EVALUATION OF MORPHO-PHYSIOLOGICAL AND METAL ACCUMULATION POTENTIAL OF *Salix alba* L. AND *Toona ciliata* M. Roemer GROWN ON HEAVY METAL CONTAMINATED SOILS

Dissertation

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY
in
BOTANY
(Minor Subject: Forestry)

By

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CERTIFICATE-I

This is to certify that the dissertation entitled **"EVALUATION OF MORPHO-PHYSIOLOGICAL AND METAL ACCUMULATION POTENTIAL OF *Salix alba* L. AND *Toona ciliata* M. Roemer GROWN ON HEAVY METAL CONTAMINATED SOILS",** submitted for the degree of **Ph.D.**, in the subject of **Botany** (Minor subject: **Forestry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ravneet Kaur (L-2018-BS-82-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE-II

This is to certify that the dissertation entitled, **"EVALUATION OF MORPHO-PHYSIOLOGICAL AND METAL ACCUMULATION POTENTIAL OF *Salix alba* L. AND *Toona ciliata* M. Roemer GROWN ON HEAVY METAL CONTAMINATED SOILS"** submitted by **Ravneet Kaur (L-2018-BS-82-D)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Ph.D.,** in the subject of **Botany** (Minor subject: **Forestry**) has been approved by the Student's Advisory Committee after an oral examination on the same in collaboration with External Examiner.

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ABSTRACT

The present investigations were carried out to study the effect of lead (Pb), cadmium (Cd) and their combinations (Pb+Cd) on morpho-physiological and metal accumulation potential of *Salix alba* and *Toona ciliata* during 2020 and 2021. Heavy metals induce negative effect on morphological and biomass attributes of both species, but *Toona* showed better survival percentage (>95%) than *Salix* (<55%) even at higher concentrations of Pb_{300} and Cd_{25}. The accumulation of proline, total soluble sugars, total soluble proteins along with increased antioxidant enzyme activities are responsible to enhance tolerance in both species. On the basis of phytoremediation efficiency evaluation factors such as bioconcentration factor (BCF) and translocation factor (TF), *Salix alba* can be categorized as excluder plant for Pb and Cd with higher metal accumulation in roots than shoots (BCF>1, TF<1), while *Toona ciliata* can be categorized as hyperaccumulator with more Pb and Cd accumulation in the shoots than roots (BCF>1 and TF>1). Further, heavy metal translocation and accumulation decreased in combinations as compared to single element application suggesting the antagonistic relationship among both metals. Pb and Cd negatively affect the plant nutrient content either by affecting their translocation or by decreasing nutrient availability in soils. Anatomical studies showed significant alterations in stomatal pore size, stomatal density and trichome density due to heavy metal toxicity in both *Salix* and *Toona*. Field emission scanning electron microscopy and energy dispersive x-ray spectroscopy (FESEM-EDS) results confirmed the Pb and Cd accumulation sites in the leaves and root tissues of plants. Fourier transformed infrared (FTIR) spectroscopy analysis revealed that Pb and Cd accumulation in plants induced changes in carboxy, amino, hydroxyl and phosphate groups that ultimately caused alteration in physiological functioning in plants. Conclusively, both *Salix alba* and *Toona ciliata* have potential to be used as remediation species for Pb and Cd contaminated soils.

Keywords: Heavy metals, translocation, antioxidants, phytoremediation, nutrients, anatomy

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Signature of Major Advisor **Signature of the Student**

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ਸਾਰ ਅੰਸ਼

ਮੌਜੂਦਾ ਜਾਂਚਾਂ 2020 ਅਤੇ 2021 ਦੌਰਾਨ ਸੈਲਿਕਸ ਐਲਬਾ ਅਤੇ ਟੂਨਾ ਸਿਲਿਆਟਾ ਦੀ ਮੋਰਫੋ-ਫਿਜ਼ਿਓਲੋਜੀਕਲ ਅਤੇ ਧਾਤੂ ਸੰਚਵ ਸੰਭਾਵੀ 'ਤੇ ਲੈੱਡ (Pb), ਕੈਡਮੀਅਮ (Cd) ਅਤੇ ਉਨ੍ਹਾਂ ਦੇ ਸੰਜੋਗ (Pb+Cd) ਦੇ ਪ੍ਰਭਾਵ ਦਾ ਅਧਿਐਨ ਕਰਨ ਲਈ ਕੀਤੀਆਂ ਗਈਆਂ ਸਨ। ਭਾਰੀ ਧਾਤਾਂ ਦੋਵਾਂ ਸਪੀਸੀਜ਼ ਦੇ ਮੋਰਫੋਲੋਜੀਕਲ ਅਤੇ ਬਾਇਓਮਾਸ ਗੁਣਾਂ 'ਤੇ ਨਕਾਰਾਤਮਕ ਪ੍ਰਭਾਵਿਤ ਕਰਦੀਆਂ ਹਨ, ਪਰ ਟੂਨਾ ਨੇ Pb_{300} ਅਤੇ Cd_{25} ਦੀ ਉੱਚ ਗਾੜ੍ਹਾਪਣ 'ਤੇ ਵੀ ਸੈਲਿਕਸ (<55%) ਨਾਲੋਂ ਬਿਹਤਰ ਬਚਾਅ ਪ੍ਰਤੀਸ਼ਤ (>95%) ਦਿਖਾਇਆ ਹੈ। ਪ੍ਰੋਲਾਈਨ, ਕੁੱਲ ਘੁਲਣਸ਼ੀਲ ਸ਼ੂਗਰ, ਕੁੱਲ ਘੁਲਣਸ਼ੀਲ ਪ੍ਰੋਟੀਨ ਦੇ ਨਾਲ-ਨਾਲ ਵਧੀਆਂ ਐਂਟੀਆਕਸੀਡੈਂਟ ਐਂਜ਼ਾਈਮ ਗਤੀਵਿਧੀਆਂ ਦੋਵਾਂ ਸਪੀਸਜ਼ ਵਿੱਚ ਸਹਿਣਸ਼ੀਲਤਾ ਨੂੰ ਵਧਾਉਣ ਲਈ ਜ਼ਿੰਮੇਵਾਰ ਹਨ। ਫਾਈਟੋਰੀਮੀਡੀਏਸ਼ਨ ਕੁਸ਼ਲਤਾ ਮੁਲਾਂਕਣ ਕਾਰਕਾਂ ਜਿਵੇਂ ਕਿ ਬਾਈਓਕੈਂਸਟਰੇਸ਼ਨ ਫੈਕਟਰ (BCF) ਅਤੇ ਟ੍ਰਾਂਸਲੋਕੇਸ਼ਨ ਫੈਕਟਰ (TF) ਦੇ ਆਧਾਰ 'ਤੇ ਸੈਲਿਕਸ ਐਲਬਾ ਨੂੰ Pb ਅਤੇ Cd ਲਈ ਐਕਸਕਲੂਡਰ ਪਲਾਂਟ ਵਜੋਂ ਸ਼੍ਰੇਣੀਬੱਧ ਕੀਤਾ ਜਾ ਸਕਦਾ ਹੈ, ਜਿਸ ਵਿੱਚ ਸ਼ੂਟ (BCF>1, TF<1) ਨਾਲੋਂ ਜੜ੍ਹਾਂ ਵਿੱਚ ਉੱਚ ਧਾਤ ਇਕੱਠਾ ਹੁੰਦਾ ਹੈ, ਜਦੋਂ ਕਿ ਟੂਨਾ ਸਿਲੀਏਟਾ ਨੂੰ ਜੜ੍ਹਾਂ (BCF>1 ਅਤੇ TF>1) ਨਾਲੋਂ ਸ਼ੂਟ ਵਿੱਚ ਵਧੇਰੇ Pb ਅਤੇ Cd ਇਕੱਠਾ ਕਰਨ ਵਾਲੇ ਹਾਈਪਰਐਕਯੂਮੂਲੇਟਰ ਵਜੋਂ ਸ਼੍ਰੇਣੀਬੱਧ ਕੀਤਾ ਜਾ ਸਕਦਾ ਹੈ। ਇਸ ਤੋਂ ਇਲਾਵਾ, ਦੋਨਾਂ ਧਾਤਾਂ ਵਿਚਕਾਰ ਵਿਰੋਧੀ ਸਬੰਧਾਂ ਦਾ ਸੁਝਾਅ ਦੇਣ ਵਾਲੇ ਸਿੰਗਲ ਐਲੀਮੈਂਟ ਐਪਲੀਕੇਸ਼ਨ ਦੀ ਤੁਲਨਾ ਵਿੱਚ ਹੈਵੀ ਮੈਟਲ ਟਰਾਂਸਲੋਕੇਸ਼ਨ ਅਤੇ ਇਕੱਠਾ ਹੋਣਾ ਸੰਜੋਗਾਂ ਵਿੱਚ ਘਟਿਆ ਹੈ। Pb ਅਤੇ Cd ਪੌਦਿਆਂ ਦੇ ਪੌਸ਼ਟਿਕ ਤੱਤ ਨੂੰ ਨਕਾਰਾਤਮਕ ਤੌਰ 'ਤੇ ਪ੍ਰਭਾਵਿਤ ਕਰਦੇ ਹਨ ਜਾਂ ਤਾਂ ਉਹਨਾਂ ਦੇ ਟ੍ਰਾਂਸਲੇਸ਼ਨ ਨੂੰ ਪ੍ਰਭਾਵਿਤ ਕਰਕੇ ਜਾਂ ਮਿੱਟੀ ਵਿੱਚ ਪੌਸ਼ਟਿਕ ਤੱਤਾਂ ਦੀ ਉਪਲਬਧਤਾ ਨੂੰ ਘਟਾ ਕੇ। ਅਨੈਟਮੀ ਅਧਿਐਨਾਂ ਨੇ ਸੈਲਿਕਸ ਅਤੇ ਟੂਨਾ ਦੋਵਾਂ ਵਿੱਚ ਭਾਰੀ ਧਾਤੂ ਦੇ ਜ਼ਹਿਰੀਲੇਪਣ ਦੇ ਕਾਰਨ ਸਟੋਮੈਟਲ ਪੋਰ ਦੇ ਆਕਾਰ, ਸਟੋਮੈਟਲ ਘਣਤਾ ਅਤੇ ਟ੍ਰਾਈਕੋਮ ਘਣਤਾ ਵਿੱਚ ਮਹੱਤਵਪੂਰਨ ਤਬਦੀਲੀਆਂ ਦਿਖਾਈਆਂ ਹਨ। ਫ਼ੀਲਡ ਐਮੀਸ਼ਨ ਸਕੈਨਿੰਗ ਇਲੈਕਟ੍ਰੌਨ ਮਾਈਕ੍ਰੋਸਕੋਪੀ ਅਤੇ ਇਨਰਜੀ ਡਿਸਪਰਸਿਵ ਐਕਸ-ਰੇ ਸਪੈਕਟ੍ਰੋਸਕੋਪੀ (FESEM-EDS) ਦੇ ਨਤੀਜਿਆਂ ਨੇ ਪੌਦਿਆਂ ਦੇ ਪੱਤਿਆਂ ਅਤੇ ਜੜ੍ਹਾਂ ਦੇ ਟਿਸ਼ੂਆਂ ਵਿੱਚ Pb ਅਤੇ Cd ਇਕੱਠਾ ਕਰਨ ਵਾਲੀਆਂ ਥਾਵਾਂ ਦੀ ਪੁਸ਼ਟੀ ਕੀਤੀ ਹੈ। ਫੌਰੀਅਰ ਟਰਾਂਸਫਾਰਮਡ ਇਨਫਰਾਰੈੱਡ (FTIR) ਸਪੈਕਟ੍ਰੋਸਕੋਪੀ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਖੁਲਾਸਾ ਕੀਤਾ ਕਿ ਪੌਦਿਆਂ ਵਿੱਚ Pb ਅਤੇ Cd ਇਕੱਠਾ ਹੋਣ ਕਾਰਨ ਕਾਰਬਾਕਸੀ, ਅਮੀਨੋ, ਹਾਈਡ੍ਰੋਕਸਿਲ ਅਤੇ ਫਾਸਫੇਟ ਸਮੂਹਾਂ ਵਿੱਚ ਤਬਦੀਲੀਆਂ ਆਉਂਦੀਆਂ ਹਨ ਜੋ ਆਖਿਰਕਾਰ ਪੌਦਿਆਂ ਵਿੱਚ ਫਿਜ਼ੀਓਲੋਜੀ ਕਾਰਜਾਂ ਵਿੱਚ ਤਬਦੀਲੀ ਦਾ ਕਾਰਨ ਬਣਦੀਆਂ ਹਨ। ਸਿੱਟੇ ਵਜੋਂ, ਸੈਲਿਕਸ ਐਲਬਾ ਅਤੇ ਟੂਨਾ ਸਿਲਿਆਟਾ ਦੋਵਾਂ ਵਿੱਚ Pb ਅਤੇ Cd ਦੂਸ਼ਿਤ ਮਿੱਟੀ ਲਈ ਉਪਚਾਰਕ ਕਿਸਮਾਂ ਵਜੋਂ ਵਰਤੇ ਜਾਣ ਦੀ ਸੰਭਾਵਨਾ ਹੈ।

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LIST OF ABBREVIATIONS AND SYMBOLS

µmol	:	Micromole
mg		Milligram
µg		Microgram
Kg		Killogram
APX	:	Ascorbate peroxidase
BCF	:	Bioconcentration Factor
BIS	:	Bureau of Indian Standards
EC	:	Electrical conductivity
CEC	:	Cation Exchange Capacity
CAT	:	Catalase
CPCB	:	Central Pollution Control Board
CRD	:	Completely randomized design
dSm^{-1}	:	Deci Siemens per meter
DW	:	Dry weight
LSD	:	Least Significant Difference
EDS	:	Energy Dispersive X-ray Spectroscopy
FESEM	:	Field Emission Scanning Electron Microscopy
FTIR	:	Fourier Transformed Infra-red Spectroscopy
FW	:	Fresh weight
FYM	:	Farm yard manure
GR	:	Glutathione reductase
GSH	:	Reduced Glutathione
HPI	:	Heavy Metal Pollution Index
IAA	:	Indole Acetic Acid
ICP-MS	:	Inductively Coupled Plasma-Mass Spectroscopy
Mha	:	Million hectare
min	:	Minute
MLD	:	Minimal liquid discharge
MRE	:	Metal removal efficiency
MT	:	Million tonnes
MTs	:	Metallothioneins
NS	:	Non-Significant
OD	:	Optical density
PC	:	Phytochelatins
pH	:	Power of hydrogen

POD	:	Peroxidase
Ppm	:	Parts per million
ROS	:	Reactive Oxygen Species
SOC	:	Soil Organic Carbon
SOD	:	Superoxide Dismutase
TF	:	Translocation Factor
TSS	:	Total soluble sugar
WHO	:	World Health Organization

CHAPTER - I

INTRODUCTION

Heavy metals (HMs) are ubiquitous environmental pollutants owing to their toxic, bio accumulative nature and prolonged persistence in the environment (Rzymski *et al* 2014). They are metals possessing a specific density of more than 5 gcm^{-3} and have adverse impacts on environment (Järup 2003). Some metal elements are called micronutrients, such as copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn), plays a vital role in the normal functioning of plant cells such as biosynthesis of nucleic acids, chlorophyll, carbohydrates, secondary metabolites, stress resistance, maintenance of biological membranes as well as overall growth of the plants (Rengel 2004), however, when their internal concentration transcends a certain threshold limit, they negatively influence plant growth. As per the World Health Organization (WHO), the common toxic 'heavy metals' of public health concern are arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), manganese (Mn), copper (Cu) and molybdenum (Mo). Moreover, the concentration of heavy metals is generally location-specific, subjected to the source of individual pollutants (Briffa *et al* 2020).

Rapid industrialization and an exponential increase in population have increased the discharge of massive loads of heavy metal pollutants in the environment (Zhang *et al* 2020). Globally, around 500 M ha of our land resources are facing the problem of soil contamination ended up with higher concentrations of heavy metals compared to the regulatory levels (Liu *et al* 2018). Over the past few years, the annual global release of heavy metals has surpassed 0.2 lacs MT for Cd, 9.3 lacs MT for Cu, 7.83 lacs MT for Pb and 1.35 lacs MT for Zn (Thambavani and Prathipa 2012). As per Indian central pollution control board (CPCB 2021), approximately 26,164 MLD of sewage and 25,000 MLD of untreated industrial wastewater generated from urban areas are released in the surface water bodies, wreaking degradation of the quality of water resources. Until now over thousands of acres of farmland in Punjab is contaminated by heavy metals, among them Cd, Cr, Pb and Ni are the major heavy metal pollutants found in the Malwa region of Punjab (Sharma and Dutta 2017). A total of eight districts of Malwa region has been studied and maximum lead contamination in ground water has been found in Bathinda followed by Mansa > Ferozpur > Faridkot > Moga > Muktsar > Barnala > Sangrur; whereas, maximum cadmium concentration was found in Ferozpur followed by Faridkot > Muktsar > Moga > Mansa > Barnala > Sangrur > Bathinda (Sharma and Dutta 2017).

Buddha Nullah is an old rivulet flowing through Ludhiana, Punjab (India), and its roadside soil was contaminated due to poor solid/liquid waste disposal and industrial

activities. A total of seven metals (Cd, Cr, Co, Cu, Pb, Ni, Zn) was estimated by Kaur *et al* (2022) and reported that Pb, Cd, Cu, Zn and Co were found above the permissible limits. The higher concentration these metals implies that the soil of the studied area was polluted with heavy metals, which arise from industrial activities and may have a direct influence on human health, groundwater, terrestrial and therefore ecological systems (Hamzah *et al* 2016). These heavy metals entering the environment through leather, electroplating, metal processing, dying hosiery, smelting industries, attrition of automobile tires, burning of automobile oils, sewage sludge and use of phosphate fertilizers on agricultural lands (Rafique and Tariq 2016). These heavy metals are major public concern as highlighted by the WHO for their potential to be strong carcinogens (Briffa *et al* 2020).

Soil heavy metal pollution is not caused by single element; it is multi-element problem due to interaction of several heavy metal elements viz., Pb, Cd, Zn, As, Ni, Cr etc. (Clabeaux *et al* 2013). Among all the toxic heavy metals, Cd ranks highest in terms of damage to plant growth and human health. Moreover, its uptake and accumulation in plants poses a serious health threat to humans via the food chain (Haider *et al* 2021). Exposure to Pb in the environmental and occupational settings continues to be a serious public health problem (Balali-Mood 2021). Pb and Cd as non-essential elements, can be readily taken up by plant roots and induce adverse effects on plant metabolism, growth and development (Hatamian *et al* 2020). Heavy metal pollution affects crop productivity, soil microbial activity and deteriorates the soil-water quality, therefore the remediation of these metal polluted soils become prerequisite. Hence, remediation procedures are required to prevent heavy metals from entering the terrestrial, atmospheric and aquatic environments, as well as to ameliorate contaminated land (Hasan *et al* 2019).

Phytoremediation is an ecofriendly, cost-effective approach that involves use of plants for the extraction, immobilization and degradation of elemental pollutants from the polluted sites (Yan *et al* 2020). Pulford and Watson (2003) reported five different phytoremediation techniques viz., phytoextraction (uptake of contaminants by plant roots and translocation within the aerial plant parts), rhizofiltration (both terrestrial and aquatic plants are used to absorb and concentrate contaminants in their roots), phytostabilization refers to the use of plants to reduce the bioavailability of pollutants in the environment), phytovolatilization (uptake of contaminants from soil and waste water, transforming them into volatilized form and transpiring into the atmosphere), phytodegradation (plants and associated microbes degrade organic pollutants). Utilizing perennial plants for restoring the balance seems to be an effective *in-situ* remediation technology, as it utilize inherent plant physiological mechanisms to degrade, immobilize or selectively uptake the contaminants from polluted soil and water interface (Ahmadi *et al* 2020). Phytoremediation of heavy metal polluted sites by utilizing trees may be preferred over annuals on account of their ability to

produce higher biomass; have extensive root system and potential to sequester high quantities of metal for a longer time span (Mleczek *et al* 2017). On the basis of plant's response to heavy metal contaminated soil, plants are divided into three categories by Cioica *et al* (2019): excluder plants (limits the heavy metal accumulation in the roots only and prevents the movement of heavy metals into their aerial plant parts), bioindicators (plants having accumulation of heavy metals in their aerial plant parts with low metal extraction capability, used for biological monitoring of contaminated soil), accumulators (allows the uptake, translocate and accumulation of heavy metals in aerial plant parts without affecting normal development). Among them, hyperaccumulator plants can accumulate enormous amounts of heavy metal in their aerial plant parts, thus can be utilized to remediate heavy metal polluted soil.

Alterations in plant morphology, physiology and anatomy in response to adverse environmental conditions indicate the tolerance and adaptability of plant against abiotic stress (Huang et al 2019, Khosropour *et al* 2019). Lead (Pb) and cadmium (Cd) are phytotoxic in nature as they induces alterations in plant at all physiological, biochemical and genetic aspects, which inhibits plant growth by suppressing respiration rate, photosynthetic rate and water nutrient uptake in spinach (Alia *et al* 2015). The phytotoxic nature of cadmium is due to its high mobility inside the plant that adversely affects the plant growth by reducing new cell production and root growth, also induce oxidative stress through generation of reactive oxygen species (ROS) (Zhao *et al* 2021). Pb affects the uptake and translocation of magnesium (Mg) and iron (Fe) that is responsible for chlorophyll synthesis, hence it affect photosynthesis by inducing alteration in enzymatic activities inside the photosynthetic apparatus (Aslam *et al* 2021).

Among all the toxic heavy metals, Cd and Pb are considered as most harmful as they have the potential to negatively affect the plant growth and development by altering the cell division, photosynthesis, respiration, protein synthesis, redox balance, membrane permeability and anatomical modifications (Kanwal *et al* 2020, Maestri *et al* 2010). Plants growing on heavy metal contaminated soils showed visible symptoms such as stunted growth, necrosis, chlorosis, root browning and ultimately plant death (Ozturk *et al* 2015). Furthermore, heavy metal stress results in over-production of ROS in plants that causes oxidative damage, ultimately leading to cell death. Moreover, plants have efficient antioxidant defense mechanism to scavenge ROS during heavy metal toxicity (Khan *et al* 2021). Some plants cope up with certain heavy metal concentration in contaminated soil by reducing the uptake, promoting the toxic ions compartmentalization into vacuoles, heavy metal sequestration through phytochelatins (PC) or metallothioneins (MT) and activation of plant antioxidant defense mechanism (Hasan *et al* 2017). However, the effectiveness and induction of a specific defense response are dependent on the plant species, type of heavy

metal as well as their concentration and exposure time (Riyazuddin *et al* 2022). Thus, in order to understand the plant tolerance mechanisms it is crucial to investigate the response and adaptability of plant physiological and anatomical traits in response to heavy metal toxicity.

Roots, being the first plant organ exposed to heavy metals, either acts as storage part in tolerant plants or as an intermediate to facilitate transfer of heavy metals from soil media to shoots in susceptible plants (Cheng *et al* 2006). Root cell walls has efficient mechanism of metal ion exchange that limits its translocations to aerial plant parts by modifying their anatomical structures and reduces the heavy metal uptake and translocation (Gomes *et al* 2011). Increase in the thickness of casparian strips of root endodermis also inhibits the heavy metal translocation through apoplast and thereby reducing the metal concentration in pericycle and vascular region (Maksimović *et al* 2007). Although, this anatomical strategy reduces the water and nutrient uptake, it also blocks the toxic metal ion uptake, translocation and accumulation, thus reduces the heavy metal stress in plants (Li *et al* 2019).

