant correlations. This heterogeneity suggests that Cu uptake and accumulation mechanisms may be highly species-specific, potentially influenced by factors such as root morphology and physiological adaptations. Zinc correlations exhibited the most diverse patterns among the studied heavy metals. While most species showed strong positive correlations (e.g., *Shorea robusta*, r = 0.99; $p<0.01$), *Ficus semicordata* displayed a moderate negative correlation (r = -0.43). This variability underscores the complexity of Zn uptake and translocation processes in plants, which may be influenced by soil chemistry, plant physiology, and environmental factors. As demonstrated by the correlation in the current study, several studies (Ogundele *et al.*, 2015; Suryawanshi *et al.*, 2016; Roy *et al.*, 2020) reported a significant positive correlation between soil and bark metals concentration.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The study showed that plants accumulate heavy metals from the contaminated air and underlying soil dust. The concentration of these metals is higher in the soil dust compared to the leaves and bark of trees in the study area. Some plants can tolerate or even be benefited from the presence of these metals, for e.g. *Mallotus philippensis* and *Mangifera indica*, which accumulated more concentration of heavy metals among studied species. Leaves of *Mangifera indica* and *Ficus religiosa* showed high accumulation of lead, copper and zinc. Bark of *Ficus religiosa* showed high accumulation of lead and copper while bark of *Mallotus philippensis* and *Mangifera indica* showed high accumulation of copper and zinc. The concentration of heavy metals in the leaves and bark of trees and soil dust decreased with an increase in distance from factory pollution, so trees can be used to map the extent of pollution.

The concentration of heavy metals in the leaves and bark of different tree species also varied, so trees can be used to identify which species are most effective at absorbing heavy metals. Species with consistently high correlations across all three metals (e.g., *Ficus religiosa* leaves and *Mangifera indica* bark) could be suitable for multi-metal phytoremediation efforts. Among selected tree species, *Shorea robusta* showed less accumulation of heavy metals. This suggests that this particular species may have avoidance 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.

6.2. Recommendations

i. This study included a limited number of plant species and only 3 heavy metals, so further research is needed considering more plant species and more heavy metals.
ii. A detailed study should be carried out on the physiological mechanism of *Mallotus philippensis* and *Mangifera indica* for efficient heavy metal accumulation.
iii. As evidenced by the heavy metal concentrations in soil dust by our study, the

possible environmental effects of industrial activities on soil quality should be considered appropriately in time.

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APPENDICES

Table A1. Heavy metal concentrations with triplicate values (mg/kg) in bark, including their mean and standard deviation values.