Plants are composed of macromolecules viz., nucleic acids, proteins, lipids and carbohydrates, with unique functional groups that interact with heavy metals (Usman *et al* 2019). These functional groups react to certain infrared light frequencies and their interactions with the heavy metal can be investigated using Fourier Transformed Infrared Spectroscopy (FTIR) (Griffiths and Haseth 2007) which will reflect the presence of heavy metals in plant tissues using metal cation binding in treated plant samples. Similarly, heavy metals confer significant impact on numerous soil aspects including their biological characterstics (Karaca *et al* 2010).

Yan *et al* (2020) reported five best plants for phytoremediation are Indian mustard (*Brassica juncea* L.), willow (*Salix* sp.), poplar (*Populus deltoids*), Indian grass (*Sorghastrum nutans* L.) and Sunflower (*Helianthus annuus* L.). Among these plants, *B. juncea* known to accumulate heavy metal (Cd, Pb, Se, Zn, Hg and Cu) in their plant tissues with better heavy metal removal ability from contaminated soil (Rathore *et al* 2019). *Salix* have also ability to grow on heavy metal polluted soil (Pb, Cd and Ni), even in mixed heavy metals like diesel fuel polluted sites (Wani *et al* 2020). *P. deltoids* is also considered as best plant for phytoremediation purposes due to its naturally well-developed root system which is able to remediate Cu, Cd, Zn along with 95 per cent of organic pollutants such as trichloroethylene and carcinogenic carbon tetrachloride from contaminated lands (Guerra *et al* 2011). Indian grass, which is frequently found growing along roadsides, has a remarkable ability to detoxify typical agrochemical residues, including widely used pesticides and herbicides relevant to atrazine and metalochlor (Nedunari *et al* 2009). Sunflower also showed greater potential to extract heavy metals (Pb and Zn) from contaminated sites due to its fast growth with less effect on its productivity (Rivelli *et al* 2012). Moreover, tree species exhibit characterstics like enormous biomass productivity, rapid growth, well-established extensive root system,

significantly higher transpiration rate, high genetic variability and have innate capacity to remediate a variety of contaminants that make them suitable for successful phytoremediation practices (Suman *et al* 2018). Numerous studies have been conducted with *Populus*, *Salix*, *Picea* and *Pinus* that confirm their remarkable potential to remediate polluted sites (Gomez *et al* 2019). Hence, present study has been conducted on *Salix alba* and *Toona ciliata* to study their phytoremediation potential and associated mechanisms.

Arborescent willows are good candidates for phytoremediation owing to their perennial nature, high biomass under short rotation, ease of vegetative propagation coupled with high coppicing ability and absence of linkage with food chain (Thakur *et al* 2015, Malik *et al* 2020). *Salix* holds huge potential for nutrient cycling, phyto-remediation of sewage/polluted water along with biological control of soil erosion and siltation (Marmiroli *et al* 2011, Mleczek *et al* 2017). Willow species/clones that are adapted to soil with various levels of metal contamination, have been documented to exhibit remarkable genetic variability (Yang *et al* 2015). However, a systematic research approach to explore the untap phytoremedial potential in *Salix alba* (L.) of Salicaceae family is highly desirable. *Toona ciliata* M. Roemer is a fast growing and large deciduous tree belongs to family Meliaceae, known to produce high biomass and its versatile timber used for building houses and ships, musical instruments, furniture and carvings (Kundal *et al* 2020). Although *T.ciliata* has some medicinal properties, but their alternative use in the timber production will prevent the phytoremediated heavy metal entry into food chain. Since, the phytoremediation potential of *T. ciliata* has not been reported yet, the present study was undertaken to study the uptake, translocation and metal accumulation efficiency of *T. ciliata.*

Holistic studies encompassing uptake, translocation and accumulation coupled with central thrust on validation of plant species and heavy metal interactions is highly decisive, because many morpho-physiological and anatomical traits are expected to be linked by trade-off relationships where expression of one trait will govern the expression of another. Hence, an attempt has been made to analyze the phytoremediation efficiency of *S. alba and T. ciliata* raised on heavy metal contaminated soils so as to investigate the interactive effect of Pb and Cd on morpho-physiological and anatomical characteristics of plant. The present study was taken up with the following objectives:

i. To study the effect of heavy metals on morpho- physiological and anatomical traits of *Salix alba* and *Toona ciliata.*
ii. To analyze the partitioning of heavy metals in different plant parts i.e. root, stem and leaves.
iii. To analyze the physico-chemical properties and heavy metal content in soils at the beginning and termination of the experiments.

CHAPTER - II

REVIEW OF LITERATURE

Industrial and anthropogenic activities are the major reason for heavy metal pollution. To date, thousands of hectares of farmland globally and in India specifically have been contaminated by heavy metals (Riyazuddin *et al* 2021). This has adversely affected the crop productivity, soil microbial diversity and eventually deteriorated the soil quality. Soil quality is closely associated with crop quality, human health and welfare (Nagajyoti *et al* 2018). Therefore, the remediation of these metal-polluted soils becomes imperative. Phytoremediation, or the use of plants to extract or eliminate metal contaminants from contaminated soil, has proven to be a successful and cost-effective technical solution (Cioica *et al* 2019). Tree species have been recommended for phytoremediation of heavy metal contaminated soil because they are perennial and have a substantial biomass, genetic variability, established management strategies, economic value, public acceptance, and site stability (Pulford and Waston 2003).

To gain the good phytoremedial potential, plants must accumulate considerable amount of heavy metal ions along with large biomass production under heavy metal contamination (Sharma *et al* 2012). Thus, understanding the whole mechanism of phytoremediation along with bioavailability, uptake, translocation, sequestration and different defense mechanisms will help to select heavy metal stress-resistant and highly efficient plant species for phytoremediation. Plants develop various morpho-physiological and anatomical adaptation mechanisms in response to heavy metal stress. The morphological changes are usually preceded by changes in the physiological attributes of plants, thus long term tolerance to heavy metal stress may be related to the degree at which physiological functions are affected (Seth *et al* 2012). A review of literature relevant to the present study entitled "Evaluation of morpho-physiological and metal accumulation potential of *Salix alba* L. and *Toona ciliata* M. Roemer grown on heavy metal contaminated soils" has been documented under the following headings:

2.1 Heavy metal pollution status in India and Punjab

2.2 Phytoremediation strategies and associated mechanism

2.3 Effect of heavy metals on growth and biomass attributes of plants

2.4 Effect of heavy metals on plant physiological, biochemical and anatomical traits

2.5 Effect of heavy metals on uptake and transport of macro- and micro-nutrients

2.6 Effect of heavy metals on soil physico-chemical properties

2.1 Heavy metal pollution status in India and Punjab

As per the World Health Organization (WHO), the common toxic 'heavy metals' of public health concern are arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr),

mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), manganese (Mn), copper (Cu) and molybdenum (Mo), The standards for heavy metals in soil, plant and water as per Bureau of Indian standards (BIS) and WHO have been presented in Table 2.1.

Table 2.1 Normal and critical range of heavy metals in soil, plant and water

Heavy metals	Normal range in soil (mg/kg)	Critical soil total concs (mg/kg)	Normal range in plant (mg/kg)	Critical concentration in plants (mg/kg)	Permissible limit in water (mg/kg)
Arsenic	0.1-40	20-50	0.02-7	5-20	0.01
Cadmium	0.01-2.0	3-8	0.1-2.4	5-3	0.003
Cobalt	0.5-65	25-50	0.02-1	15-50	0.05
Chromium	5-1500	75-100	0.03-14	5-30	0.05
Mercury	0.01-0.5	0.3-15	0.005-0.17	1-3	0.001
Nickel	2-750	100	0.02-5	10-100	0.02
Lead	2-300	100-400	0.2-20	30-300	0.01
Selenium	0.1-5	5-10	0.0001-0.2	5-30	0.01
Manganese	20-10000	1500-3000	20-1000	300-500	0.3
Copper	2-250	60-125	5-20	20-100	1.5
Molybdenum	0.1-40	2-10	0.03-5	10-50	0.07

Data from BIS (2012), Briffa *et al* (2020)

In India, the hazardous metals viz., Cr, Cd, Hg, Zn, Pb and Ni and minerals such as As and F contamination is become an important national issue (Shanker and Sahni 2014). The persistence of toxic heavy metals in the environment results in their interference into human system, primarily through contaminated food, water and air, which causes both visible and hidden health issues. Kaur *et al* (2021) reported the heavy metal contamination (Cd, Cr, Co, Cu, Pb, and Zn) by assessing toxic ion concentration in untreated and treated effluents of two textile industries along with agricultural soil samples in the vicinity of these industries located in Ludhiana, Punjab (India). However, the hazard index was observed to be less than 1, indicating there was no potential health risk of heavy metals in soil samples.

Krishan *et al* (2021) reported through heavy metal pollution index (HPI) spatial distribution map that main hazardous zones have been found in the Firozepur and Ludhiana districts. Kumar *et al* (2019) reported that heavy metal concentration in agricultural soil

were lower than the maximum permissible limits due to intensive agricultural practices, sandy texture of soil and leaching of metals to lower ground layers. The reduced heavy metal contamination in agricultural soil was also due to the cultivation of crops such as sugarcane and sorghum which were able to accumulate heavy metals (Cr, Cd and Pb) and have bioaccumulation factor (BAF) near 1 (Bhatti *et al* 2016). Thus, cost-effective, efficient and environmentally acceptable remediation approaches to reclaim heavy metal-contaminated soil are needed.

Geogenic and anthropogenic activities are mainly responsible for heavy metal pollution; hence the major contributors of heavy metal pollution in the environment are listed in Table 2.2

Table 2.2 Major sources of heavy metals

Heavy metal (s)	**Contributors of heavy metals in the environment**
Arsenic	Volcanic eruptions, semiconductors, smelting coal mines, power plants, petroleum refining, metal adhesives, ammunition, wood preservatives, pesticides and herbicides, animal feed additives
Copper	Biosolids electroplating, mining activities, petroleum refining and smelting operations
Cadmium	Geogenic sources, metal smelting and refining process, combustion of fossil fuels, fertilizers, sewage sludge
Chromium	Sewage sludge, solid wastes, electroplating, tanning industries
Lead	Mining and smelting of metalliferous ores, leaded gasoline combustion, sewage and industrial waste, paints
Mercury	Volcanic eruptions, wild forest fires, emissions from industries producing caustic soda, combustion of coal, peat and wood
Selenium	Coal mining, oil refineries, fossil fuels, glass manufacturing industry, varnish and pigment formulation
Nickel	Volcanic eruptions, forest fire, landfilling operations, oceanic gaseous exchange, weathering of soils and geological processes
Zinc	Smelting and refining industries, mining operations, electroplating industry, bio solids

Source: Briffa *et al* (2020), Kaur *et al* (2021)

Geogenic processes such as biogenic, terrestrial, volcanic processes, erosion, leaching and meteoric are the main sources of heavy metals in the environment (Muradoglu *et al*

2015). While, industrialization, urbanization and modernization of the agricultural sector are substantially contributed to the release of heavy metal pollutants into the surrounding which get deposited on the soil through natural processes of sedimentation and precipitation (Dixit *et al* 2015). In addition, anthropogenic processes such as irrigation with sewage and industrial wastewater, mining activities, excessive application of pesticides and fertilizers, have disturbed the natural balance of geochemical cycles, which in turn has resulted in the entry of heavy metal into the soil (Zhang *et al* 2011).

2.2 Phytoremediation strategies and associated mechanism

Plants remediate the heavy metal polluted soil by adopting different strategies such as phytoextraction, rhizofiltration, phytostabilization, phytovolatilization, phytostimulation or phytodegradation (Pathak *et al* 2020, Pulford and Watson 2003). These are described as:

Phytoextraction: Phytoextraction is the process of the uptake and storage of heavy metals from the soil by the plants, there are two fundamental ways of phytoextraction:

i) **Natural**: The natural way of removal of heavy metals by the plants, also known as unassisted phytoremediation.

ii) **Assisted**: Microbes, plant hormones and chelating agents assist the plant in the remediation of heavy metal polluted soils.

Natural phytoremediation can be accomplished by either 1) hyperaccumulator plants or 2) genetic engineering of the plant with certain characteristics of hyperaccumulators for the accomplishment of phytoextraction (Chaney *et al* 2005). The hyperaccumulator plants are the plants whose tissues can contain certain heavy metals from 1,000 to 10,000 mg kg^{-1} (Bakers 1981). They can collect and concentrate the heavy metals in the harvestable tissues, biomass without affecting the plant growth. The heavy metal concentration in the hyperaccumulator plants is approximately 100 times higher compared to the ordinary plants. It is approximately 1,000 mg kg^{-1} for arsenic and nickel, 100 mg kg^{-1} for cadmium, and 10,000 mg kg^{-1} for zinc and manganese. The most prominent example of hyperaccumulator plants are *Arabidopsis, Alyssum, Noccaea* and the members of Brassicaceae family.

Rhizofiltration: Rhizofilteration, also called phytofiltration, a remediation approach in which plant roots are mainly responsible to remove contaminants from polluted or waste or water (Mesjasz-Przybyłowicz *et al* 2004). During this process, heavy metals adsorbed onto the root surface and can be absorbed by roots. Root exudates can alter rhizosphere pH, resulting in heavy metal precipitation on plant roots and a reduction in heavy metal transport to subterranean water (Javed *et al* 2019). The plants used for rhizofiltration are initially hydroponically cultivated in clean water to build a big root system, and then waste water is utilized to acclimatize the plants. After that these plants are transferred to the polluted site for heavy metal removal, and the roots are harvested and disposed of once they becomes saturated (Wuana and Okieimen 2011). Plants utilised for rhizofiltration should ideally have a

dense root system, produce a lot of biomass, and be resistant to heavy metals. Rhizofiltration can be done with both terrestrial and aquatic plants. Commonly used aquatic plants for remediation of polluted water are hyacinth, duckweed, azolla, cattail and poplar due to their high heavy metal accumulation, high tolerance to heavy metals along with fast growth and high biomass production (Hooda 2007). When compared to aquatic plants, terrestrial plants like Indian mustard (*B. juncea*) and sunflower (*H. annuus*) with longer and dense hair root systems have good heavy metal accumulating abilities during rhizofiltration (Dhanwal *et al* 2017).

Phytostabilization: Phytostabilization involves complexation, precipitation, sorption or metal reduction (Ghosh and Singh 2005). Plants restrict the movement of the metals in the roots by the assimilation, aggregation, adsorption and precipitation. They also help to avoid movement of the metals through water, wind, drainage and dispersion of soil (Marques *et al* 2009). Heavy metal precipitation or valence reduction in the rhizosphere, absorption and sequestration within root tissues, or adsorption onto root cell walls can all contribute to phytostabilization (Gerhardt *et al* 2017). In this, there is the aggregation of metal by roots or root exudates that immobilise and lower the accessibility of the soil pollutants. Chromium and lead are toxic metals that are remediated by phytostabilization. The proficiency of the phytostabilization is increased by the addition of nutrients to soil *viz.* lime and phosphate. *Brassica juncea* has been reported to be an efficient phytostabilizer as it accumulates chromium in the roots (Bluskov and Arocena 2005).

Phytovolatilization: The release of metal pollutants to the atmosphere by the plants in altered or unaltered form after metabolic and transpirational pull is called phytovolatilization (USEPA 1999). Selenium, arsenic and mercury are the main metal pollutants that can be remediated through phytovolatilization (Dietz and Schnoor 2001, Yan *et al* 2020).

Phytodegradation: Plants and associated microbes are used in phytodegradation (also known as phytotransformation) to decompose organic contaminants in the soil or within the plant's body (Govere 2021). The plant roots secrete enzymes that break down the organic chemicals, which are subsequently taken in by the plant and expelled by transpiration. This method works best for remediation of organic pollutants such as herbicides, trichloroethylene and methyl tert-butyl ether (Pulford and Watson 2003).

Mechanism of phytoremediation

The phytoremedial mechanism involves bioavailability, uptake, translocation, sequestration and different defence mechanism that can help to develop heavy metal stress-resistant cultivars (Fig. 2.1) and highly efficient plant species for phytoremediation through genetic engineering technologies.

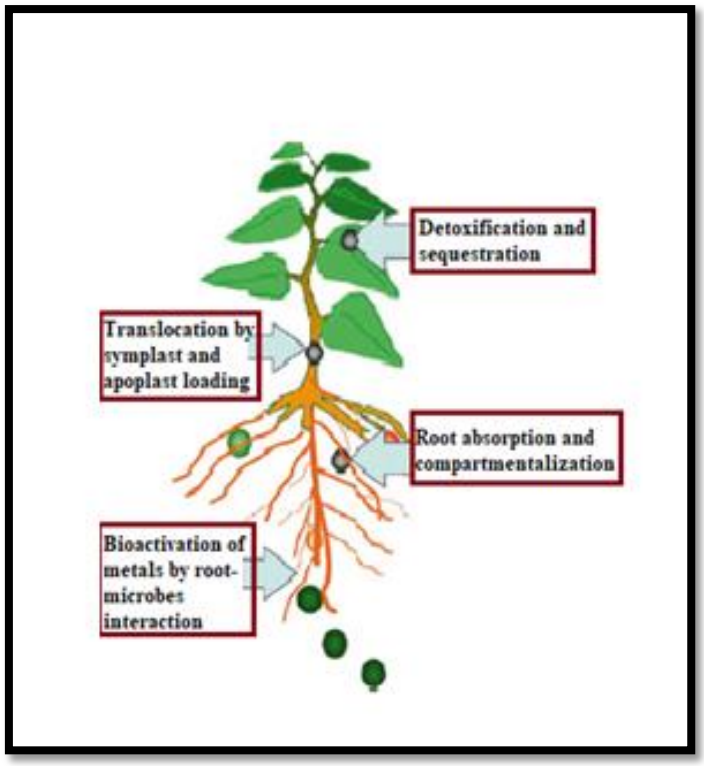


Fig 2.1: Series of processes involved in phytoremediation

2.4.1 Heavy metal bioavailability in soil: It is defined as a part of the total elemental concentration available to plants that determines the uptake and accumulation of heavy metal ions in plants. Heavy metals exist in soils with several degrees of fractions i.e. soil solution form, soluble metal complexes and free metal ions forms. Several factors that determine the bioavailability of heavy metal elements are environmental conditions (moisture, temperature and oxidation state), soil properties (pH and organic matter) and enhanced biological activity by microbes (Yang *et al* 2012, Bravin *et al* 2012).

2.4.2 Heavy metal uptake by plants: The movement of heavy metals in soils depends upon precipitation, redox potential, absorption/ adsorption and its complexation/methylation responses mediated by microbes along with plants (Kumar *et al* 2017). The mechanism of plant metal uptake, rejection, translocation and sequestration is specific and highly variable within the plant varieties (Lone *et al* 2008). Plants adopt two main strategies to combat heavy metal stress by either reduce metal uptake or increase vacuolar sequestration.

i) Bioactivation of metals by root-microbe interaction

Several studies depicted the positive interaction of microorganisms with plant species in the rhizosphere (Dakora and Phillips 2002, Kuiper *et al* 2004). Plant growth promoting rhizobacteria increase the bioavailability of metal ions by dissolving them *via* changing the chemical properties (pH, redox state, organic matter) of soils in the rhizosphere and modify the heavy metal speciation in the root zone. They solubilize the ions like phosphate, siderophore and increase acid production (Jing *et al* 2007).

ii) Root absorption and compartmentalization

The transport of nutrients and heavy metals from soils to plant roots occurs via symplastic and apoplastic transport. In symplastic transport heavy metals enters the root cells through the plasma membrane of the endodermis of the root. While in apoplastic transport, it enters the root apoplast *via* spacing within the cells. Generally, heavy metals and nutrient ions cross the membranes only with the aid of naturally occurring membrane transport proteins. The abundance of these proteins depends upon tissue type and environmental conditions. If a small amount of nutrients is present in soils, then the plant requires high-affinity transporters for uptake; whereas if the nutrients in the soil are present in high concentrations (e.g. agricultural soils with fertilizers), then low-affinity transporters would be more useful for plant uptake (Cailliatte *et al* 2010). Several transporter families have been reported in plants such as heavy metal ATPase (HMA), natural resistance and macrophage proteins (NRAMP), Zrt, Irt-like proteins (ZIP) etc. In the cytosol, toxic metals rapidly bind to chelators and are transferred to the vacuole for sequestration (Kumar *et al* 2017).

iii) **Translocation:** Heavy metal transporters are required for translocation of metallic ions from root symplast to xylem apoplast due to endodermal barrier (casparian strips) in the root. The translocation of heavy metal ions depends on two factors: root pressure and leaf transpiration (Ali *et al* 2016).

a. Root symplast to apoplast through xylem tissues: Xylem loading of metals from root symplast is an important phenomenon making the plant to tolerate heavy metal toxicity instead of promoting its accumulation in root cells that would inactivate the enzymes involved in metabolic processes (Yan *et al* 2020).

b. Root apoplast to aerial (stem and leaves) tissues: Hyper accumulator plants rapidly translocate the absorbed metal ions from the root to the above-ground parts, while non-accumulators accumulate heavy metals only in their root portions. Heavy metals can be stored in root vacuoles, but due to the limited space and high heavy metal concentration in the soil matrix, it gets translocated to shoot tissue where sequestration and detoxification rate is comparatively high (Kumar *et al* 2017). Generally, metals are stored in only chelated form but are transported from one cellular compartment to other in free ionic state according to the selectivity of transporter proteins. Research experiments showed that hyperaccumulator plants accumulate high concentration of heavy metals in stem and leaf vacuoles than the root tissues. In the leaf tissues, high amount of metals accumulates in epidermal tissues compared to the cortical and vascular tissues (Kupper *et al* 2001).

The ability of plant to uptake, translocate and accumulate heavy metals in different tissues can be determined by bioconcentration factors (BCF) and translocation factors (TF). Bioconcentration factor (BCF) and translocation factor (TF) are the two important parameters that provides an insight towards the phytoremediation potential of a plant (Baker 1981). BCF

defines the relationship between the concentration of element in plant parts and the substrate, thus reflects the elemental accumulation (Yoon *et al* 2006). Further, depending on the BCF and TF values, the plant accumulation efficiency was determined as one of four groups: BCF >1: intensive; BCF=1–0.1: medium; BCF = 0.1–0.01 weak and BCF = 0.01–0.001: no accumulation (Kabata-Pendias and Pendias 1999). TF determines the efficiency of plants to translocate any element or metal from the root to the aerial plant parts. A plant is considered efficient in metal translocation if TF is higher than one indicating an efficient metal transport system (Gajic *et al* 2018).

iv) **Sequestration/ Detoxification:** To cope up with heavy metal stress, plants adapt different survival strategies like compartmentalization, exclusion, complexation and synthesis of binding proteins such as metallothioneins and phytochelatins (Aslam *et al* 2021). Heavy metal toxicity inside the plant cell gets detoxified by complex formation and compartmentalization to make them less available to metabolic active sites. Organic acids, glutathione precursor of phytochelatins and metallothiones play a significant role in detoxification/sequestration (Zhu *et al* 2004). Phytochelatins (PC) have an imperative role to detox cadmium in fungi and plants through conjugation. Glutathione enhance the PC synthesis and thus more PC-metal complex formation in the vacuole which ultimately enhance cadmium tolerance in plants (Lee *et al* 2003). Metallothiones are metal-binding proteins that modulate the concentration of metals inside the cell by binding heavy metal ions to cysteine and thiol groups (Khan *et al* 2004).

Heavy metal toxicity hindered the functional group of important molecules that disrupt the metabolic enzyme activity and consequently inhibit or suppress photosynthetic rate, respiration rate and all physiological and biochemical processes of plants (Gupta *et al* 2015, Ali *et al* 2013). Naturally, plants develop various defense mechanisms against heavy metal stress inside the plant body which include compartmentalization reduction, suppression of high-affinity phosphate transport system, sequestration and translocation (Zhao *et al* 2009). When metal ions cross enters into plant tissues by crossing these barriers then various cellular defense mechanisms (as a second line of defense *viz.*, ROS production, antioxidants) are initiated to detox the adverse effect of noxious heavy metals (Silva and Matos 2016).

2.3 Effect of heavy metals on growth and biomass attributes of plants

Lead and cadmium are considered as non-essential element for plants, because their exposure causes general growth abnormalities and inhibition along with biomass reduction in many plant species (Zhang *et al* 2019). The effect of heavy metal induced toxicity can be observed in plant tissues at all developmental stages starting from seed germination to senescence; however the Pb and Cd toxicity reported to be more pronounced during germination and root growth (Aslam *et al* 2021).

As seed germination is depend upon rhizosphere conditions, heavy metal contamination has been linked to decrease in seed germination, seed vigour as well as

subsequent seedling growth (Adrees *et al* 2015). As previously reported by Zulifiqar *et al* (2019), Pb-toxicity has been shown to have negative effects on seed morphology, physiology, germination and early crop growth in a various crops such as *Lens culinaris*, *Oryza sativa*, *Hordeum vulgare* and *Zea mays*. Exposure of plants to other heavy metals, in addition to Pb and Cd known to had similar effects on seed physiology and germination (Yan *et al* 2020). Previous research studies evidenced that heavy metal toxicity cause inhibition of certain enzymes such as amylase and protease that are important for seed germination, thus their inhibition affects the seed germination along with hypocotyl and radical growth (Sengar *et al* 2010).

Changes in root architecture have also been reported in plants growing in heavy metal contaminated soils. After long-term Cd exposure, roots become necrotic, decaying and mucilaginous, limiting root and shoot elongation and producing leaf rolling and chlorosis (Abbas *et al* 2017). In the presence of heavy metal such as Cu, Pb, Cr, Zn, and Cd stress, decreased root elongation and increased lateral root formation have been observed in *Arabidopsis thaliana, Triticum aestivum, Sesbania cannabina, Pinus sylvestris* and *Lupinus luteus* (Sofo *et al* 2017, Riyazuddin *et al* 2022). Cd contamination in the soil rhizosphere inhibits the production of lateral roots and causes the formation of rigid, twisted and brown roots due to disruption in cortical and epidermal cell enlargement (Krantev *et al* 2008). Cd toxicity also reported to reduce the mitotic division of meristematic cells that leads to reduction in root length, density and ultimately affects root biomass (Gratao *et al* 2009). In contrary, Ismael *et al* (2018) reported that under Cd stress, increased root diameter due to increase in cortical cell size provide plant resistance to solute movement and water.

Pb and Cd accumulation in plants alters the leaf phenology with modification in chlorophyll ultra-structure having low pigment concentration that leads chlorosis and reduced photosynthetic activity in rice (Miyadate *et al* 2011). A considerable reduction in total leaf area and dry weight of numerous plant components has also been reported under Cd stress, which confers the decreased potential of resource storage i.e. mineral, nutrient and water (Jinadasa *et al* 2016). Similarly, Pb accumulation negatively affects the germination, root and shoots biomass due to poor nutrient uptake and transport (Ali *et al* 2014). Under heavy metal toxicity, a decrease in leaf number, area, and biomass has been observed in a variety of plants, including *Albizia lebbeck* (Tripathi and Tripathi 1999), *Arabidopsis thaliana* (Sofo *et al* 2017), *Acacia holosericea* (Shanker *et al* 2005), *Prosopis laevigata* (Buendía-González *et al* 2010), *Phyllostachys pubescens* (Liu *et al* 2015) and *Leucaena leucocephala* (Riyazuddin *et al* 2022)

2.4 Effect of heavy metals on plant physiological, biochemical and anatomical traits

Under heavy metal stress, almost all the plant tissues show alteration in their morphology, ultra structure along with their physiological mechanisms (Aslam *et al* 2021).

Plants under heavy metal stress have stunted growth due to reduced nutrition and water uptake, photosynthesis, respiration, nitrogen and carbon assimilation, and antioxidant activity (Rizwan *et al* 2017), which is responsible for reduction in crop quality and production (Rizwan *et al* 2016). In case of poplar, jatropha (Shu *et al* 2012), maize (Hayat *et al* 2012), wheat and rice (He *et al* 2014), it has been reported that heavy metals transported from contaminated soils via roots to aerial parts and their accumulation in plant cells interfere directly with the cellular metabolism of shoots, resulting in a reduction in height.

In response to heavy metal stress, the normal physiological mechanism of plants affected that is responsible for further morphological changes and ultimately affect plant survival rate. Heavy metal accumulation in endodermis and pericycle triggers the production of lateral roots (Muszynska *et al* 2019). However, the lateral root generation is considered as first indication of heavy metal stress, which then inhibits water absorption and conduction, resulting in decreased photosynthate transfer to roots (Zhao *et al* 2021). During heavy metal stress, growth of primary and secondary roots is inhibited because roots get low levels of photosynthates, resulting in a shorter root length and high root/shoot area ratio (Rucinska-Sobkowiak 2016). Under heavy metal toxicity, genes involved in IAA production were shown to be upregulated, resulting in increased auxin content and auxin/cytokinin ratio, considered as essential variables to determine morphological alterations in roots (Sofo *et al* 2013).

The accumulation of heavy metals in plants causes negative alterations in the principal photosynthetic organ leaves, implying a decrease in photosynthesis rate. Furthermore, heavy metal accumulation in leaves has been found to disassemble chlorophyll molecules by interacting with the central Mg atom of the porphyrin ring, resulting in photosynthesis suppression and affecting overall growth in sunflower seedlings (Yadav *et al* 2014). According to Gao *et al* (2020), the effect of heavy metals on photosynthetic machinery is influenced by the reactivity and concentration of heavy metals in leaves, which impacts light capture, electron transport and the activities of photosynthetic enzymes like RuBisCO. Based on the evidence in the literature, it can be concluded that heavy metals can target chlorophyll in one of three ways: (1) by increasing the activity of the chlorophyllase enzyme (Latif *et al* 2020); (2) by causing the oxidation/reduction of chlorophyll molecules due to heavy metal toxicity-induced ROS (Moradi and Ehsanzadeh 2015); and (3) inhibition of chlorophyll biosynthesis because some heavy metals, such as Hg, Cu, Pb, Ni, Cd, and Zn, can replace the central Mg atom of the porphyrin ring (Sharma *et al* 2019).

Heavy metal toxicity causes the accumulation of reactive oxygen species (ROS), which interact with different plant macromolecules including DNA, lipids and proteins, and lipids, resulting in a cascade of processes known as oxidative stress (Sharma and Dietz 2009). Heavy metal stress-induced ROS production not just provokes the peroxidation of chloroplast membranes, but it also reduces the uptake of critical elements required for the synthesis of

photosynthetic pigments like Mg, K, Ca, and Fe (Sharma *et al* 2012), as reported under different heavy metal stress including Pb, Cd, and Cr in *Brassica napus* (Afshan *et al* 2015), Cu, Pb, and Cd toxicity in wheat (Rizwan *et al* 2016). A large number of studies reported that heavy metals change the plant cellular redox state in five different ways (Hossain *et al* 2012, Shahid *et al* 2014):

a. Modification in cellular redox potential by shifting to more oxidized form
b. Direct generation of ROS through Haber-weiss cycle or Fenton's reactions
c. Consumption of reduced glutathione (GSH) that is considered necessary for heavy metal chelation and detoxification or sequestration of toxic metals into vacoules through ABC transporters
d. Direct inhibition of antioxidant enzymes activity by altering their binding sites
e. By increasing NADPH oxidase activity

Heavy metal interferes with several proteins and further cause their down regulation and inhibition of essential physiological activities such as respiration and photosynthesis (Anjum *et al* 2016). Heavy metal stress in plants further induces oxidative stress which leads to activation of antioxidants and osmoregulation system through accumulation of osmolytes and compatible solutes to keep stability in cell osmoticum (Riyazuddin *et al* 2022). These compatible solutes include proline, total soluble sugars and some ammonium compounds (glycine betaine, proline betaine, alanine betaine, and polyamines) to provide osmotic adjustment, macromolecule stability, metal chelation and detoxification (Sharma *et al* 2019). In case of *Brassica juncea* (Chowardhara *et al* 2020) and hybrid poplar (*Populus trichocarpa deltoides*) (Nikolic *et al* 2008), increased level of proline content has been reported under heavy metal stress. Research experiments revealed that exogenous proline application also helps to restore the membrane integrity and growth inhibition under Cd toxicity in cultured tobacco Bright Yellow (BY-2) cells (Islam *et al* 2009). De carvalho *et al* (2013) reported the significant relationship between proline and enzymatic antioxidants through their study on transgenic *Swingle citrumelo* plants having pyrroline-5-carboxylate synthetase (P5CS112A) gene showed higher endogenous proline production which further increased the expression of various antioxidant isoforms such as chloroplastic glutathione reductase, cytosolic ascorbate peroxidase and superoxide dismutase, hence increased tolerance to particular heavy metal stress conditions.

Phytochelatins and metallothioneins are biochelators that can bind with several heavy metal elements like Cd, Zn, Ni, Cu etc. thus reduces their toxicity by prevent their ion entrance to plant metabolism (Aslam *et al* 2022). These biochelators also enhance the plant tolerance to heavy metal stress by increasing the activity of antioxidant enzymes such as SOD and POD, thus decreasing the generation of hydrogen peroxide and protect the plants from heavy metal induced oxidative stress (Zhou *et al* 2014). Under Cd toxicity, Chowardhara *et al*

(2020) found accumulation of non-enzymatic antioxidants like ascorbate, proline and glutathione as well as enhanced antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX) and peroxidase (POD) in *B. juncea* cultivars implying that these antioxidants plays a key role in heavy metal stress tolerance.

Heavy metal toxicity induces ultra-structural changes in all plant parts, viz. root, stem and leaves which is responsible for modification in their normal functioning. It has be reported that Cd and Cu toxicity has been linked to increased lignification in both roots and shoots resulting in lower biomass accumulation due to impaired cell development, and a larger production of trichomes on the leaves and stems to sequester excess of toxic ions (Nair and Chung 2015, Martins *et al* 2020). Su *et al* (2020) reported that lignin plays a crucial role to provide heavy metal stress tolerance in rice and suggested that under heavy metal stress enhanced lignin synthesis considered as an adaptive strategy of plant. Heavy metal toxicity affects the leaf area, number, pigmentation concentration and thickness by disrupting plant-water relations, which in turn impacts physiological processes like transpiration and photosynthesis (Alsokari and Aldesuquy 2011). The stomatal index of *Beta vulgaris* (Sagardoy *et al* 2010) and *Sorghum vulgaris* (Kasim 2006) was shown to decrease under Zn and Cd-Cu stress, whereas increased number of stomata has been recorded in the presence of Pb and As in several plants, such as *Helianthus annuus* and *Vigna radiata*, at the early stages of metal toxicity, which was followed by the formation of fused and deformed stomata in *Vigna radiata* (Gupta *et al* 2015). In *Zea mays*, exposure to Zn and Cd resulted in the development of abnormal and non-functional stomata (Souza *et al* 2005). Furthermore, as seen in *Brachiaria decumbens* under Cu stress, heavy metal exposure causes reduction in parenchymatous tissues and xylem vasculature, as well as the production of relatively smaller mesophyll tissue (Gomes *et al* 2011). All these heavy metal toxicity induced alterations in the stomata, xylem vessels, parenchymatous, and mesophyll cells impair the plant-water relationship, resulting in decreased leaf growth (Riyazuddin *et al* 2022).

2.5 Effect of heavy metals on uptake and transport of macro and micro-nutrients

The rhizospheric soil is an optimal growing medium for plants which includes essential necessities like micro- and macronutrients, as well as water that are required for plant growth and development. Since, the water and nutrient availability in soil depends upon soil chemistry, thus heavy metal contamination can degrade the nutritional status of soil and water that further affect ecological stability (Siyar *et al* 2020). Heavy metal contaminated soil also affect the ability of plants to absorb water and nutrients which further leads to mineral and nutrient deficiencies in plants (Ali *et al* 2015). Heavy metal and nutrient interrelationship is a complex phenomenon, which depends on the type of soil and varies among species to species. Further, it also depends on the type of mineral and heavy metal element, nutrients

having cationic potential interact with anionic heavy metals and form complex which then unable to fulfill the nutrient requirements of plants, hence, cause nutritional deficiency (Hossain *et al* 2012).

The presence of one heavy metal in the soil as well as in plant has a significant impact on the availability of another nutrient element which suggests that heavy metal has both antagonistic and synergistic behaviours (Chibuike and Obiora 2014). Ahmad *et al* (2011) reported that Pb concentration (0.01-1.0mg/ L) significantly reduces K and Cu concentration in root, shoot and leaves of maize genotypes. Li *et al* (2016) reported that heavy metal interacts with other nutrients such as Zn, Fe, Cu, Ca, N, P and K, and malfunctioned the absorption of nutrients by roots either through immobilization or decreased uptake which results in nutrient deficiency in plants. Roots and root hairs are primary organs that directly come in contact with soil heavy metals, which get injured due to high concentration of heavy metal that may also results to abnormalities in optimum water and nutrient uptake and ultimately cause nutrient deficiency and water deficit (Singh *et al* 2016). Thus, all these abnormalities further responsible for reduced plant growth and limited production. Gupta *et al* (2013) reported that cadmium contamination induce inhibitor affect for Mn and total amount of mineralized carbon status of plants and soil. Similarly, it has been demonstrated that Cu, Zn, Ni and Cd compete for same membrane carriers for translocation in plants. Lamhamdi *et al* (2013) reported that Pb stress reduced that uptake of micronutrients such as Na, K, Ca, P, Mg, Fe, Cu and Zn in *Triticum aestivum* and *Spinacia oleracea*. Similarly, Wang *et al* (2011) found that Pb contamination leads to decreased concentration of P, K and Mn in *Vallisneria natans* and suggests antagonistic relationship among them. Liu *et al* (2010) found that Cd stress negatively correlates with Mn^{2+} ions and further Mn deficiency leads to chlorosis and necrotic lesions in leaves of *Oryza sativa.* Yoshihara *et al* (2006) reported that Cd stress induce Fe deficiency along with reduced uptake of K and Ca ions in *Nicotiana tabacum*.

Heavy metal toxicity not only disturbs the nutrient uptake and translocation, but it also affects the water status in soil as well as in plants (Siyar *et al* 2020). Heavy metal concentration above the threshold level in soil and plants results in water deficit conditions in soil as well as in plant tissues, thus, creates osmotic stress conditions which cause difficulty for plants to absorb optimum amount of water (Rucińska-Sobkowiak 2016), however, high accumulation of heavy metals in plant tissues results in irregular osmosis, transpiration and stomatal activities. Furthermore, Rucińska-Sobkowiak (2016) described that heavy metal induced water deficit and nutrient deficiency is a quite complex process which involves series of steps such as metal-mineral complex formation, competition with respective nutrients for active absorption by roots, osmotic deficit, root injury, accumulation in roots and subsequently in shoots and xylem blockage.

2.6 Effect of heavy metals on soil physico-chemical properties

Soil heavy metal pollution cause long-term hazardous effects on soil ecosystems and adversely affects physico-chemical properties such as pH, electrical conductivity, organic carbon and cation exchange capacity (Jacob *et al* 2018). Similarly, heavy metal accumulation in soil negatively influenced biological processes including soil microbial biomass carbon and soil enzyme activities (Kabata-Pendias 2010). Heavy metals have also been found to have significant positive relationships with various soil physical characteristics such as moisture content and water holding capacity. Meanwhile, heavy metal availability in soils has been demonstrated to be influenced by soil aeration, microbial activity, and mineral composition (Sharma and Raju 2013).

Metal behavior in soils is a dynamic process, once heavy metal enters the soil undergoes various processes that may exist as free ion or form complex with organic/ inorganic ligands in soil solution, adsorbed to soil surface or precipitates as oxides, hydroxides and carbonates (Bolan *et al* 2014). The available form of heavy metals in soil is depends on several physical and chemical processes like ion exchange, adsorption-desorption, complexation, precipitation and dissolution, oxidation-reduction, diffusion and migration, metal competition, biological mobilization and plant uptake (Wuana and Okieimen 2011). Soil parameters that influence the heavy metal binding and their bioavailability includes pH, soil texture, organic matter, cation exchange capacity (CEC), microbial activity, macro and micro nutrient content (Xu *et al* 2022).

Soil pH had great influence on metal sorption capacity that it increases with rise in pH, whereas at pH less than 5, metal mobility increased due to elevation in proton concentration (Zhong *et al* 2020). Thus, at low pH value the most of the metals can be found in solution and able to mobilize. Generally, at low pH values, free aqueous cations predominates, but with increase in pH these cations underdoes hydrolysis to form hydroxylated cation and then simple uncharged complex, whereas during alkaline conditions anionic hydroxyl species were found (Chaney *et al* 2010). Trace elements that predominantly exists in cationic form are Pb, Cu, Zn, Ni, Cd, Hg, Cr(III), Co, while other trace elements that present in anionic form includes As, Se, Cr(VI), Mo and B (Kabata-Pendias 2010). Soil pH is the most important factor influencing metal availability in soil, the availability of Cd and Zn to *Thlaspi caerulescens* roots decreased when soil pH increased (Wang *et al* 2006).

The physical, chemical, and biological properties of soil, as well as environmental functions, are highly dependent on soil organic matter (Li *et al* 2018). Soil organic matter is the total amount of organic carbon-containing substances in soil, which consist a plant and animal residues in various stages of decomposition, microbiological synthesized substances and/or chemically from of breakdown products, and the living and dead microorganisms and their decomposing remains. Soils with higher amount of clay particle and humus has high

buffer capacity, thus metal contamination does not affect the soil sorption capacity and further do not cause adverse biological effects (Siyar *et al* 2020).

Cation exchange capacity (CEC), dominant factor to study heavy metal retention in soil, defined simply as the sum total of exchangeable cations that a soil can adsorb or the number of cation adsorption sites per unit weight of soil expressed as centimoles per kg (cmol(+)/kg) (Aprile and Lorandi 2012). The CEC of soils depends on soil types, amounts and types of different colloids present and on the CEC of the colloids. Fine-textured (clay) soils tend to have higher cation exchange capacity and high total capacity towards metals than sandy soils (Moghimian *et al* 2017). Heavy metals are found in soil as individual constituents or in combinations with other soil components. These components constitute exchangeable ions sorbed on inorganic solid surfaces, nonexchangeable ions and insoluble inorganic metal compounds such as carbonates and phosphates, soluble metal compounds or free metal ions in the soil solution, metal complexes of organic materials, and metals attached to silicate minerals (Marques *et al* 2009). The immobilization of heavy metals by organic materials and hydrous ferric oxide has been shown to reduce their availability. The capacity of the soils for adsorbing heavy metals is correlated with their CEC, i.e. greater the CEC values more exchange sites on soil minerals will be available for metal retention (Zhen *et al* 2019). Competing ions can have marked effect on ion sorption by soils. In solution, metal cations such as Cu, Zn, Cd, and Pb compete with more abundant soil cations such as Ca^{2+} and Mg^{2+} for both nonspecific and specific exchange sites. Chen (2012) found that the presence of Pb did significantly reduce the adsorption of Cd on soils.

CHAPTER - III

MATERIAL AND METHODS

The present investigations entitled "Evaluation of morpho-physiological and metal accumulation potential of *Salix alba* L. and *Toona ciliata* M. Roemer grown on heavy metal contaminated soils" was carried out at Research Farm, Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana (30 º90'N, 75º85E) for two consecutive years 2020 and 2021. Detailed information of the experimental location, methodology adopted for conducting the field and laboratory experiments and observations recorded are discussed as follows:

3.1 Location

The experimental site is located at an elevation of 247 m above mean sea level in the central zone of Punjab and lies between 30º-50'N latitude and 75º-52'E longitude.

3.2 Climate

The climate of the region is typically subtropical to tropical, with a long dry season lasting from early October to June and wet season from July to mid-September. The region also experiences hot dessicating winds in the summer (May-June), severe cold in winters with intensive evapo-transpirational loss, meanwhile December and January are among the coldest months. From October to July, generally dry conditions prevail in the region of the experimental site, except for few light showers from northern-western depressions during the winters. Monthly meteorological data on rainfall (mm), relative humidity (RH%) and air temperature (°C) for the year 2020 and 2021 was obtained from School of Agricultural Meteorology, Punjab Agricultural University, Ludhiana (Fig. 3.1).