Sites	**Control**			**200 m**			**1000 m**		
Species	**Lead**	**Copper**	**Zinc**	**Lead**	**Copper**	**Zinc**	**Lead**	**Copper**	**Zinc**
Shorea robusta	0.42	9.5	13.3	0.8	11.8	26.7	0.92	13.48	16.2
	0.5	10	14.5	1.5	12.6	27.5	1	15	17
	0.58	11.2	15.2	1.3	13.7	28.4	1.1	15.7	17.6
Mean	**0.50**	**10.23**	**14.33**	**1.20**	**12.70**	**27.53**	**1.01**	**14.73**	**16.93**
S.D.	**0.08**	**0.87**	**0.96**	**0.36**	**1.00**	**0.85**	**0.09**	**1.13**	**0.70**
Mangifera indica	0.41	2.2	18.6	1.98	8.8	66.5	0.98	4.2	29.5
	0.5	2.5	20	2	10	67.8	1	5	30.5
	0.58	2.9	21.4	2.6	10.7	68.9	1.13	5.6	31.8
Mean	**0.50**	**2.53**	**20.00**	**2.19**	**9.83**	**67.73**	**1.04**	**4.93**	**30.60**
S.D.	**0.09**	**0.35**	**1.40**	**0.35**	**0.96**	**1.20**	**0.08**	**0.70**	**1.15**
Ficus semicordata	0.98	4.4	23.8	2.4	6.28	15.58	1.42	7.37	13.7
	1	5	25.3	2.5	6.49	17.5	1.5	7.5	16.2
	1.2	5.2	26	2.64	6.63	18.3	1.71	7.63	19.9
Mean	**1.06**	**4.87**	**25.03**	**2.51**	**6.47**	**17.13**	**1.54**	**7.50**	**16.60**
S.D.	**0.12**	**0.42**	**1.12**	**0.12**	**0.18**	**1.40**	**0.15**	**0.13**	**3.12**
Ficus religiosa	1.2		14.96	2.81	11.6	48.4	2.1	7.6	29.3
	1.5	5	17	3	12.5	50	2.8	8.5	30.5
	1.75	5.7	19	3.6	13.8	51.2	3.1	8.8	31.6
Mean	**1.48**	**4.97**	**16.99**	**3.14**	**12.63**	**49.87**	**2.67**	**8.30**	**30.47**
S.D.	**0.28**	**0.75**	**2.02**	**0.41**	**1.11**	**1.40**	**0.51**	**0.62**	**1.15**
Mallotus philippensis	0.42	11.6	34.2	2.38	24.5	114.6	1.86	43.2	70.6
	0.5	12.5	35.5	2.5	25	117.5	2	45.5	72
	0.61	13.2	36.3	2.59	25.8	119.8	2.3	45.8	74.4
Mean	**0.51**	**12.43**	**35.33**	**2.49**	**25.10**	**117.30**	**2.05**	**44.83**	**72.33**
S.D.	**0.10**	**0.80**	**1.06**	**0.11**	**0.66**	**2.61**	**0.22**	**1.42**	**1.92**

Table A2. Heavy metal concentrations with triplicate values (mg/kg) in soil dust including their mean and standard deviation values.

Sites		**Control**			**200 m**			**1000 m**	
Metals	**Lead**	**Copper**	**Zinc**	**Lead**	**Copper**	**Zinc**	**Lead**	**Copper**	**Zinc**
	7.5	15.6	25.3	42.5	38.6	63.3	13.6	14.5	28.2
	9	18	27.5	45	40	65	15	17	30
	10.5	20.8	29.2	47	42.4	67.4	17.5	18.7	32.4
Mean	**9.00**	**18.13**	**27.33**	**44.83**	**40.33**	**65.23**	**15.37**	**16.73**	**30.20**
S.D.	**1.50**	**2.60**	**1.96**	**2.25**	**1.92**	**2.06**	**1.98**	**2.11**	**2.11**

Table A3. Heavy metal concentrations with triplicate values (mg/kg) in leaves, including their mean and standard deviation values.

Sites		**Control**			**200 m**			**1000 m**	
Species	**Lead**	**Copper**	**Zinc**	**Lead**	**Copper**	**Zinc**	**Lead**	**Copper**	**Zinc**
Shorea robusta	0.35	2.38	12.2	1.3	4.38	21.38	1.2	2.24	15.6
	0.5	2.5	14	2	5	22.5	1.5	2.3	17
	0.62	2.64	16.8	2.2	5.56	23.6	1.7	2.46	18.4
Mean	**0.49**	**2.51**	**14.33**	**1.83**	**4.98**	**22.49**	**1.47**	**2.33**	**17.00**
S.D.	**0.14**	**0.13**	**2.32**	**0.47**	**0.59**	**1.11**	**0.25**	**0.11**	**1.40**
Mangifera indica	0.98	2.2	11.2	1.58	7.3	18	1.2	7.38	24.3
	1	2.5	12	2	7.5	18.2	1.5	7.5	25.7
	1.8	3.6	12.8	2.32	7.6	18.5	1.53	7.66	26.5
Mean	**1.26**	**2.77**	**12.00**	**1.97**	**7.47**	**18.23**	**1.41**	**7.51**	**25.50**
S.D.	**0.47**	**0.74**	**0.80**	**0.37**	**0.15**	**0.25**	**0.18**	**0.14**	**1.11**
Ficus semicordata	0.44	2.36	8.8	1.3	2.3	33.4	0.97	2.3	22.5
	0.5	2.5	10.6	2	2.5	35	1	2.5	24
	0.64	2.66	11.4	2.02	2.58	35.8	1.3	2.56	24.7
Mean	**0.53**	**2.51**	**10.27**	**1.77**	**2.46**	**34.73**	**1.09**	**2.45**	**23.73**
S.D.	**0.10**	**0.15**	**1.33**	**0.41**	**0.14**	**1.22**	**0.18**	**0.14**	**1.12**
Ficus religiosa	0.98	2.33	12.2	2.22	7	23.6	1.6	4.4	17.2
	1	2.5	13	2.5	7.5	25.5	1.7	5	18
	1.2	2.77	13.8	2.6	8.2	26.6	1.84	5.8	18.7