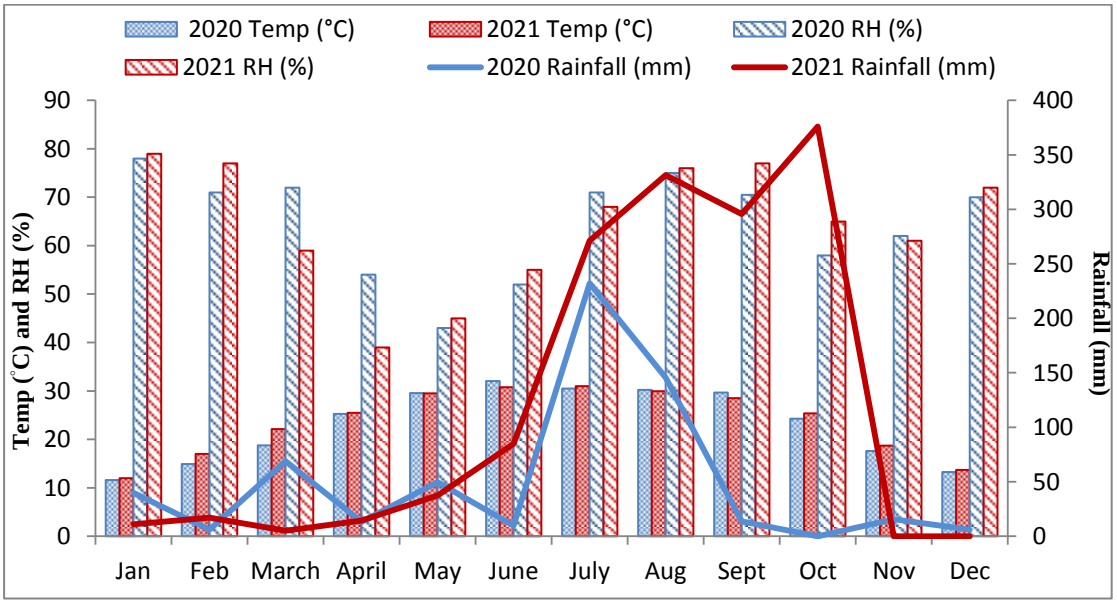


Fig. 3.1: Mean monthly meteorological data of study area during 2020 and 2021

3.3 Experimental design and Methodology

Salix alba (L.) and *Toona ciliata* M. Roemer were the two tree species selected for the present study. The germplasm of *S. alba* was obtained from Dr. Yashwant Singh Parmar University of Horticulture & Forestry, Nauni, Himachal Pradesh and *T. ciliata* seeds were collected from Research Farm, Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana. The physico-chemical characteristics of soil used for present study are given below (Table 3.1):

Table 3.1: The physico-chemical properties of soil used for experimentation

Characteristics	Content
Soil Texture	Loamy sand
pH	7.65
EC (dS/m)	0.483
Organic carbon (%)	0.42
Cation exchange capacity (cmol(+)/kg)	5.69
Available-N (mg/kg)	183
Available-P (mg/kg)	50.9
Available-K (mg/kg)	148
Total Pb (mg/kg)	17.1
Available-Pb (mg/kg)	0.91
Total Cd (mg/kg)	0.16
Available-Cd (mg/kg)	0.003

For each species, 480 pots filled with five kg of tested soils and were arranged in completely randomized design (CRD) for 16 treatment levels of lead (Pb) and cadmium (Cd), and three replications with a plot size of ten plants per replication. The different concentrations of Pb (100 mg/kg, 200 mg/kg and 300 mg/kg) and Cd (5 mg/kg, 15 mg/kg and 25 mg/kg) were applied as per the layout given below:

Table 3.2: Detailed list of heavy metal treatments used in present study

Treatments (mg/kg)	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}
Cd_0	Pb_0Cd_0 (T1)	$Pb_{100}Cd_0$ (T2)	$Pb_{200}Cd_0$ (T3)	$Pb_{300}Cd_0$ (T4)
Cd_5	Pb_0Cd_5 (T5)	$Pb_{100}Cd_5$ (T6)	$Pb_{200}Cd_5$ (T7)	$Pb_{300}Cd_5$ (T8)
Cd_{15}	Pb_0Cd_{15} (T9)	$Pb_{100}Cd_{15}$ (T10)	$Pb_{200}Cd_{15}$(T11)	$Pb_{300}Cd_{15}$ (T12)
Cd_{25}	Pb_0Cd_{25} (T13)	$Pb_{100}Cd_{25}$ (T14)	$Pb_{200}Cd_{25}$(T15)	$Pb_{300}Cd_{25}$ (T16)

	Control
	Pb concentrations
	Cd concentrations
	Combination (Pb+Cd) concentrations

The detail of experiment is as mentioned below:

Plant Species: *Salix alba* (L.) and *Toona ciliata* M. Roemer

Total plants = Treatments × Replications × Plants per replication × Plant species

= 16 × 3 × 10 × 2

= 960

For the preparation of heavy metal-enriched media, stock solutions of lead from $Pb(NO_3)_2$ and cadmium from $Cd(NO_3)_2$ were freshly prepared in the laboratory, and soil in pots treated with different levels of Pb and Cd. For uniform distribution of the heavy metals, the treated soil was equilibrated for about one month with mild irrigation as per the field capacity and samples were collected for analysis. A hole was drilled in all pots for proper aeration and plates were placed under every pot to collect the leachate; once pot plates filled with leachate it was again transferred in same pot to prevent the loss of metals from treated soils.

Fresh cuttings of 15-20cm length of *Salix alba* were prepared from one year plants and were planted in (one cutting/pot) treated soil in the month of February during 2020 and 2021, whereas nursery of *Toona ciliata* was first raised in small polybags and then transplanted when they attained the height of approx. 15cm in the pots containing treated soils in the month of February in the year 2020 and 2021. The experimental setup in nursery is shown in Plate I.

Experiment No. 1 Growth performance, physiological and biochemical analysis of tree species raised on different concentrations of heavy metals contaminated soils.

In this experiment, five plants per replication for each species (*Salix alba* and *Toona ciliata*) were uprooted at the intervals of three and six months for analysis of growth, physiological and biochemical parameters.

a. Growth and biomass attributes such as plant height, collar diameter, number of branches, number of leaves per plant, root length, root number, fresh and dry weight of both root and shoots were recorded.
b. Physiological and biochemical parameters from leaves such as pigment concentration, total soluble sugars, proteins, phenols, proline content and activity of antioxidant enzymes (peroxidase, catalase, superoxide dismutase) were estimated by standard methods.
c. The anatomical changes induced due to heavy metal stress in roots and leaves of *Salix alba* and *Toona ciliata* were determined by Lieca Bright Field Research Microscope and Field Emission Scanning Electron Microscopy (FE-SEM). Along with this, differential Pb and Cd accumulation sites in different plant tissues were analyzed using Energy Dispersive X-ray Spectroscopy (EDS).

Experiment No.2 To study the effect of heavy metals on its uptake, translocation and metal

accumulation potential of tree species

a. Pb and Cd concentrations were estimated from different plant parts (root, stem and leaves) of *Salix alba* and *Toona ciliata* and their soil (before planting and after uprooting of plants) after six months.
    i. Three plants per replication were uprooted, washed with normal tap water followed by 0.1 N HCl and deionized water. The washed root, stem and leaf samples were air dried followed by oven drying at 60^0C. After that these samples were grounded by stainless steel grinder and stored in paper bags for further heavy metal and nutrient analysis.
    ii. Approximately five grams of soil in replications were collected in the beginning (before plantating) and at experiment termination. The processed soil samples passed through 2mm sieve and used for heavy metal (Pb and Cd) and nutrient analysis.
b. The effect of heavy metals on macro-nutrient (N, P, K, Ca, Mg, S) and micro-nutrient profile (Mn, Zn, Cu, Fe) of different plant parts (root, stem and leaves) of *Salix alba* and *Toona ciliata* were investigated.
c. Fourier transformed infrared spectroscopy (FTIR) analysis was carried out to study the effect of heavy metal accumulation on functional group binding of plant (*Salix alba* and *Toona ciliata*) and soil samples ($Pb_{300}Cd_{25}$) with respect to control (Pb_0Cd_0).

Experiment No. 3 To investigate the effect of heavy metals on physico-chemical properties of soil.

a. The physico-chemical properties such as pH, electrical conductivity (EC), soil organic carbon (SOC) and cation exchange capacity (CEC) was determined from air dried soil before planting and at termination of experiment by using standard methods.
b. The effect of heavy metals on macro-nutrients (N, P, K, Ca, Mg, S) and micro-nutrient profile (Mn, Zn, Cu, Fe) of soils (before plantation and after uprooting of *Salix alba* and *Toona ciliata*) were investigated.

3.4 Observations recorded

The observations were recorded from three- and six-month old plants. Five plants per replication were selected for analysis of morpho-physiological traits, and recorded mean data is presented in tables.

3.4.1 Morphological data

a. Survival percentage (%): Plant survival percentage was recorded from the date of planting (Feb, 2020-21) till the end of experiment (Aug, 2020-21) at interval of 3 and 6 months by using formula:

$$\text{Survival percentage (\%)} = \frac{\text{Number of plants survived}}{\text{Total number of plants}} \times 100$$

Plate I: Experimental setup in nursery

b. Lead and cadmium spiked pots before plantation

c. Six month old *Salix alba* grown in lead and cadmium contaminated soil

d. Six month old *Toona ciliata* grown in lead and cadmium contaminated soil

e. Plant height (cm)

The height of plant was measured from the base till the tip of aerial plant part and expressed in centimeters (cm).

f. Collar diameter (cm)

Collar diameter of plant shoot was measured at the collar region using vernier caliper.

g. Number of branches

Total number of branches at the main stem of plant was counted manually.

h. Number of leaves

The average number of leaves of plant was counted on each plant.

i. Root length (cm)

The soil was removed by washing the roots gently in running water with utmost care so as to avoid root damage. Root length was measured from collar region upto the root tip using measuring tape.

j. Root number

Number of roots of each plant was counted manually.

3.4.2. Biomass characteristics

a. Fresh shoot weight (g): Fresh shoot weight of plants was measured after separating the roots from shoots. The weight of shoots was recorded by using electronic weighing balance in grams.

b. Fresh root weight (g): Fresh roots separated from the selected plants were weighed using electronic weighing balance in grams.

c. Dry shoot weight (g): The (fresh weighed) shoots were placed in brown paper envelope and labeled properly. Thereafter it was placed in oven at 40° C till a stable weight was obtained and recorded in grams.

d. Dry root weight (g): The (fresh weighed) roots were placed in brown paper envelope and labeled properly. Thereafter it was placed in oven at 40° C till a stable weight was obtained and recorded in grams.

3.4.3 Physiological and biochemical parameters

a. Total chlorophyll and carotenoids (Hiscox and Israeltam 1979)

Reagent

Dimethyl sulfoxide (DMSO) reagent

Extraction and Estimation

100 mg of fresh leaves were finely macerated in 5 ml of DMSO and were centrifuged at 5000 rpm to facilitate the pigment extraction. After the time period, the absorbance of the extract was recorded at 480 nm, 645 and 663 nm in the spectrophotometer against reagent blank. The chlorophyll and carotenoid content was calculated using the following equation:

$$\text{Chlorophyll a (mg/g FW)} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{\text{Volume}}{1000 \times \text{Weight}}$$

$$\text{Chlorophyll b (mg/g FW)} = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times \frac{\text{Volume}}{1000 \times \text{Weight}}$$

$$\text{Total chlorophyll content (mg/g FW)} = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times \frac{\text{Volume}}{1000 \times \text{Weight}}$$

$$\text{Carotenoid content (mg/g FW)} = \frac{1000 \times A480 - 1.29 \times \text{Chla} - 53.78 \times \text{Chlb}}{220} \times \frac{\text{Volume}}{1000 \times \text{Weight}}$$

Where,

A_{480} = Absorbance at 480 nm

A_{645} = Absorbance at 645nm

A_{663} = Absorbance at 663 nm

Total chlorophyll and carotenoid content was expressed in units as mg/g FW.

b. Total soluble sugars (Dubois *et al* 1956)

Reagents

i. Ethanol (80%)

ii. Conc. Sulphuric acid (H_2SO_4)

iii. Phenol (5%)

iv. D-Glucose (Standard)

Extraction

0.1g of plant leaf sample was homogenized with 20 ml 80% ethanol twice by placing the glass test tubes in the water bath with water condensers, boiling at 60°C for 20 minutes. The supernatant from all extractions were pooled. The supernatant was then again placed in the water bath boiling at 50ºC. When the ethanol gets evaporated, a fruity smell develops. The extractant volume was made upto 10 ml and then the extract solution was used for further quantification of total soluble sugars.

Estimation

1 ml of sugar extract was pipetted out in test tube, 1 ml of 5% phenol was added to added and incubated in dark for 10 min. To this, 5 ml conc. chilled H_2SO_4 was added to the centre of test tube. To ensure that the solution is mixed properly, it was vortexed and again incubated for 30 min at room temperature. The absorbance of the orange-red complex formed was recorded at 490 nm against blank. The amount of the total soluble sugars was quantified using a glucose standard (10-50 µg) and expressed in mg/g DW.

c. Total soluble proteins (Lowry *et al* 1951)

Reagents

i. 2% Sodium carbonate in 0.1 N NaOH (Reagent A)

ii. 0.5% Copper sulphate in 1% sodium potassium tartrate (Reagent B)

iii. Reagent A and reagent B mixed in 50:1 ratio (Reagent C)

iv. Folin-Ciocalteau's phenol reagent (2N) diluted with water in 1:1 ratio (Reagent D)

v. 20% Trichloroacetic acid (TCA)

vi. Bovine serum albumin (Standard BSA)

Extraction

Sample (0.2g) was extraced in 10 ml of 0.1 N NaOH and centrifuged for 15 min at 5000rpm. The extraction was done twice and total volume was made to 10ml. The supernatant was collected. From this, 1 ml of supernatant aliquot was pipetted out in test tube and 1 ml of 20 % TCA was added to it and kept at 4^{o}C for 24 hrs. Later, the extractant was centrifuged for 20 min at 5000 rpm. After that the obtained precipitates were dissolved in 10 ml of 0.1 N NaOH, which further used as protein extract.

Estimation

From the freshly prepared protein extract, 0.1 ml of aliquot taken in test tube and 0.9 ml of distilled water was poured for dilution. Then, reagent C (5ml) was added to the mixture, vortexed and placed at room temperature for 10 min. After that reagent D (0.5 ml) was added to the mixture and again kept at room temperature (37°C) for 30 min. Following the appearance of blue colour, results were recorded at 520 nm against blank. The standard curve was prepared using bovine serum albumin (BSA) in the range of 20-100 µg. Hence, the proteins were quantified from standard curve and the protein content was expressed as mg/g DW.

d. Proline content (Bates *et al* 1973)

Reagents

i. 3% Sulphosalicylic acid

ii. Acid ninhydrin reagent: It was prepared by mixing 0.3g ninhydrin, 7.5ml glacial acetic acid, 5 ml orthophosphoric acid (6M) and the solution was placed in oven at 70ºC till the solution becomes clear.

iii. Toulene

Extraction

Proline was extracted by crushing 200 mg of sample in 2 ml of 3% chilled sulphosalicylic acid. The centrifugation tubes containing reaction mixture were centrifuged at 3000 rpm for 10 min, after that tubes were again centrifuged by adding 2ml of sulphosalicylic acid. Supernatents were pooled, 10 ml of volume was made by adding distilled water and the extract was used for assay.

Estimation

One ml of diluted supernatant was incubated with one ml of acid ninhydrin and one ml of acetic acid (glacial) at 100º C for one hour in water bath. After one hour, the reaction was terminated in ice-water bath. Then, toluene (4ml) was added and tubes were vortexed for15-20 seconds. The different phases were separated by placing the test tubes containing

mixture undisturbed at room temperature for 24 hours. The pinkish-red upper layer of chromphore containing toluene was measured at 520nm and toluene was taken as blank. Further, the proline content was quantified from a standard curve prepared by using proline in the concentration range of 10-50µg/ml. Proline content was expressed as µg/g FW and calculated as follows:

$$\frac{\text{Concentration of standard} \times \text{OD of sample} \times \text{Volume made after extraction}}{\text{OD of standard} \times \text{Volume of extract taken for estimation} \times \text{Weight of sample taken}}$$

e. Total antioxidant activity (Patel *et al* 2010)

Reagents

i. 90% Ethanol
ii. 0.2 M Phosphate buffer
iii. 1% Potassium ferricyanide
iv. 10% Trichloroacetic acid
v. 0.1% Ferric chloride

Extraction

0.2 g of plant sample was macerated in 10 ml of 90% ethanol and kept for 4 hrs; then filtered using WhatmanNo. 1 filter paper and the filtrate solution were used for antioxidant assay.

Estimation

To 2 ml of filtrate, 1ml of phosphate buffer (0.2 M) and 1 ml of potassium ferricyanide (1 %) were added. The obtained reaction mixture was incubated at 60°C in a water bath for 10 min. Later, the solution was cooled down and 2.5 ml of trichloroacetic acid (10%) was added to terminate the reaction. After that the mixture was centrifuged at 2000rpm for 15 min. After centrifugation, 2.5 ml of aliquot was pipette out in test tube and 2.5 ml distilled water and 0.5 ml ferric chloride (0.1%) was added to it. The colour of the mixture changes to green and absorbance was recorded at 593 nm spectrophotometrically. The standard was prepared by using ascorbic acid solution.

3.4.3.7 Antioxidant enzymatic assays

a. Peroxidase (EC1.11.1.7) (Shannon *et al* 1966)

Reagents

i. 0.05M guaiacol prepared in 0.1M potassium phosphate buffer (PPB) (pH 6.5)
ii. 0.8M H_2SO_4
iii. 1 % Polyvinylpyrrolidone (PVP)
iv. 10mM β- mercaptoethanol
v. 1 mM Ethylenediamine tetraacetic acid (EDTA)

Extraction

The enzyme extraction was done from fresh sample using 0.1M PPB containing 1 percent PVP, 10mM β- mercaptoethanol and 1 mM EDTA. After that, the enzyme extract was centrifuged for 15 minutes at 10,000 rpm. The obtained supernatant solution was used for estimating protein content by Lowry method and following enzymatic activity.

Estimation

The enzyme activity was assayed upon appearance of brown discoloration induced from oxidation of guaiacol to tetraguaiacol in the presence of hydrogen peroxide. The reaction mixture contained 3 ml of 0.05 M guaiacol in 0.1 M PPB and 0.2 ml of enzyme extract in the cuvette. The reaction was initiated by adding 0.1 ml of H_2SO_4 and the change in absorbance was measured at 470 nm for 2 minutes with 30 sec of interval. The blank was prepared simultaneously containing all reagents except enzyme extract. The enzymatic activity of peroxidase was calculated using the formula:

$$\text{Activity} = \frac{\Delta\, \text{A} \times \text{Vol. after centriguge (ml)}}{\text{Enzyme extract taken (ml)x fresh wt. (g)}}$$

$$\text{Specific activity} = \frac{\text{Enzyme activity}}{\text{Protein concentration}}$$

Where, Δ A is change in optical density and the peroxidase specific activity was expressed in units min^{-1} mg^{-1} protein.

b. Catalase (EC 1.11.1.6) (Chance and Mahley 1955)

Reagents

i. **0.1M Sodium phosphate buffer (pH 7.0):** 0.2M sodium phosphate buffer (SPB) (25ml) was prepared by mixing 0.2M sodium phosphate monobasic (61 ml) and 0.2M sodium phosphate dibasic (39 ml), and the final volume was made to 100 ml.

ii. **H_2O_2**: 825µl of hydrogen peroxide (H_2O_2) was dissolved in the 100ml of double distilled water

Extraction

Fresh tissue (0.1 g) was crushed with 2 ml of chilled 50 mM sodium phosphate buffer (pH 7.5) containing 1% polyvinyl pyrrolidone. The extracted mixture was centrifuged at 10000 rpm at 4°C in cooling centrifuge for 20 min. The clear supernatant was collected, used for estimation of protein content by Lowry method and following enzymatic activity.

Estimation

For estimation, 1 ml of 0.12% H_2O_2 solution, 1.9 ml of 50 mM phosphate buffer (pH 7.5) was added in the cuvette. After that enzyme extract (0.1 ml) was added to the cuvette and decomposition/utilization of H_2O_2 was recorded for 3 min at 30 seconds intervals and decrease in absorbance was recorded at 240 nm. Catalase activity is calculated as µ moles of H_2O_2 decomposed $min^{-1}g^{-1}$FW and can be expressed as units min^{-1} mg^{-1} protein.

c. Superoxide Dismutase (EC 1.15.1.1) (Marklund and Marklund 1974)

Reagents

i) 6 mM Pyrogallol

ii) 6 mM Ethylenediaminetetra-acetic acid (EDTA)

iii) 1 % PVP

iv) 10 mM β- mercaptoethanol

v) 0.1 M Potassium phosphate buffer (PPB) (pH 6.5)

vi) 0.1M Tris-HCl buffer (pH 8.2)

Extraction

0.1g of fresh plant leaf sample was extracted using 0.1 M PPB containing 1 percent PVP, 10 mM β- mercaptoethanol and 6 mM EDTA. The obtained supernatant was centrifuged for 15 min at 15,000 rpm in cooling centrifuge and then used for estimation of enzymatic activity and protein content by Lowry method.

Estimation

1.5 ml of 0.1M Tris-HCl buffer, 0.5 ml of 6 mM EDTA and 1ml of 6 mM freshly prepared pyrogallol solution added to cuvette. After that 0.1 ml enzyme extract was pipette out in the same cuvette for inhibition of half-time (50%) oxidation of pyrogallol. The rate at which pyrogallol was auto-oxidized was determined by the increase in optical density (OD) measured at 420 nm for 3 min at 30 sec interval using spectrophotometer. The test tube without enzyme extract was taken as blank. Superoxide dismutase activity was expressed as units min^{-1} mg^{-1} protein.

3.4.4 Anatomical analysis

The anatomical analysis was performed by using Lieca Bright Field Research Microscope, Field Emission Scanning Electron Microscope (FESEM) and Energy Dispersive X-ray Spectroscopy (EDS), the adopted methodology is given below:

a. Light microscopy

The fresh, acid-water washed roots and leaves of control (Pb_0Cd_0) and highest treated plant ($Pb_{300}Cd_{25}$) were selected for anatomical analysis. To calculate leaf stomatal density, or the number of stomata per unit leaf area, the impression approach was utilized (Radoglou and Jarvis 1990). The adaxial surface of leaf in the middle, between the central vein and leaf edge was cleansed with degreased cotton boll and after that Quickfix was applied for approximately 5 min. After meantime, thin film was peeled off, mounted on microscopic glass slide and covered with the cover slip. The total no. of stomata (s) along with epidermal cell (e) from each film strip was counted manually under a Leica Bright Field Research Microscope (10X). At the end, the leaf stomatal index was calculated using the formula given as: $[s/(e + s)]\times100$. In order to determine the capability of stomatal pore opening, the distance between the guard cells at junctions from the stoma end was measured (Xu and Zhou 2008).

Similarly, total number of trichomes per unit area represents the trichome density, which was calculated on the adaxial leaf surface of control and heavy metal treated plants.

b. Field emission scanning electron microscopy (FE-SEM)

To compare the ultra-structure of control (Pb_0Cd_0) and heavy metal treated plants with highest concentration ($Pb_{300}Cd_{25}$), Field Emission Scanning Electron Microscopy (FESEM) was performed. The roots and leaves from treated and untreated plants were cut into small and equal sections of 0.5-1 cm^2 size and fixed in 2.5% (v/v) glutaraldehyde for overnight at 4°C. After that, the fixative solution was discarded upon atleast three to four washings have been performed with 0.1 M calcium cobalt (CaCo) buffer at 15 min intervals and thereafter, the prepared sample kept in 1% osmium tetraoxide (OsO_4) for approximately 2 hour at 4°C. After that OsO_4 was decanted and the samples were washed with 0.1 M CaCo buffer for continuous three times. The samples were subjected to dehydration in ethanol (30%, 50%, 70%, 80%, 90%, 100 % v/v) series and kept in vacuum desiccator for overnight. After proper dehydration process, gold coating of dehydrated samples were done in sputter coater and samples were sent to Sophisticated Analytical Instrumentation Facility at Punjab University, Chandigarh for FESEM imaging.

c. Energy Dispersive X-ray Spectroscopy (EDS)

The differential Pb and Cd accumulation sites in different plant tissues were marked by using Energy Dispersive X-ray Spectroscopy (EDS) at Sophisticated Analytical Instrumentation Facility (SAIF) at Punjab University, Chandigarh. The sample preparation methodology is similar with FESEM, meanwhile EDS results are obtained in tables and spectra, Pb and Cd values expressed in weight per cent in different tissue sites.

3.4.5 Macro and micronutrient analysis

The estimation of macro and micronutrients from both plant species (root, stem and leaves) along with soils before plantation and phytoremediated soils were performed. Macronutrients such as phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) except nitrogen (N) and sulphur (S), and micronutrients viz., manganese (Mn), copper (Cu), iron (Fe), zinc (Zn), heavy metals such as lead (Pb) and cadmium (Cd) was estimated by using Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS) at Natural resource management (NRM) laboratory in the Department of Soil Science, Punjab Agricultural University, Ludhiana. The estimation of nitrogen (N) and sulphur (S) was performed separately due to different digestion procedure of nitrogen, and volatile nature of sulphur element as well as due to high sensitivity of ICP-MS.

A. Plant samples

0.5g dried plant sample of roots, stem and leaves were weighed and placed in acid-washed dried conical flasks. The powdered plant samples were digested with 10ml di-acid (HNO_3 and $HClO_4$ in 3:1) and kept overnight for pre-digestion. On the following day, flasks

containing samples were placed on hot plate and heated till whitish, transparent solution obtained. When the digested samples cooled down, 50ml volume was made with Millipore deionized water, filtered and stored in polypropylene bottles. The estimation of macronutrients (P, K, Ca, Mg), micronutrients (Mn, Cu, Fe, Zn) and heavy metals (Pb and Cd) in the aliquot of digested samples was made by using Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS).

Calculations

Weight of digested sample	= 0.5 g
Vol. made after digestion	= 50ml
First Dilution	= 100 times
Volume of aliquot taken for estimation	= 1 ml
Volume made for estimation	= 25ml
Second Dilution	= 25 times
Total dilution	= 1250 times
Readings of ICP-MS	= X ppb
	=X/ 1000 = Y ppm
	= Y ppm × 1250
	= mg/Kg

The values of macro and micronutrients were expressed in mg/Kg.

Sulphur: The total sulphur was estimated by turbidity method. For this, 1ml of di-acid digested aliquot of plant samples was pipette in 25 ml volumetric flask. After that 1g of $BaCl_2$ was added and shaken for 1 minute, then 1ml of gum-acacia (0.25%) was added and the volume was made upto 25ml. The turbidity was generated with sulphate precipitation as barium sulphate was estimated calorimetrically at 420nm wavelength. The standard curve was prepared with 100ppm sulphur solution i.e. potassium sulphate. The values of plant sulphur content were expressed as mg/kg.

Nitrogen: Total nitrogen in the plant samples were analyzed by Kjeldahl method. For extraction procedure, One gram of plant sample was put in Kjeldahl's digestion flask, and 10 g of catalyst mixture (20g $CuSO_4$. $6H_2O$, 1g Se-powder and 3g HgO and 480 g of potassium sulphate) and 25-30 ml of conc. H_2SO_4 was added to it. After that, flask containing mixture was placed on low heat until frothing stopped further temperature was increased upto the achievement of boiling point, so mixture was heated continuously until solution colour turns to clear light yellow/grey color. After digestion, samples were allowed to cool and made volume 100ml with distilled water. For distillation, 10 ml of 0.02N H_2SO_4 pipette out in a conical flask; then two to three drops of methyl-red indicator was added to them and fitted to the delivery tube of condenser. Thereafter, 5 ml of aliquot was taken in distillation flask and

connected to mouth of distillation flask. Further, 25 ml of 45%NaOH was poured in the distillation flask by using funnel which is already attached to distillation apparatus, after that 30 ml of distillate was collected. The excess of 0.02N H_2SO_4 in the conical flask was titrated against the standardized 0.02N NaOH solution and the end point was pink to yellow colour change was noted.

Calculations

Weight of sample taken for digestion = 1 g

Volume made after digestion = 100 ml

Aliquot used for distillation = 5 ml

0.02N H_2SO_4 taken in flask for titration = A ml

0.02N NaOH used for titration = B ml

Volume of 0.02N H_2SO_4 used for NH_3 absorption = A-B ml

1 ml of 0.02N H_2SO_4 = 0.00028 g of nitrogen

% N in the given plant sample $= (A-B) \times 0.00028 \times \frac{100}{5} \times \frac{100}{1}$

B. **Soil samples**

a. Total nutrient analysis (mg kg^{-1})

For total macro and micronutrient analysis, 2 g of air dried soil samples were taken in conical flask and 10 ml of aqua-regia (HNO_3 and HCl) was added for digestion. On the following day, flasks containing samples were placed on hot plate and heated till less than 1 ml of solution was left in tube. After digestion, appropriate dilutions were made with Millipore deionized water, filtered via Whatman No.1 filter paper and stored in polypropylene bottles. The estimation of total macronutrients (P, K, Ca, Mg), micronutrients (Mn, Zn, Cu, Fe) and heavy metals (Pb and Cd) in the aliquot of digested samples was made by using Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS). Further, total sulphur content from soils was estimated calorimetrically as mentioned above.

b. Available nutrient analysis (mg kg^{-1})

Available macro- and micronutrients from sampled soil was estimated by AB-DTPA (ammonium bicarbonate diethylenetriaminepentaacetic acid) method as explained by Lindsay and Norvell (1978). Ten gram of soil samples were extracted in 20 ml DTPA after shaking them for 2 hours in reciprocating shaker. After that solution was filtered using Whatman No.1 filter paper and stored in polypropylene bottles. For estimation of available macronutrients (P, K, Ca, Mg), micronutrients (Mn, Cu, Fe, Zn) and heavy metals (Pb and Cd) from the extracted aliquot of samples, appropriate dilutions were made and fed to ICP-MS. Further, total sulphur content from soils was estimated calorimetrically as mentioned above.

Available nitrogen was estimated by Kjeldahl's method, for this 5 g of soil is

oxidized by 0.32 % $KMnO_4$. Ammonia released was absorbed in 4% Boric acid that was then titrated against N/200 H_2SO_4.

c. Soil physico-chemical properties

i. pH (Jackson 1967)

The soil pH was measured by pH meter in suspension by mixing 25g of soil mixture with 50 ml distilled water as explained by Jackson (1967).

ii. Electrical conductivity (Jackson 1967)

The EC (dS/m) was measured in the same pH suspension after 24hrs in supernatant solution by conductivity meter.

iii. Soil organic carbon (Walkley and Black 1934)

The soil organic carbon was determined by rapid titration method, 1g of soil was oxidized with $K_2Cr_2O_7$ (5ml) in the presence of H_2SO_4 (10ml). After 30 min, oxidation reaction was stopped with addition of distilled water (100ml) and sodium fluoride (NaF) was added to absorb the impurities. Then 2-3 drops of diphenylamine (indicator) was added and titrate it against 0.5N FAS (Ferrous ammonium sulphate). Soil organic carbon (%) was calculated as below:

$$\text{SOC \%} = \frac{(X - Y)}{2} \times 0.003 \times \frac{100}{1}$$

Where, X = ml of FAS used, Y = soil sample

iv. Cation exchange capacity (Bower *et al* 1952)

The soil cation exchange capacity (CEC) was determined with sodium and ammonium acetate centrifuge method. For this, 4 g of soil along with 33 ml of 1N CH_3COONa (pH 8.2) in polypropylene bottles was shaken for 5 minutes in reciprocating shaker and centrifuged at 2000 rpm for 10 minutes. Thereafter, the obtained clear supernatant liquid was decanted and samples were treated four times in same manner. Further, samples were suspended with 33ml of 95% ethanol, shaken for five minutes and supernatant was drained off, same process was repeated thrice. Then samples were washed with ethanol 3 times, at the time of third washing EC of supernatant was recorded, which must be lower than 40micromhos/cm. For replacement of absorbed Na, samples were shaken with 33ml of neutral ammonium acetate for 5 min in reciprocating shaker and centrifuged until clear supernatant was obtained. Three extracts of ammonium acetates were collected in 100ml volumetric flasks and volume was made. The estimation of sodium acetate from extract was determined on flame photometer and standard curve was prepared with standardized sodium solution. The value of soil CEC expressed as cmol (+)/kg.

3.4.6. Phytoremediation evaluation factors

Metal uptake, translocation and bioaccumulation potential of *Salix alba* and *Toona ciliata* was evaluated by bioconcentration factor (BCF), translocation factor (TF), metal

removal efficiency (MRE %) and tolerance index (TI %) by using following formulae (Shukla *et al* 2010, Zhang *et al* 2019):

$$BCFr = \frac{\text{Concentration of HM in root}}{\text{Concentration of HM in soil}}$$

$$BCFs = \frac{\text{Concentration of HM in stem}}{\text{Concentration of HM in soil}}$$

$$BCFl = \frac{\text{Concentration of HM in leaf}}{\text{Concentration of HM in soil}}$$

$$TF = \frac{\text{Concentration of HM in leaves}}{\text{Concentration of HM in root}}$$

$$TI\ (\%) = \frac{\text{Biomass of HM treated plants}}{\text{Biomass of control plants}} \times 100$$

$$MRE\ (\%) = 100 - \left(\frac{\text{Biomass of HM treated plants}}{\text{Biomass of control plants}} \times 100 \right)$$

Where, HM is heavy metals (Pb and Cd); BCFr, BCFs and BCFl are bioconcentration factor of root, stem and leaves respectively. TF is translocation factor of plants from root to aerial plant parts. Further, depending on the BCF and TF values, the plant accumulation efficiency was determined and categorized in four groups such as, BCF >1: intensive; BCF=1–0.1: medium; BCF = 0.1–0.01 weak and BCF = 0.01–0.001: no accumulation; the similar criteria is followed for translocation factor (Kabata-Pendias and Pendias 1999). Audet and Charest (2007) developed criteria to determine the plant tolerance against stress on the basis of plant biomass, thus if tolerance index (TI) values < 100, represents the plant growing on heavy metal contaminated soils undergoes stress with net decrease in the plant biomass. Meanwhile, if TI values >100, indicated that plants have been developed tolerance against heavy metal stress with a net increase in biomass which is noticeable in hyper accumulators. Further, if TI equals to 100, the plant can be unaffected by induced metal stress.

3.4.7. FTIR analysis

Fourier transformed infrared spectroscopy (FTIR) analysis was performed to determine the effect of heavy metal accumulation on functional group binding of plant and soil. For this, dried, fine powdered plant root, stem and leaf samples along with soils of control (Pb_0Cd_0) and highest treated plant ($Pb_{300}Cd_{25}$) were used. These samples were analyzed at ATR mode by using Raman spectrophotometer at electron microscopy and nanoscience (EMN) laboratory, Department of Soil Science, Punjab Agricultural University, Ludhiana; data recorded within 400-4000 Cm^{-1} range.

3.4.8. Statistical analysis

The data was analyzed statistically using Tukey's HSD test. One way ANOVA (analysis of variance) was performed to confirm the variability among all treatments and two way ANOVA to investigate the interaction among two metal elements (Pb and Cd) using statistical analysis software (SAS) version 9.3 for Windows.

CHAPTER - IV

RESULTS AND DISCUSSION

The present investigation on "Evaluation of morpho-physiological and metal accumulation potential of *Salix alba* L. and *Toona ciliata* M. Roemer grown on heavy metal contaminated soils" was carried out for two consecutive years 2020 and 2021 with the objective to study the effect of heavy metals on morphological, physiological and anatomical traits of plants as well as physico-chemical properties of soil. Along with this, alterations in macro and micro nutrient profile of both plants and soils due to heavy metal stress were also analyzed. The study was categorized in three main experiments and results are presented under following headings:

4.1 Growth performance, physiological and biochemical analysis of tree species raised on different concentrations of heavy metal contaminated soils

4.1.1 Morphological (Growth and biomass) attributes

4.1.2 Physiological and biochemical parameters

4.1.3 Anatomical traits

4.2 Effect of heavy metals on its uptake, translocation and metal accumulation potential of tree species

4.2.1 Heavy metals accumulation in plant parts (root, stem and leaves)

4.2.2 Phytoremediation efficiency evaluation factors

4.2.3 Plant nutrient analysis

4.2.4 FTIR analysis

4.3 Effect of heavy metals on physico-chemical properties of soil

4.3.1 Physico-chemical properties of soil

4.3.2 Soil nutrient analysis

Experiment No. 1

4.1 Growth performance, physiological and biochemical analysis of tree species raised on different concentrations of heavy metal contaminated soils

4.1.1 Morphological (Growth and biomass) attributes: Effect of heavy metals on plant's survival percentage, height, collar diameter, number of branches, number of leaves, root length, root number, fresh and dry weight of both root and shoots were recorded and are discussed below:

a. Survival percentage

Heavy metals negatively affect the plant growth which is also manifested in the survival percentage of plants grown on heavy metal polluted soils. The survival percentage of *Salix* and *Toona* as affected by heavy metal concentrations is presented in Fig. 4.1.

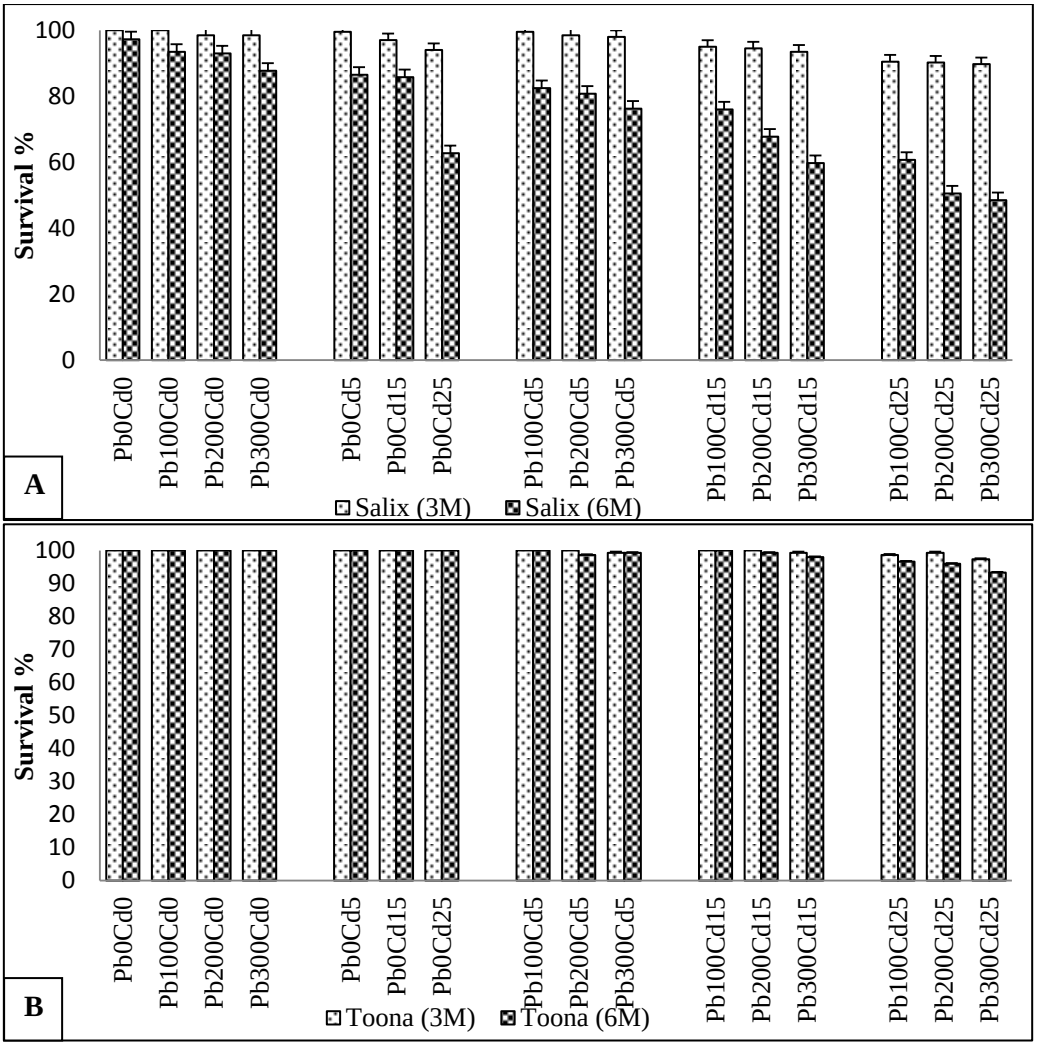


Fig. 4.1: Effect of heavy metals on survival percentage of *Salix alba* and *Toona ciliata*

In case of *Salix*, after three and six months, significantly higher survival percentage was observed in control plants (100% and 97.3% respectively) which decreased with increase in concentrations of Pb, Cd and their combinations in soil (Fig. 4.1A). Among different Pb concentrations, Pb_{100} treatment showed no significant effect on survival percentage, but with increase in Pb concentration significant decrease in survival percentage was recorded and lower survival percentage was observed with highest Pb concentration i.e. Pb_{300} (87.7%) after six months. Among different Cd concentrations, the decrease in survival percentage was observed with increase in Cd concentration from Cd_5 (86.5%) to Cd_{25} (62.8%). Moreover, among combinations (Pb+Cd), the maximum negative effect on survival was observed with highest combination concentration ($Pb_{300}Cd_{25}$) i.e. 89.8 % after three months and 48.5% after six months. Thus, the effect of heavy metals on survival percentage of *Salix* was more pronounced after six months than three months.

In case of *Toona*, maximum survival percentage (100%) was recorded in control (Pb_0Cd_0) after both three and six months (Fig. 4.1B). The effect of different Pb and Cd concentrations on survival percentage was same, i.e. they do not affect survival percentage

even at higher concentrations (Pb_{300} and Cd_{25}). Moreover, with highest combination concentration i.e. $Pb_{300}Cd_{25}$ the sufficient survival percentage was recorded after three and six months (97.3% and 93.3% respectively).

Thus, the reduction in survival percentage of *Salix* was observed due to heavy metals, but *Toona* plants showed better survival percentage (> 97%) than *Salix* (< 50%) at higher Pb and Cd ($Pb_{300}Cd_{25}$) concentrations. However, *Salix* showed sufficient survival percentage (> 85%) with Pb_{200} and Cd_{15} concentrations, further increase in concentration leads to drastic reduction in survival percentage. Thus, it is speculated that survival percentage of *Salix* decreased with time as well as with increase in heavy metal concentration. The effect of Cd concentrations on survival percentage of *Salix* was recorded to be more than the Pb concentrations, but the effect of their combination concentrations was more pronounced in an order as Pb+Cd > Cd > Pb.

Plant survival rate is a critical metric for determining how well a species has adapted to particular environmental conditions and it also influence average biomass production (Chibuike and Obiora 2014). In the present study, the reduction in plant survival percentage in response to Pb and Cd treatments has been recorded and these results are in accordance with observations of Ismail *et al* (2013), who also reported that heavy metal toxicity adversely affect plant growth which further affects the survival as in case of *Delonex regia*, *Leucaena leucocephala* and *Thespesia populneoides* raised under different concentrations Pb and Cd contaminated soils.

b. Plant height (cm)

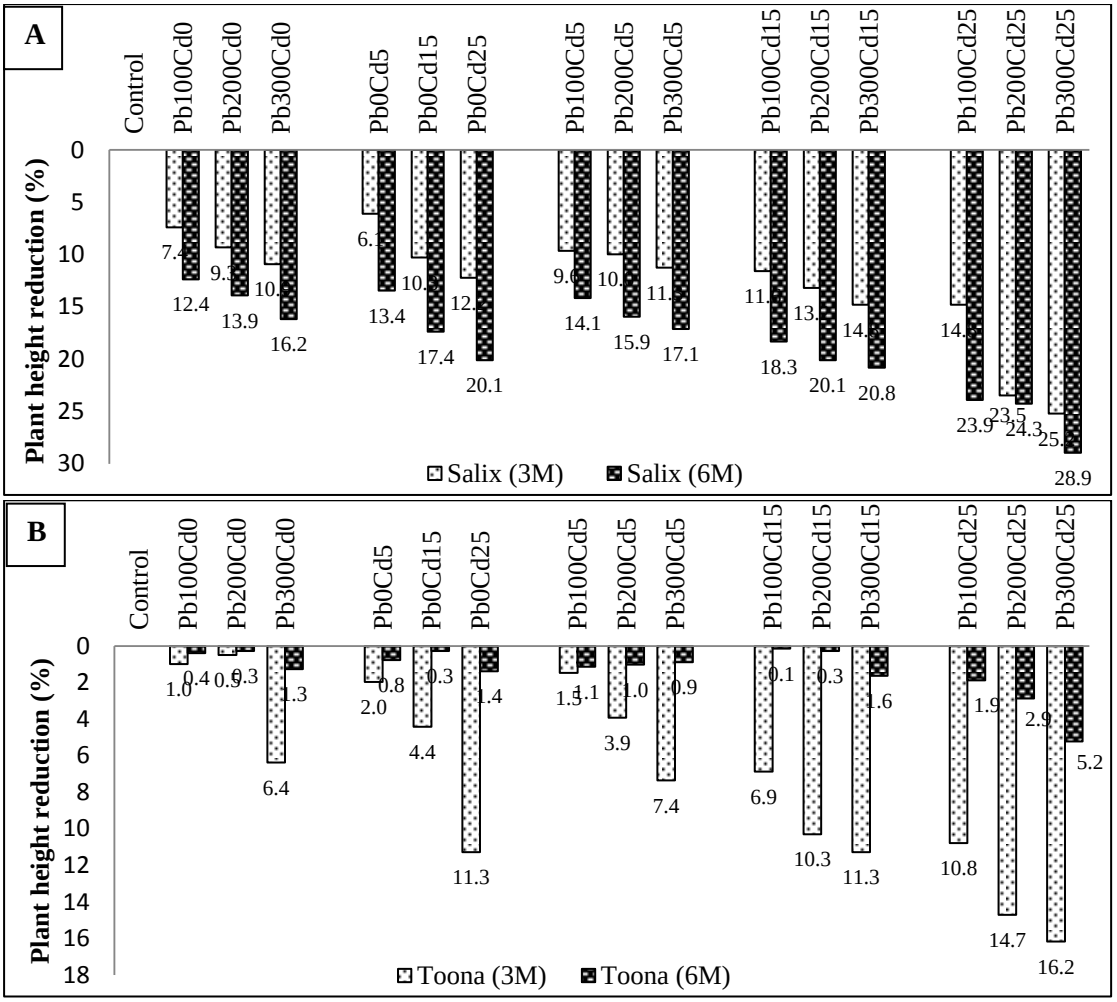
The data on plant height shows response of *Salix* and *Toona* with different concentrations of Pb, Cd and their combinations (Table 4.1). With increasing concentrations of Pb and Cd in soil (individual as well as combinations) significant reduction in plant height of both *Salix* and *Toona* was recorded and is expressed in Fig. 4.2.

In *Salix*, the significant reduction in plant height was observed after three and six month in response to Pb and Cd concentration, but the Pb x Cd interaction was significant only at six months (Table 4.1). After three months, mean plant height was observed to be higher in control (Pb_0, 72.2cm), which decreased significantly with increase in Pb concentration in following order Pb_{100} (69.3cm), Pb_{200} (66.9cm) and Pb_{300} (64.9 cm). Similar trend was observed in Cd that higher mean plant height was observed in control Cd_0 (72.4 cm) which significantly decreased with increase in Cd concentrations i.e. Cd_5 (70.6cm), Cd_{15} (68.1cm) and Cd_{25} (62.3cm). Among Pb and Cd combinations, minimum plant height was recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (55.3cm) with maximum reduction (25.2%) as shown in Fig. 4.2A. The reduction in plant height increased with increase in heavy metal concentrations, however maximum reduction was recorded in combinations followed by individual Cd and Pb concentrations in order Pb+Cd > Cd > Pb.