Mean	**1.06**	**2.53**	**13.00**	**2.44**	**7.57**	**25.23**	**1.71**	**5.07**	**17.97**
S.D.	**0.12**	**0.22**	**0.80**	**0.20**	**0.60**	**1.52**	**0.12**	**0.70**	**0.75**
Mallotus philippensis	0.6	2.9	14.6	2.42	6.8	43.25	1.2	4.8	35.5
	0.8	3	15	2.5	7.5	45.5	1.5	5.5	37.7
	1.4	3.7	15.54	2.7	8.2	46.4	1.58	6.4	38.4
Mean	**0.93**	**3.20**	**15.05**	**2.54**	**7.50**	**45.05**	**1.43**	**5.57**	**37.20**
S.D.	**0.42**	**0.44**	**0.47**	**0.14**	**0.70**	**1.62**	**0.20**	**0.80**	**1.51**

Table A4. Metal Accumulation Index in bark samples

Species/ sites	**Control**	**200 m**	**1000 m**
Shorea robusta	10.96	16.40	16.10
Magnifera indica	9.11	24.28	15.52
Ficus semicordata	14.22	23.00	24.44
Ficus religiosa	6.77	18.22	14.99
Mallotus philippensis	18.06	35.60	26.11

Table A5. Metal Accumulation Index in leaf samples

Species/ sites	**Control**	**200 m**	**1000 m**
Shorea robusta	9.75	10.86	12.83
Magnifera indica	7.15	42.22	28.04
Ficus semicordata	9.85	16.60	15.04
Ficus religiosa	12.13	13.86	15.12
Mallotus philippensis	13.83	18.70	12.88

Table A6. Correlation between Soil Pb-leaves Pb, soil Cu- leaves Cu and soil Zn- leaves of following tree species with r and *p*-values.

		Shorea robusta	***Mangifera indica***	***Ficus semicordata***	***Ficus religiosa***	***Mallotus philippensis***
Soil (Pb)	**r**	.779*	.773*	.908**	.942**	.958**
	p	.013	.015	.001	.000	.000
Soil (Cu)	**r**	.986**	.456	.089	.836**	.804**
	p	.000	.218	.819	.005	.009
Soil (Zn)	**r**	.925**	.034	.872**	.937**	.750*
	p	.000	.932	.002	.000	.020

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table A7. Correlation between Soil Pb-Bark Pb, soil Cu- bark Cu and soil Zn- bark Zn of following tree species with r and *p*-values.

		Shorea robusta	***Mangifera indica***	***Ficus semicordata***	***Ficus religiosa***	***Mallotus philippensis***
Soil (Pb)	**r**	.734*	.974**	.983**	.780*	.786*
	p	.024	.000	.000	.013	.012
Soil (Cu)	**r**	.068	.926**	.098	.878**	-.165
	p	.861	.000	.802	.002	.671
Soil (Zn)	**r**	.993**	.988**	-.430	.939**	.920**
	p	.000	.000	.248	.000	.000

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Photo Plates

Photo 1. Collecting bark using a steel knife of *Mangifera indica* tree

Photo 2. Periphery around Hongshi Shivam Cement Factory

Photo 3. Drying samples in a hot air oven during sample preparation

Photo 4. Sample filtration

Photo 5. Chemito-201 AAS

Photo 6. Sample preparation

Photo 7. Digested sample

Photo 8. Control site (Deurali)