Table 4.1: Effect of heavy metals on plant height of *Salix alba* and *Toona ciliata*

Plant height (cm)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	77.8^{a}	72.0^{a}	70.5^{ab}	69.3^{ab}	72.4^{A}	210.3^{a}	184.2^{b}	181.0^{bc}	176.2^{bcd}	187.9^{A}
Cd_5	73.0^{a}	70.3^{ab}	70.0^{ab}	69.0^{ab}	70.6^{AB}	182.0^{bc}	180.5^{bc}	176.7^{bcd}	174.2^{bcdef}	178.4^{B}
Cd_{15}	69.8^{ab}	68.7^{ab}	67.5^{ab}	66.3^{ab}	68.1^{AB}	173.7^{bcdef}	171.7^{bcdef}	168.0^{bcdef}	166.5^{cdef}	170.0^{C}
Cd_{25}	68.3^{ab}	66.2^{ab}	59.5^{bc}	55.3^{c}	62.3^{B}	168.0^{bcdef}	160.0^{def}	159.2^{ef}	157.2^{f}	161.1^{D}
Mean	72.2^{A}	69.3^{AB}	66.9^{BC}	64.9^{C}		183.5^{A}	174.1^{B}	171.25^{B}	168.56^{B}	
LSD($p \leq 0.05$)	Pb	3.36				Pb	5.08			
	Cd	3.36				Cd	5.08			
	Pb×Cd	NS				Pb×Cd	10.1			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	68.0^{a}	67.3^{ab}	67.7^{a}	63.7^{bcde}	66.7^{A}	268.7^{a}	267.7^{a}	268.0^{a}	265.3^{bc}	267.4^{A}
Cd_5	66.7^{abc}	67.0^{abcd}	65.3^{abcd}	63.1^{de}	65.5^{A}	266.7^{a}	265.7^{ab}	266.0^{ab}	266.3^{a}	266.2^{A}
Cd_{15}	65.0^{abcd}	63.3^{cde}	61.0^{ef}	60.3^{efg}	62.4^{B}	268.0^{a}	268.3^{a}	268.0^{a}	264.3^{ab}	267.2^{A}
Cd_{25}	60.3^{efg}	60.7^{efg}	58.0^{fg}	57.1^{g}	59.0^{C}	265.0^{ab}	263.7^{ab}	261.0^{b}	254.7^{c}	261.1^{B}
Mean	65.0^{A}	64.6^{A}	63.0^{B}	61.2^{C}		267.1^{A}	266.3^{A}	265.7^{A}	262.7^{B}	
LSD($p \leq 0.05$)	Pb	1.17				Pb	1.55			
	Cd	1.17				Cd	1.55			
	Pb×Cd	NS				Pb×Cd	NS			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)
(NS: Non-significant)



(3M: After three months, 6M: After six months)

Fig. 4.2: Per cent reduction in plant height of *Salix alba* and *Toona ciliata* as affected by heavy metals with respect to control

Similar trend was observed after six months, maximum *Salix* height was observed in control plants (Pb_0Cd_0, 210.3cm) which decreased with increase in heavy metal concentrations and recorded to be minimum in highest combination concentration i.e. $Pb_{300}Cd_{25}$ (157.2cm) treated plants with maximum reduction of 28.9% (Fig. 4.2A).

Toona plants showed significant decrease in plant height under different Pb and Cd concentrations at three and six months, but the Pb x Cd interactions were recorded to be non-significant (Table 4.1). At three months, for different Pb concentrations higher mean plant height was observed in control i.e. Pb_0 (65cm) which was at par with Pb_{100} (64.6cm) and minimum with Pb_{300} (61.2cm) concentration. Among different Cd concentrations, higher mean plant height was in control (Cd_0, 66.7cm) which was at par with Cd_5 (65.5cm) and minimum with Cd_{25} (59cm) concentration. Among combinations, higher plant height was recorded in control (Pb_0Cd_0, 68 cm) and these values decreased significantly with increase in heavy metal concentrations, hence, minimum plant height (57.1cm) was observed in highest concentrations of Pb and Cd i.e. ($Pb_{300}Cd_{25}$) with 16 per cent reduction as compared to control (Fig.4.2B).

Similar trend was recorded after six months, higher plant height (268.7cm) was recorded in control which was at par with $Pb_{200}Cd_0$ (268 cm), $Pb_{100}Cd_0$ (267.7cm) and Pb_0Cd_5 (266.7cm) concentrations. However, after six months, the reduction in plant height was only five per cent in plants grown on highest heavy metal concentration i.e. $Pb_{300}Cd_{25}$.

Hence, Pb, Cd and their combination concentrations negatively affect plant height of both species, however, *Toona* plants showed minimum reduction under higher heavy metal concentrations ($Pb_{300}Cd_{25}$) than *Salix*. Overall, the reduction in *Salix* height was increased from three months (6% - 25%) to six months (12% - 28.9%), whereas in case of *Toona* the reduction in plant height was decreased from three months (1% - 16%) to six months (0.4% - 5.2%) which might be due to enhanced tolerance against heavy metal stress with time. The effect of Cd concentrations on plant height of *Salix* and *Toona* was recorded be more than the Pb concentrations, but the effect of their combination concentrations was more pronounced as following order Pb+Cd > Cd > Pb.

c. Collar diameter (cm)

Data on collar diameter reveals the response of *Salix* and *Toona* with different concentrations of Pb, Cd and their combinations (Table 4.2). The significant decrease in collar diameter of both species was recorded with increase in heavy metal concentrations as shown in Fig. 4.3.

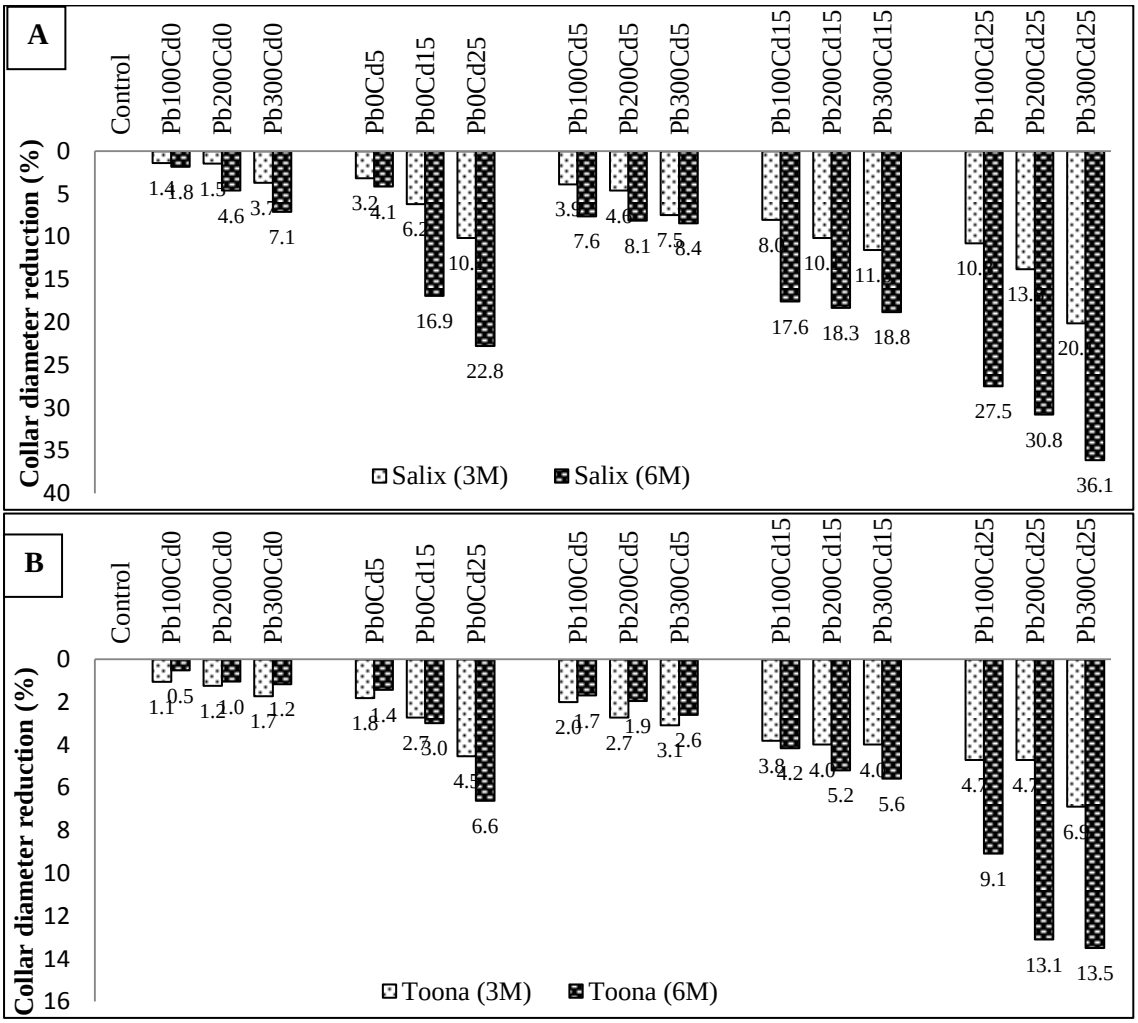
In *Salix*, the significant decrease in collar diameter was observed in response of Pb and Cd concentrations after three and six months, but Pb x Cd interaction was non-significant at three months only, further the significant results were found after six months (Table 4.2). After three months, among Pb concentrations higher mean collar diameter was observed in control (Pb_0, 0.75 cm), Pb_{100} (0.74cm) and Pb_{200} (0.73cm) and significantly minimum collar diameter was recorded at highest Pb concentration i.e. Pb_{300} (0.71 cm). Similarly for Cd concentrations, higher mean collar diameter was observed in control (Cd_0,0.78 cm) which decreased significantly with increase in Cd concentration from Cd_5 (0.76cm to Cd_{25} (0.68 cm). Among Pb and Cd combinations, minimum collar diameter was recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (0.63cm) with maximum reduction (20%) as shown in Fig. 4.3A. The reduction in collar diameter increased with increase in heavy metal concentrations; however, the maximum reduction was recorded with combinations (Pb+Cd) than individual Cd and Pb in order Pb+Cd > Cd > Pb.

Similar trend was observed after six months where maximum collar diameter was observed in control (2.22cm), which decreased with increase in heavy metal concentrations and recorded to be minimum with $Pb_{300}Cd_{25}$ (1.41cm) which was statistically equivalent with $Pb_{200}Cd_{25}$ (1.53cm) concentration.

Table 4.2: Effect of heavy metals on collar diameter of *Salix alba* and *Toona ciliata*

Collar diameter (cm)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.79^{a}	0.78^{ab}	0.78^{ab}	0.76^{abc}	0.78^{A}	2.22^{a}	2.18^{ab}	2.12^{bcd}	2.06^{cde}	2.43^{A}
Cd_5	0.77^{abc}	0.76^{abc}	0.75^{abc}	0.73^{abcd}	0.76^{B}	2.13^{bc}	2.05^{de}	2.04^{e}	2.03^{e}	2.06^{B}
Cd_{15}	0.74^{abcd}	0.73^{abcd}	0.71^{bcd}	0.70^{cd}	0.72^{C}	1.84^{f}	1.83^{f}	1.81^{f}	1.80^{f}	1.82^{C}
Cd_{25}	0.71^{bcd}	0.71^{cd}	0.68^{de}	0.63^{e}	0.68^{D}	1.71^{g}	1.61^{h}	1.53^{i}	1.41^{j}	1.56^{D}
Mean	0.75^{A}	0.74^{A}	0.73^{A}	0.71^{B}		1.97^{A}	1.92^{B}	1.87^{C}	1.56^{D}	
LSD($p \leq 0.05$)	Pb	0.020				Pb	0.024			
	Cd	0.020				Cd	0.024			
	Pb×Cd	NS				Pb×Cd	0.048			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	1.84^{a}	1.82^{ab}	1.82^{ab}	1.81^{bc}	1.82^{A}	2.57^{a}	2.56^{a}	2.54^{ab}	2.54^{ab}	2.55^{A}
Cd_5	1.81^{bcd}	1.80^{bcd}	1.79^{cde}	1.78^{de}	1.79^{B}	2.53^{ab}	2.53^{ab}	2.52^{abc}	2.50^{abcd}	2.52^{B}
Cd_{15}	1.79^{cde}	1.77^{ef}	1.77^{ef}	1.77^{ef}	1.77^{C}	2.49^{abcd}	2.46^{bcde}	2.44^{cde}	2.43^{de}	2.45^{C}
Cd_{25}	1.76^{f}	1.75^{f}	1.75^{f}	1.71^{g}	1.74^{D}	2.40^{ef}	2.34^{f}	2.23^{g}	2.22^{g}	2.29^{D}
Mean	1.79^{A}	1.78^{B}	1.78^{B}	1.76^{C}		2.49^{A}	2.47^{A}	2.43^{B}	2.42^{B}	
LSD($p \leq 0.05$)	Pb	0.007				Pb	0.027			
	Cd	0.007				Cd	0.027			
	Pb×Cd	0.014				Pb×Cd	0.054			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)
(NS: Non-significant)



(3M: After three months, 6M: After six months)

Fig. 4.3: Per cent reduction in collar diameter of *Salix alba* and *Toona ciliata* as affected by heavy metals with respect to control

Toona plants also showed significant decrease in collar diameter with increasing concentration of Pb, Cd and their combinations after three and six months. After three months, among Pb concentrations higher mean collar diameter was recorded in control (Pb_0, 1.79cm) which significantly decreased with increase in Pb concentrations from Pb_{100} (1.78cm) to Pb_{300} (1.76cm). Similar trend was observed in Cd that higher mean collar diameter was recorded in control (Cd_0, 1.82cm) which decreased significantly with increase in Cd concentration and recorded to be minimum with Cd_{25} (1.74cm) treatments. Among combinations of Pb and Cd, higher collar diameter was observed in control (1.84cm) and minimum in plants grown on highest concentration i.e. $Pb_{300}Cd_{25}$ (1.71cm) with maximum reduction (6.8 %) as expressed in Fig. 4.3B.

Similar trend was recorded after six months that maximum collar diameter was observed in control (2.57cm) and minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (2.22cm)

with maximum reduction (13.5 %).

Thus, the negative effect of Pb and Cd concentrations on collar diameter was recorded in both species, however, *Toona* plants showed minimum reduction (0.5% -13.5%) in comparison to *Salix* (1.4% -36%). Overall, the reduction in collar diameter was recorded more after six months than three months in both species. Similar to plant height, combination (Pb+Cd) treatments showed more pronounced effect than individual Cd and Pb concentrations in both *Salix* and *Toona* with following order Pb+Cd >Cd > Pb.

Plant height, collar diameter, biomass are the primary determinants of plant growth, Pb and Cd being non-essential elements inhibits plant growth at higher concentration (Haider *et al* 2021). The reduction in plant height due to Cd toxicity in *Poplar* species and Pb toxicity in *Jatropha curcas* is reported by Kieffer *et al* (2019) and Shu *et al* (2012) respectively. Guerrea *et al* (2011) reported that heavy metals such as Pb, Cd and Zn negatively affect the plant height and collar diameter of different *Poplar* species raised under controlled conditions. In case of *Leucaena leucocephala* and *Thespesia populneoides*, the significant reduction in collar diameter due to Pb and Cd induced toxicity has also been reported by Ismail *et al* (2013).

d. Number of branches

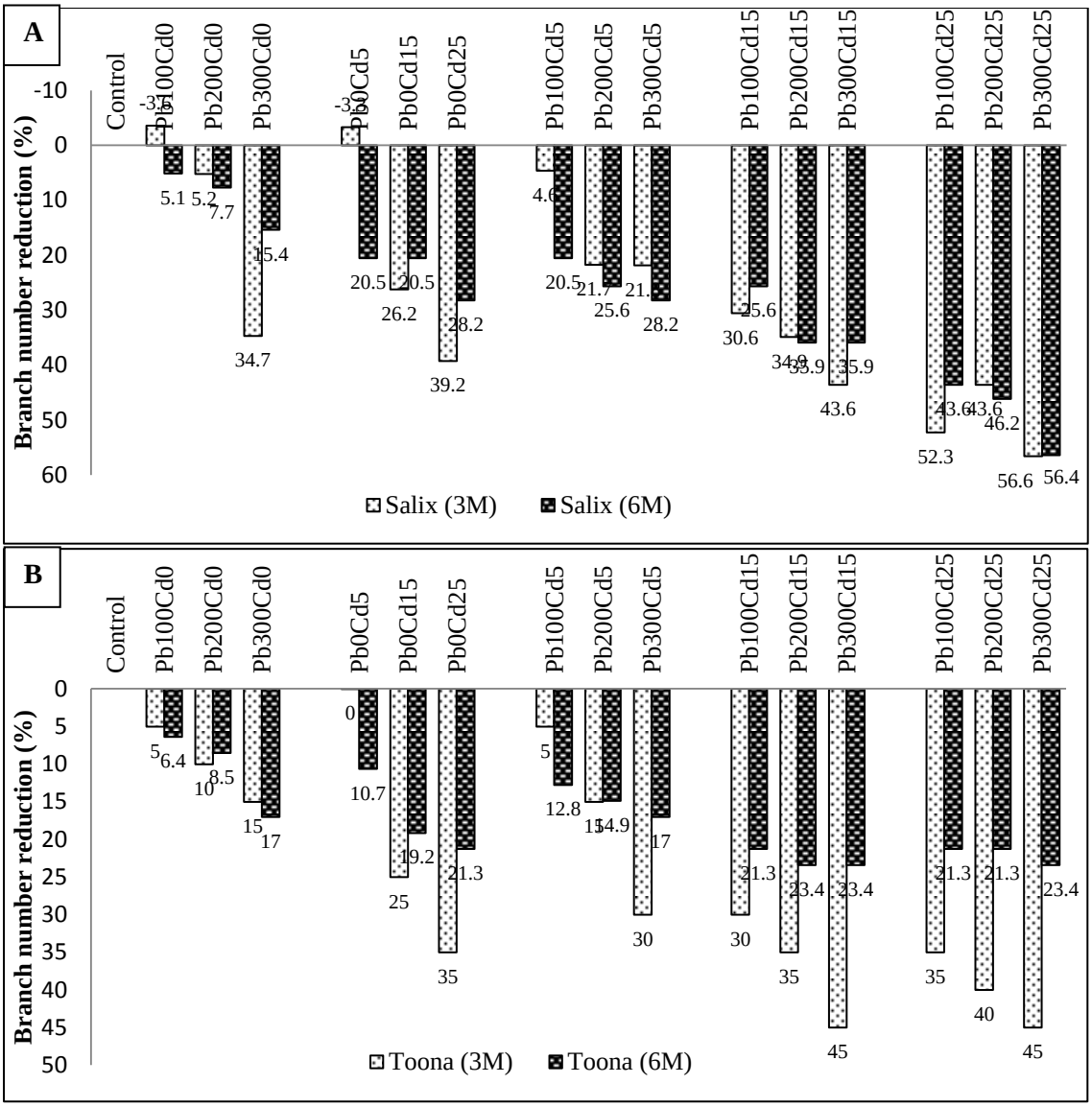
An appraisal of Table 4.3 showed significant differences in number of branches of *Salix* and *Toona* grown on different Pb and Cd concentrations, meanwhile the Pb x Cd interaction showed non-significant differences after three and six months.

In *Salix*, the significant decrease in number of branches was observed with increase in Pb and Cd concentrations (Table 4.3). After three months, among Pb concentrations maximum mean number of branches were recorded in control (Pb_0, 4.86) which was at par with Pb_{100} (4.53), Pb_{200} (4.24) and minimum with Pb_{300} (3.50) concentrations. Similar trend was observed with Cd concentrations that maximum mean number of branches observed in control (Cd_0, 5.24) which was statistically equivalent with Cd_5 (5.11) and minimum at Cd_{25} treatment level (3.0). Among combinations, maximum number of branches was observed with $Pb_{100}Cd_0$ (5.97) and minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (2.5) with maximum reduction of 56.7% as expressed in Fig. 4.4A. Similar trend was observed after six months, the significant decrease in number of branches with increase in Pb and Cd concentrations was recorded. Hence, maximum number of branches was observed in control (9.75) and minimum in highest concentration i.e. $Pb_{300}Cd_{25}$ (4.25) with maximum reduction of 56.4% as compared to control.

Table 4.3: Effect of heavy metals on number of branches of *Salix alba* and *Toona ciliata*

Number of branches										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	5.76^{ab}	5.97	5.46^{abcd}	3.76^{cde}	5.24^{A}	9.75^{a}	9.25^{ab}	9.00^{ab}	8.25^{abc}	9.06^{A}
Cd_5	5.95^{a}	5.50^{abc}	4.51^{abcd}	4.50^{abcd}	5.11^{A}	7.75^{bcd}	7.75^{bcd}	7.75^{cd}	7.00^{cde}	7.44^{B}
Cd_{15}	4.25^{abcde}	4.00^{bcde}	3.75^{cde}	3.25^{de}	3.81^{B}	7.75^{bcd}	7.25^{cd}	6.25^{def}	6.25^{def}	6.87^{B}
Cd_{25}	3.50^{de}	2.75^{de}	3.25^{de}	2.50^{e}	3.00^{C}	7.00^{cde}	5.50^{efg}	5.25^{fg}	4.25^{g}	5.50^{C}
Mean	4.86^{A}	4.53^{A}	4.24^{A}	3.50^{B}		8.06^{A}	7.44^{B}	6.94^{B}	6.44^{C}	
LSD($p\leq0.05$)	Pb	0.561				Pb	0.526			
	Cd	0.561				Cd	0.526			
	Pb×Cd	NS				Pb×Cd	NS			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	6.67^{a}	6.33^{ab}	6.00^{abc}	5.67^{abcd}	6.17^{A}	15.7^{a}	14.6^{ab}	14.3^{abc}	13.0^{bcd}	14.4^{A}
Cd_5	6.67^{a}	6.33^{ab}	5.67^{abcd}	4.67^{bcde}	5.83^{A}	14.0^{abcd}	13.7^{bcd}	13.3^{bcd}	13.0^{bcd}	13.5^{B}
Cd_{15}	5.00^{abcde}	4.67^{bcde}	4.33^{cde}	3.67^{e}	4.42^{B}	12.7^{bcd}	12.3^{cd}	12.0^{d}	12.0^{d}	12.2^{C}
Cd_{25}	4.33^{cde}	4.33^{cde}	4.00^{de}	3.67^{e}	4.08^{B}	12.3^{cd}	12.3^{cd}	12.3^{cd}	12.0^{d}	12.2^{C}
Mean	5.67^{A}	5.42^{A}	5.00^{AB}	4.42^{B}		13.7^{A}	13.2^{AB}	13.0^{AB}	12.5^{B}	
LSD($p\leq0.05$)	Pb	0.563				Pb	0.600			
	Cd	0.563				Cd	0.600			
	Pb×Cd	NS				Pb×Cd	NS			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)
(NS: Non-significant)



(3M: After three months, 6M: After six months)

Fig. 4.4: Per cent reduction in number of branches of *Salix alba* and *Toona ciliata* as affected by heavy metals with respect to control

Toona plants also showed significant decrease in number of branches due to Pb and Cd concentrations, but the Pb x Cd interactions was recorded to be non-significant (Table 4.3). After three months, among Pb concentrations the maximum mean number of branches were recorded in control (Pb_0, 5.67) which was at par with Pb_{100} (5.42) and decreased significantly with increase in Pb concentrations, thus minimum number of branches with highest concentration i.e. Pb_{300} (4.42). Similar trend was followed for Cd concentrations that maximum mean number of branches were recorded in control (Cd_0, 6.17) which was at par with Cd_5 (5.83) and minimum in highest concentration i.e. Cd_{25} (4.08) which further statistically equivalent to Cd_{15} (4.42). Among combinations, maximum number of branches recorded in control (6.67) and Pb_0Cd_5 treated plants (6.67) which decreased significantly with increase in concentration, thus minimum number of branches were recorded at highest

concentrations of Pb and Cd i.e. ($Pb_{300}Cd_{25}$) with maximum reduction of 40% with respect to control (Fig. 4B).

Similar trend was observed after six months that maximum number of branches of *Toona* was observed in control (15.7) and minimum in plants grown in highest heavy metal concentration i.e. $Pb_{300}Cd_{25}$ (12.0) which showed maximum reduction (23%) as comparative to control (Fig. 4.4B).

Thus, Pb and Cd concentrations negatively affect branch number of both species with more reduction in *Salix* (5 - 57%) than *Toona* plants (5 - 45%). For both species, the negative effect of Cd was more than Pb concentrations (Cd > Pb) on number of branches, however combination treatments showed non-significant difference after three and six months.

e. Number of leaves

The data on total number of leaves shows variable response of *Salix* and *Toona* with different concentrations of Pb, Cd and their combinations (Table 4.4). With increasing concentrations of Pb and Cd, the significant reduction in number of leaves of both species was recorded (Fig. 4.5).

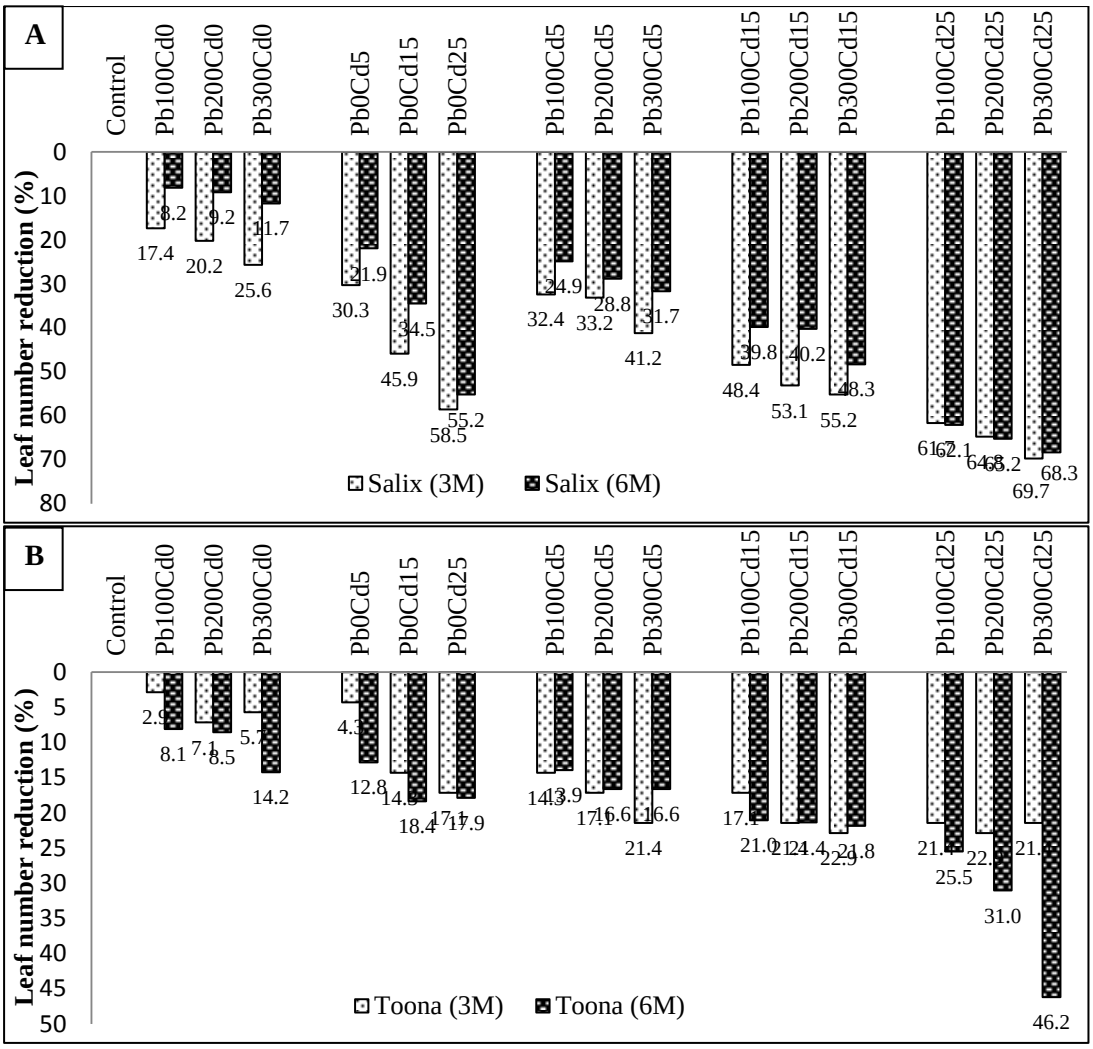
In *Salix*, the significant decrease in number of leaves was recorded after three and six months in response to Pb and Cd concentrations, but the Pb x Cd interaction was non-significant at six months. After three months, among Pb concentrations, maximum mean number of leaves recorded in control (Pb_0, 64), which decreased significantly with increase in Pb concentration in following order Pb_{100} (57.9), Pb_{200} (55.2) and Pb_{300} (50.3). Similar trend was observed in Cd that maximum mean number of leaves recorded in control (Cd_0, 81.2) which significantly decreased with increase in Cd concentrations i.e. Cd_5 (63.4), Cd_{15} (47.6) and Cd_{25} (35.1). Among Pb and Cd combinations, maximum number of leaves were recorded in control (96.5), which also decreased significantly with increase in Pb and Cd concentration, being minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (29.2) with maximum reduction (70%) as shown in Fig. 4.5A.

Similar trend was observed after six months, maximum leaf number was recorded in control (Pb_0Cd_0, 239.3) which decreased with increase in heavy metal concentrations and recorded to be minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (75.7) with maximum reduction of 68.3% (Fig. 4.5A).

Table 4.4: Effect of heavy metals on number of leaves of *Salix alba* and *Toona ciliata*

Number of leaves										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	96.5^{a}	79.7^{b}	77.0^{b}	71.8^{c}	81.2^{A}	239.3^{a}	219.7^{b}	217.2^{b}	211.2^{b}	221.8^{A}
Cd_5	67.3^{d}	65.2^{d}	64.5^{d}	56.7^{e}	63.4^{B}	186.7^{c}	179.7^{cd}	170.2^{de}	163.5^{ef}	175.1^{B}
Cd_{15}	52.3^{f}	49.7^{f}	45.3^{g}	43.3^{g}	47.6^{C}	156.7^{f}	144.0^{g}	143.0^{g}	123.7^{h}	141.8^{C}
Cd_{25}	40.0^{h}	37.0^{hi}	34.0^{ij}	29.2^{j}	35.1^{D}	107.2^{i}	90.7^{j}	83.2^{jk}	75.7^{k}	89.25^{D}
Mean	64.0^{A}	57.9^{B}	55.2^{C}	50.3^{D}		172.5^{A}	158.6^{B}	153.4^{C}	143.6^{D}	
LSD($p \leq 0.05$)	Pb	1.13				Pb	4.34			
	Cd	1.13				Cd	4.34			
	Pb×Cd	2.25				Pb×Cd	NS			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	70.0^{a}	68.1^{ab}	65.2^{b}	66.3^{b}	67.2^{A}	210.7^{a}	193.7^{b}	192.7^{b}	180.7^{cd}	194.4^{A}
Cd_5	67.0^{ab}	60.2^{c}	57.9^{cd}	55.2^{d}	60.0^{B}	183.7^{bc}	181.3^{cd}	175.6^{cde}	175.6^{cde}	179.1^{B}
Cd_{15}	60.1^{c}	57.9^{cd}	55.3^{d}	54.1^{d}	56.7^{C}	172.0^{de}	166.3^{ef}	165.7^{ef}	164.7^{ef}	167.2^{C}
Cd_{25}	57.9^{cd}	54.8^{d}	54.2^{d}	55.0^{d}	55.5^{C}	173.0^{cde}	157.0^{f}	145.3^{g}	113.3^{h}	147.2^{D}
Mean	63.7^{A}	60.3^{B}	58.0^{C}	57.5^{C}		184.8^{A}	174.6^{B}	169.8^{C}	158.6^{D}	
LSD($p \leq 0.05$)	Pb	1.23				Pb	3.39			
	Cd	1.23				Cd	3.39			
	Pb×Cd	2.47				Pb×Cd	6.78			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)



(3M: After three months, 6M: After six months)

Fig. 4.5: Per cent reduction in leaf number of *Salix alba* and *Toona ciliata* as affected by heavy metals with respect to control

Toona plants also showed significant reduction in number of leaves in response to different concentrations of Pb, Cd and their combinations as compared to control. After three months, among Pb concentrations, maximum mean number of leaves recorded in Pb_0 (63.7), which decreased significantly with increase in Pb concentration and minimum with highest Pb concentration i.e. Pb_{300} (57.5). Similarly, among Cd concentrations, maximum mean number of leaves were recorded in control (Cd_0, 67.2) which decreased significantly with increase in cadmium concentration and minimum in Cd_{25} (55.5) and at par with Cd_{15} (56.7) concentrations. Among, Pb and Cd combinations, maximum number of leaves were observed in control (Pb_0Cd_0, 70) and minimum in plants grown on highest concentration i.e. $Pb_{300}Cd_{25}$ (55) with maximum reduction (21.4%) as expressed in Fig. 4.5B.

Similar trend was observed after six months that maximum number of leaves was recorded in control (Pb_0Cd_0, 210.7) which decreased significantly with increase in heavy metal concentration. Hence, minimum number of leaves were with highest combination

concentration i.e. $Pb_{300}Cd_{25}$ (113.3) with maximum reduction of 46.2 % (Fig. 4.5B).

Hence, Pb, Cd and their combination concentrations negatively affect the total number of leaves of both species, but the reduction was more prominent in *Salix* than *Toona* at higher concentration i.e. $Pb_{300}Cd_{25}$. Overall, after three and six months, similar reduction patterns were observed in *Salix*, but in case of *Toona* higher reduction was recorded after six months than three months. The effect of Cd concentrations on number of leaves of both *Salix* and *Toona* was recorded to be more than Pb concentrations, but the effect of combination (Pb+Cd) concentrations was more pronounced in following order Pb+Cd > Cd> Pb.

The reduction in number of branches and number of leaves due to heavy metal stress is responsible for reduced biomass of the plants (Mleczek *et al* 2010). Our results are in accordance with Hatamian *et al* (2020), they also reported the reduction in total number of leaves per plants with rest of leaves having chloric and necrotic spots after application of 5mg/L Cd and 15mg/L Pb and 30mg/L Pb and single as well as in combination in European heackberry.

f. Root length (cm) and root number

Plant roots being first organ exposed to contaminated soil confers the variation in their number along with their morphological alterations induced due to heavy metal stress. Data presented in Table 4.5 vividly showed root length and root number of *Salix* and *Toona* in response to Pb and Cd concentrations after six months. With increasing concentrations of Pb and Cd in soil, significant decrease in root number and root length was recorded in both species. The Pb x Cd interaction for root attributes was non-significant in *Salix*, whereas significant interaction was recorded in *Toona*.

- **Root length (cm)**

In *Salix*, significant reduction in root length was recorded in response to Pb and Cd concentrations (Table 4.5). Among Pb concentrations, higher mean root length recorded in control (Pb_0, 73.3cm), which decreased significantly with increase in Pb concentrations in following order Pb_{100} (67.8cm), Pb_{200} (65.3cm) and Pb_{300} (61.4 cm). Similar trend was observed in Cd that higher mean root length was recorded in control (Cd_0, 79.1 cm) which decreased significantly with increase in Cd concentrations i.e. Cd_5 (74.4 cm), Cd_{15} (62.3 cm) and Cd_{25} (52.1cm). Among Pb and Cd combinations, minimum root length was recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (45.5cm) with maximum reduction (45%) as presented in Fig. 4.6A.

Similarly in case of *Toona*, significant decrease in root length was recorded with increase in Pb and Cd concentrations. Among Pb, Cd and their combinations, maximum root length was recorded in control (Pb_0Cd_0, 48.3 cm), which decreased significantly with increase in heavy metal concentration, hence minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (28.7 cm) with maximum reduction (40 %) as compared to control (Fig.4.6A).

Table 4.5: Effect of heavy metals on root length and root number of *Salix alba* and *Toona ciliata*

Root length (cm)										
	Salix alba					***Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	83.7^{a}	81.3^{ab}	80.5^{ab}	71.0^{cde}	79.1^{A}	48.3^{a}	46.0^{ab}	45.3^{b}	41.0^{c}	45.2^{A}
Cd_5	79.8^{abc}	75.0^{abcd}	72.5^{bcd}	70.25^{de}	74.4^{AB}	39.7^{cd}	38.3^{de}	34.7^{fg}	31.7^{hi}	36.1^{B}
Cd_{15}	67.7^{def}	62.2^{ef}	60.3^{fg}	58.7^{g}	62.3^{C}	37.0^{ef}	34.0^{gh}	31.0^{ij}	29.7^{ij}	32.9^{C}
Cd_{25}	62.1^{ef}	52.8^{gh}	48.1^{h}	45.5^{h}	52.1^{D}	31.0^{ij}	30.0^{ij}	29.0^{ij}	28.7^{j}	29.6^{D}
Mean	73.3^{A}	67.8^{B}	65.3^{B}	61.4^{C}		39.0^{A}	37.08^{B}	35.00^{C}	32.75^{d}	
LSD($p \leq 0.05$)	Pb	2.862				Pb	0.848			
	Cd	2.862				Cd	0.848			
	Pb×Cd	NS				Pb×Cd	1.697			
Root number										
	Salix alba					***Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	15.0^{a}	14.5^{ab}	14.5^{ab}	14.1^{abc}	14.5^{A}	15.7^{a}	14.7^{ab}	12.3^{cd}	12.0^{cde}	13.7^{A}
Cd_5	14.5^{ab}	14.2^{abc}	13.5^{abc}	12.3^{bcd}	13.5^{B}	13.7^{bc}	12.7^{cd}	11.7^{de}	11.3^{de}	12.3^{B}
Cd_{15}	14.2^{abc}	13.5^{abc}	13.1^{abc}	12.1^{bcd}	13.1^{B}	10.3^{ef}	9.67^{fg}	8.67^{gh}	8.33^{gh}	9.25^{C}
Cd_{25}	12.1^{bcd}	11.5^{bcd}	11.5^{bcd}	10.0^{d}	11.3^{C}	7.67^{h}	7.67^{h}	7.67^{h}	7.33^{c}	7.58^{D}
Mean	13.8^{A}	13.4^{A}	13.1^{A}	12.0^{B}		11.8^{A}	11.2^{B}	10.1^{C}	9.75^{C}	
LSD($p \leq 0.05$)	Pb	0.784				Pb	0.55			
	Cd	0.784				Cd	0.55			
	Pb×Cd	NS				Pb×Cd	1.10			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)
(NS: Non significant)

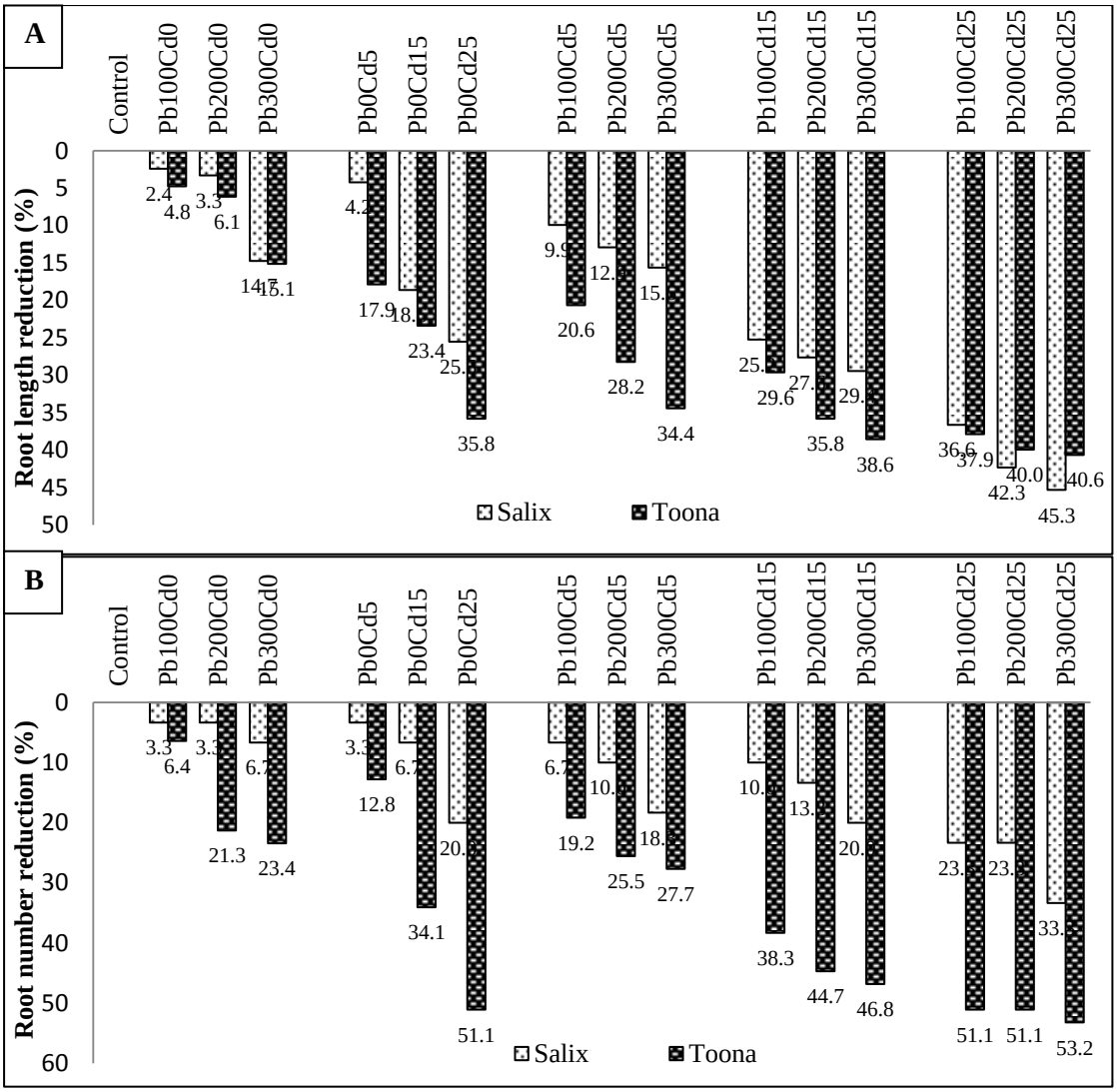


Fig. 4.6: Per cent reduction in root length and root number of *Salix alba* and *Toona ciliata* as affected by heavy metals with respect to control

- **Root number**

The significant decrease in root number of *Salix* was recorded in response of Pb and Cd concentrations (Table 4.5). Among Pb concentrations, maximum mean number of roots was recorded in control (Pb_0, 13.8) which was at par with Pb_{100} (13.4) and decreased significantly with increase in Pb concentration i.e. Pb_{200} (13.1) and Pb_{300} (12). Similar trend was observed with Cd that maximum mean root number was recorded in control (Cd_0, 14.5) which was decreased with increase in Cd concentration, thus recorded to be minimum with highest Cd concentration i.e. Cd_{25} (11.3). Among combinations, higher root number was recorded in control (Pb_0Cd_0, 15) and minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (10) with maximum reduction (10 %) as compared to control (Fig. 4.6B).

Similar trend was recorded in *Toona*, the significant decrease in root number in response to Pb and Cd concentrations. Among Pb, Cd and their combinations, maximum number of roots were recorded in control (15.7), which decreased significantly with increase

in heavy metal concentrations and recorded to be minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (7.33) with maximum reduction (53 %) as compared to control plants (Fig. 4.7B).

Hence, Pb, Cd and their combination concentrations negatively affect root length and root number of both species (*Salix* and *Toona*). Moreover, the reduction in root length was recorded to be highest in *Salix* (45%) than *Toona* (40%), whereas the reverse trend was observed in root number that maximum reduction was recorded in *Toona* (53%) than *Salix* (10%) with highest concentration i.e. $Pb_{300}Cd_{25}$. The effect of Cd concentrations on both root attributes was recorded more than the Pb concentrations, but the effect of their combination concentrations was more pronounced as in following order Pb+Cd >Cd > Pb.

In present investigations, the reduction in root length and root number was recorded in *Salix* and *Toona* grown on heavy metal contaminated soil. These results are confirmed by El-Mahrouk *et al* (2019), they reported that Pb, Cd and Cu treatments negatively affected the plant growth of *Salix mucronata* because the high concentration of metal ions in the soil media affects the elongation and meristem zone of root by altering the auxin distribution which inhibits the root growth by influencing root number, root length and root density as well as aerial plant parts. Similarly, Cd toxicity is also reported to reduce the uptake and translocation of mineral and nutrient ions by disturbing the normal root growth patterns which further deteriorate the plant metabolism and ultimately affects the plant morphology and physiology (Haider *et al* 2021).

g. Fresh and dry weight of roots (g)

Data presented in Table 4.6 shows the effect of heavy metals on fresh and dry weight of roots of *Salix* and *Toona*. With increasing concentration of Pb and Cd (individual as well as combinations), the significant reduction in fresh and dry weight of roots of both species were recorded and is expressed in Fig. 4.7.

Salix plants showed significant decrease in fresh weight of roots with different concentrations of Pb and Cd after six months. Among Pb concentrations, higher mean fresh weight of roots was recorded in control (Pb_0, 66.3g) which decreased significantly with increase in Pb concentration in following order Pb_{100} (63.4g), Pb_{200} (60.5g) and Pb_{300} (58.5g). Similar trend was observed for Cd that maximum mean fresh weight of roots recorded in control (Cd_0, 67.9g) which was at par with Cd_5 (67.5g) and minimum value with highest Cd concentration i.e. Cd_{25} (51.8g). Among Pb and Cd combinations, higher fresh weight of root was recorded in control (Pb_0Cd_0, 74.9 g) which decreased significantly with increase in heavy metal concentrations, hence, minimum fresh weight of root was observed in plants with highest concentrations of Pb and Cd i.e. $Pb_{300}Cd_{25}$ (48.4g) which was at par with $Pb_{200}Cd_{25}$ (50.7g) with maximum reduction (35 % and 32 % respectively) as compared to control (Fig. 4.7A).

Table 4.6: Effect of heavy metals on fresh and dry weight of roots of *Salix alba* and *Toona ciliata*

Fresh weight of root (g)										
	Salix alba					***Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	74.9^{a}	69.4^{b}	65.2^{d}	62.3^{e}	67.9^{A}	60.4^{a}	58.4^{a}	54.7^{b}	53.3^{b}	56.7^{A}
Cd_5	70.1^{b}	68.4^{bc}	66.5^{cd}	64.9^{de}	67.5^{A}	54.0^{b}	50.7^{c}	47.8^{cd}	44.7^{ef}	49.3^{B}
Cd_{15}	65.3^{d}	62.3^{e}	59.4^{f}	58.3^{f}	61.4^{B}	49.9^{c}	48.7^{c}	45.8^{de}	44.7^{ef}	47.3^{C}
Cd_{25}	54.6^{g}	53.4^{g}	50.7^{h}	48.4^{h}	51.8^{C}	49.6^{c}	43.8^{efg}	42.1^{fg}	41.1^{g}	44.2^{D}
Mean	66.3^{A}	63.4^{B}	60.5^{C}	58.5^{D}		53.5^{A}	50.4^{B}	47.6^{C}	45.9^{D}	
LSD($p \leq 0.05$)	Pb	0.904				Pb	0.903			
	Cd	0.904				Cd	0.903			
	Pb×Cd	1.809				Pb×Cd	1.806			
Dry weight of root (g)										
	Salix alba					***Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	36.3^{a}	33.9^{a}	30.4^{bc}	27.2^{def}	31.9^{A}	22.0^{ab}	21.8^{ab}	20.6^{abc}	20.3^{bcd}	21.2^{A}
Cd_5	31.2^{b}	29.1^{bcd}	27.9^{cde}	26.0^{efg}	28.6^{B}	22.5^{a}	20.8^{abc}	18.9^{cde}	17.5^{e}	19.9^{B}
Cd_{15}	26.2^{efg}	25.0^{fgh}	23.7^{ghi}	22.7^{hij}	24.4^{C}	20.4^{abcd}	18.9^{cde}	18.2^{e}	16.9^{e}	18.6^{C}
Cd_{25}	22.5^{hij}	21.8^{ij}	20.8^{ij}	20.3^{j}	21.4^{D}	18.5^{de}	17.3^{e}	17.3^{e}	16.8^{e}	17.5^{D}
Mean	29.0^{A}	27.5^{B}	25.7^{C}	24.06^{D}		20.8^{A}	19.7^{B}	18.7^{C}	17.8^{D}	
LSD($p \leq 0.05$)	Pb	0.889				Pb	0.635			
	Cd	0.889				Cd	0.635			
	Pb×Cd	1.778				Pb×Cd	1.271			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

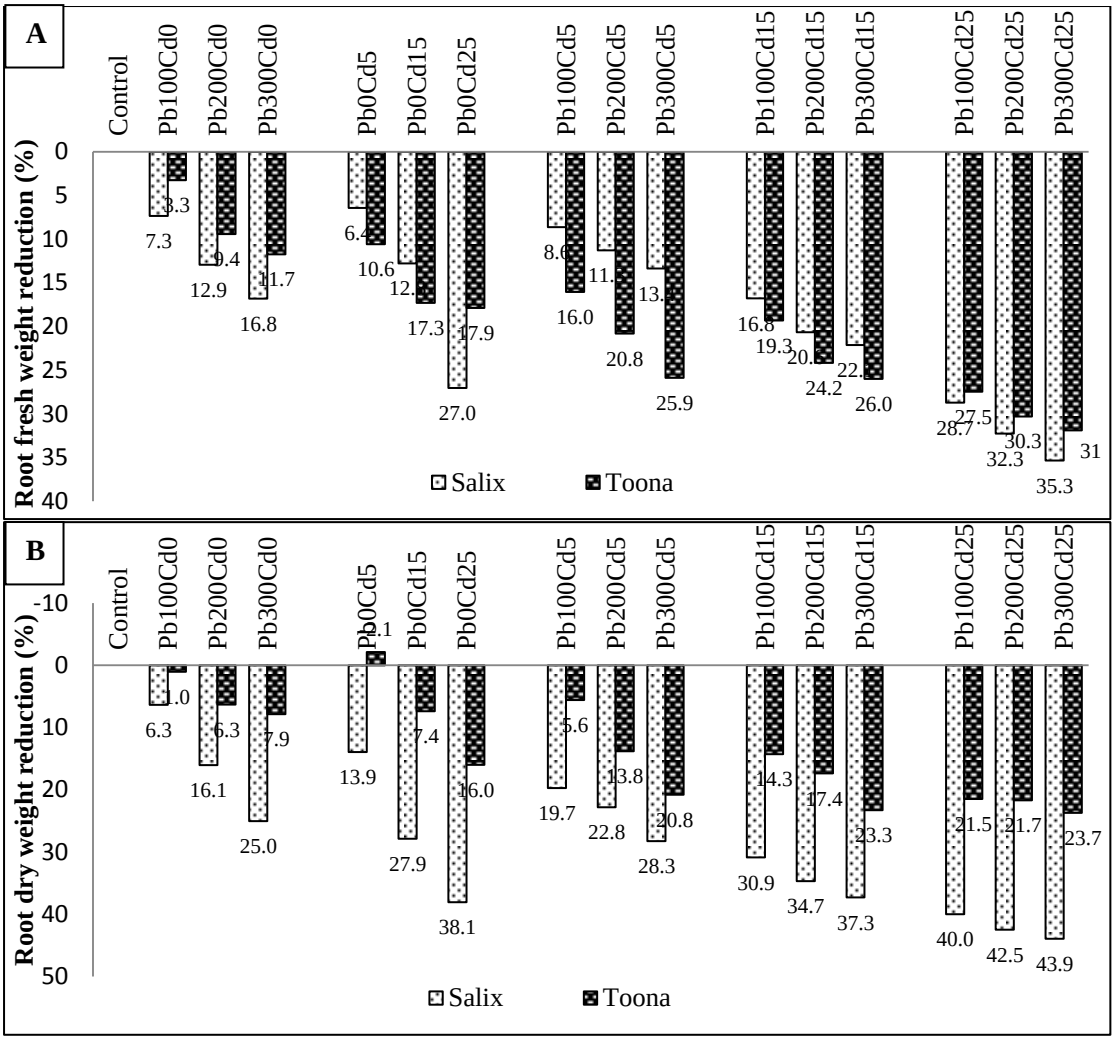


Fig. 4.7: Per cent reduction in fresh and dry weight of roots of *Salix alba* and *Toona ciliata* as affected by heavy metals with respect to control

Similar trend was recorded for dry weight of roots of *Salix* that Pb and Cd concentrations negatively affect the root biomass. Among Pb, Cd and their combinations, higher root dry weight was recorded in control (Pb_0Cd_0, 36.3g) at par with $Pb_{100}Cd_0$ (33.9g), which decreased significantly with increase in Pb and Cd concentrations, thus recorded minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (20.3) with maximum reduction (43.9%) as compared to control (Fig. 4.7B).

Toona plants also showed significant reduction in fresh weight and dry weight of roots with different Pb and Cd concentrations (Table 4.6). Among Pb concentrations, higher mean fresh weight of roots recorded in control (Pb_0, 53.5g) which decreased significantly with increase in Pb concentration, thus minimum with highest Pb concentration i.e. Pb_{300} (45.9g). Similar trend was observed with Cd concentrations that higher mean fresh weight of roots were recorded in control (Cd_0, 56.7g) which also decreased significantly with increase in concentration in following order i.e.Cd_5 (49.3g) Cd_{15} (47.3 g) and Cd_{25} (44.2g). Among Pb and Cd combinations, higher fresh weight of roots were observed in control (Pb_0Cd_0, 60.4g)

which was at par with $Pb_{100}Cd_0$ (58.4g) and recorded minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (41.1g) with maximum reduction (31 %) with respect to control (Fig. 4.7A).

Similarly, the negative effect of Pb and Cd concentrations was recorded in dry weight of roots of *Toona*. Among Pb, Cd and their combinations, higher dry weight of roots recorded in control (Pb_0Cd_0, 22g) which decreased significantly with increase in heavy metal concentrations and recorded minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (16.8g) which was statistically equivalent with $Pb_{300}Cd_{15}$ (16.9g) and $Pb_{200}Cd_{25}$ (17.3g) with maximum reduction of 23.7 %, 23.3 % and 21.7 % respectively (Fig. 4.7B).

Hence, Pb, Cd and their combinations negatively affect the root biomass (fresh and dry weight) of both species, however, *Salix* plants showed more reduction in root biomass at highest heavy metal concentrations ($Pb_{300}Cd_{25}$) as compared to *Toona*. The reduction in root biomass is correlated with root number and root length which conferred that Pb and Cd concentrations adversely affect the root system. The effect of Cd concentration on root biomass was recorded to be more than Pb concentrations, but the effect of their combinations was more pronounced and followed this order Pb+Cd > Cd > Pb.

h. Fresh and dry weight of shoots (g)

Data in Table 4.7 reveals the fresh and dry weight of shoots of *Salix* and *Toona* in response to different concentrations of Pb, Cd and their combinations. With increasing concentrations of Pb and Cd the significant decrease in shoot biomass was recorded and is presented in Fig. 4.8.

The significant reduction in fresh weight of shoots of *Salix* was recorded with different concentrations Pb and Cd after six months. Among Pb concentrations, higher mean fresh weight of shoot was observed in control (Pb_0, 98.7 g) which decreased significantly with increase in Pb concentration in following order Pb_{100} (93.1g), Pb_{200} (90.2 g) and Pb_{300} (86.1g). Similar trend was observed for Cd that higher mean fresh weight of shoot was observed in control (Cd_0, 110.3 g) which decreased significantly with increase in Cd concentration i.e. Cd_5 (102.8 g), Cd_{15} (82.6 g) and Cd_{25} (72.6 g). Among combinations, higher fresh weight of shoot recorded in control (Pb_0Cd_0, 120.5 g) which decreased significantly with increase in heavy metal concentrations, hence, minimum fresh weight of shoot was observed in plants with highest concentrations of Pb and Cd i.e. $Pb_{300}Cd_{25}$ (68.3g) with maximum reduction (43 %) as compared to control (Fig. 4.8A).

Similar trend was recorded for dry weight of shoots that Pb and Cd concentrations negatively affect the shoot biomass. Among Pb, Cd their combinations, higher shoot dry weight was recorded in control (Pb_0Cd_0, 57.3 g) which also decreased significantly with increase in Pb and Cd concentrations, thus recorded minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (37.8 g) with maximum reduction (34 %) as compared to control (Fig. 4.8B).

Table 4.7: Effect of heavy metals on fresh and dry weight of shoot of *Salix alba* and *Toona ciliata*

Fresh weight of shoot (g)										
	Salix alba					***Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	120.5^{a}	111.2^{b}	109.5^{b}	100.2^{d}	110.3^{A}	140.6^{a}	129.2^{bc}	125.8^{cd}	117.5^{ef}	128.3^{A}
Cd_5	112.2^{b}	104.4^{c}	98.5^{d}	96.4^{d}	102.8^{B}	133.3^{b}	126.2^{cd}	122.0^{de}	114.7^{fg}	124.0^{B}
Cd_{15}	85.6^{e}	83.4^{ef}	80.4^{f}	79.6^{fg}	82.6^{C}	115.6^{f}	109.8^{gh}	105.6^{hi}	95.33^{l}	106.6^{C}
Cd_{25}	76.4^{gh}	73.2^{hi}	72.3^{i}	68.3^{j}	72.6^{D}	104.3^{hij}	102.6^{ijk}	98.67^{jkl}	97.67^{kl}	100.8^{D}
Mean	98.7^{A}	93.1^{B}	90.2^{C}	86.1^{D}		123.4^{A}	116.9^{B}	113.1^{C}	106.3^{D}	
LSD($p \leq 0.05$)	Pb	1.31				Pb	1.89			
	Cd	1.31				Cd	1.89			
	Pb×Cd	2.62				Pb×Cd	3.78			
Dry weight of shoot (g)										
	Salix alba					***Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	57.3^{a}	55.1^{ab}	52.8^{b}	48.2^{c}	53.3^{A}	65.3^{a}	64.5^{a}	64.4^{a}	62.5^{ab}	64.2^{A}
Cd_5	52.0^{b}	48.3^{c}	46.5^{cde}	45.1^{cdef}	47.9^{B}	60.0^{bc}	57.7^{cd}	66.8^{cd}	55.0^{de}	57.4^{B}
Cd_{15}	47.9^{cd}	46.6^{cde}	44.7^{def}	43.1^{fg}	45.6^{C}	57.9^{cd}	55.4^{de}	52.9^{e}	47.4^{f}	53.4^{C}
Cd_{25}	44.4^{ef}	42.5^{fg}	40.9^{g}	37.8^{h}	41.4^{D}	52.5^{e}	47.8^{f}	46.9^{f}	45.2^{f}	48.1^{D}
Mean	50.4^{A}	48.1^{B}	46.2^{C}	43.6^{D}		58.9^{A}	56.4^{B}	55.3^{B}	52.5^{C}	
LSD($p \leq 0.05$)	Pb	1.03				Pb	1.13			
	Cd	1.03				Cd	1.13			
	Pb×Cd	1.06				Pb×Cd	2.26			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

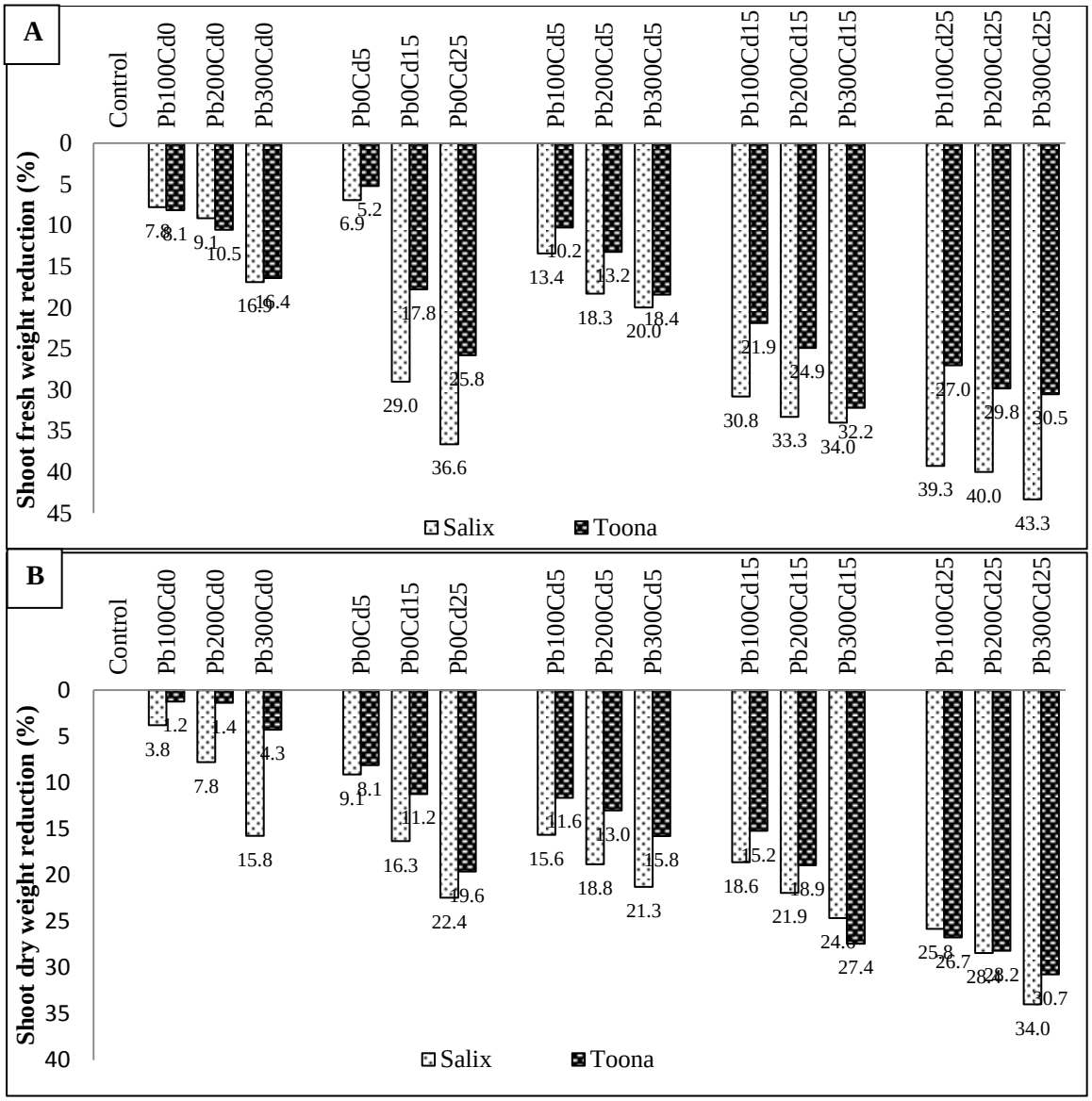


Fig. 4.8: Per cent reduction in fresh and dry weight of shoots of *Salix alba* and *Toona ciliata* as affected by heavy metals with respect to control

In case of *Toona*, significant reduction in fresh weight and dry weight of shoots was also recorded with Pb, Cd and their combination concentrations (Table 4.7). Among Pb concentrations, higher mean fresh weight of shoots was obseved in control (Pb_0, 123.4 g) which decreased significantly with increasing Pb concentration and recorded minimum with highest Pb concentration i.e. Pb_{300} (106.3 g). Similar trend was observed with Cd concentrations that higher mean fresh weight of shoots recorded in control (Cd_0, 128.3 g) which also decreased significantly with increase in concentration in following order Cd_5 (124 g) Cd_{15} (106.6 g) and Cd_{25} (100.8 g). Among combinations, higher fresh weight of shoots were observed in control (Pb_0Cd_0, 140.6) and recorded minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (97.67 g) with maximum reduction (30.5%) with respect to control. Similarly, the negative effect of Pb and Cd concentrations was recorded in dry weight of shoots of

Toona. Among Pb, Cd and their combinations, maximum dry weight of shoots was in control (Pb_0Cd_0, 65.3g) which decreased significantly with increase in heavy metal concentrations and recorded minimum with higher combination concentration i.e. $Pb_{300}Cd_{15}$ (47.4 g) $Pb_{200}Cd_{25}$ (46.9 g) and $Pb_{300}Cd_{25}$ (45.2 g) with maximum reduction of 30.7 % (Fig. 4.8B).

Hence, Pb, Cd and their combinations negatively affect the shoot biomass (fresh and dry weight) of both species, however, *Salix* plants showed more reduction in shoot biomass at highest heavy metal concentrations ($Pb_{300}Cd_{25}$) as compared to *Toona*. In present study, the reduction in shoot biomass can be correlated with reduced number of branches and number of leaves which conferred that Pb and Cd concentrations adversely affect the shoot system. The effect of Cd concentration on shoot biomass was recorded to be more than Pb concentrations, but the effect of their combinations was more pronounced and followed this order Pb+Cd > Cd > Pb.

In present study, Pb and Cd treatments reduce the biomass with average 40 % reduction in *Salix* and 25 % reduction in *Toona* at higher concentrations. The decline in root biomass is correlated with the decrease in root length and root number of plants grown on Pb and Cd contaminated soil. These results are in accordance with Günthardt-Goerg *et al* (2022) in which they reported the average 23% reduction in biomass of trees such as *Betula, Populus, Salix* and *Picea abies* raised under long term heavy metal polluted site. Mleczek *et al* (2010) reported that toxic heavy metal such as Pb, Cd and Hg reduce the biomass production ability of *Salix viminalis* and *Salix alba* var. Chermesina with an average reduction of 104 to 6.81Kg for each shrub. Under heavy metal stress, decrease in plant biomass may be linked to disrupted metabolic activity due to lower uptake of critical nutrients (Kacalkova *et al* 2015).

Conversely, it is concluded from morphological parameters that higher concentrations of heavy metals had significant negative effect on all studied traits along with reduction in biomass characteristics of *Salix* and *Toona* trees under nursery conditions. However, *Toona* plants in comparison to *Salix* showed better growth performance with minimum reduction in morphological traits at higher heavy metal concentrations. The adverse effect on morphological traits was more in combination (Pb+Cd) treatments; however, among both heavy metals the negative effect of Cd was more than Pb in both *Salix* and *Toona*.

4.1.2. Physiological and biochemical parameters

The effect of heavy metals on physiological ad biochemical parameters such as total chlorophyll content, carotenoid content, total soluble sugars, total soluble proteins, proline cotent and antioxidant enzyme activities (peroxidase, catalase and superoxide dismutase) were recorded and are discussed below:

a. Total chlorophyll (mg/g FW)

The data presented in Table 4.8 shows the total chlorophyll content of *Salix* and *Toona* leaves in response to different concentrations of Pb, Cd and their combinations. With increasing concentrations of Pb and Cd in soil (individual as well as combinations) significant decrease in chlorophyll content of both *Salix* and *Toona* was recorded.

The significant decrease in chlorophyll content of *Salix* was recorded after three and six month in response to Pb and Cd concentration, but Pb x Cd intereaction was significant at only six months. After three months, among Pb concentrations the mean chlorophyll content was recorded to be maximum in control (Pb_0, 4.05 mg/g FW), which decreased significantly with increase in Pb concentration in following order Pb_{100} (3.69 mg/g FW), Pb_{200} (3.47 mg/g FW) and Pb_{300} (3.30 mg/g FW). Similar trend was observed for Cd where maximum mean chlorophyll content was in control Cd_0 (4.17 mg/g FW) which significantly decreased with increase in Cd concentrations i.e. Cd_5 (3.87 mg/g FW), Cd_{15} (3.71 mg/g FW) and Cd_{25} (2.76 mg/g FW). Among combinations, minimum chlorophyll content was recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (2.39 mg/g FW). Thus, maximum reduction was recorded in combinations followed by individual Cd and Pb concentrations in order as Pb+Cd > Cd > Pb.

Similar trend was observed after six months that maximum chlorophyll content was recorded in control (Pb_0Cd_0, 2.87 mg/g FW) which decreased with increase in heavy metal concentrations and was minimum in highest combination concentration i.e. $Pb_{300}Cd_{25}$ (0.73 mg/g FW).

Toona plants also showed significant reduction in chlorophyll content under different Pb and Cd concentrations. After three months, among different Pb concentrations, maximum mean chlorophyll content was recorded in control i.e. Pb_0 (4.48 mg/g FW) which decreased significantly with increasing Pb concentrations and recorded to be minimum with highest Pb concentration (Pb_{300}, 3.73 mg/g FW). Among different Cd concentrations, maximum mean chlorophyll content was recorded with Cd_0 (4.60 mg/g FW) and minimum with highest Cd concentration (Cd_{25}, 3.71 mg/g FW). Among combinations, maximum chlorophyll content was recorded in control (Pb_0Cd_0, 5.70 mg/g FW) which decreased significantly with increase in heavy metal concentrations, hence, minimum chlorophyll content (3.56 mg/g FW) was observed with highest concentrations of Pb and Cd i.e. ($Pb_{300}Cd_{25}$). Similarly, after six months maximum chlorophyll content (4.47 mg/g FW) was recorded in control and minimum with highest Pb and Cd combination concentration i.e. $Pb_{300}Cd_{25}$ (2.25 mg/g FW).

Thus, Pb, Cd and their combination concentrations negatively affect chlorophyll content of both species (*Salix* and *Toona*). Moreover, the chlorophyll content also decreased from three month to six month interval. The negative effect of Cd concentrations on chlorophyll content was recorded be more than the Pb concentrations, but the effect of their combination concentrations was more pronounced as in following order Pb+Cd >Cd > Pb.

Table 4.8: Effect of heavy metals on total chlorophyll content of *Salix alba* and *Toona ciliata*

Total Chlorophyll (mg/g FW)										
Salix alba										
3 months						6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	4.47^{a}	4.25^{ab}	4.04^{abc}	3.93^{abcd}	4.17^{A}	2.87^{a}	2.65^{b}	2.44^{cd}	2.33^{de}	2.57^{A}
Cd_5	4.12^{abc}	3.98^{abcd}	3.78^{bcd}	3.59^{cd}	3.87^{B}	2.52^{c}	2.38^{de}	2.18^{f}	1.99^{g}	2.27^{B}
Cd_{15}	4.01^{abc}	3.88^{abcd}	3.58^{cd}	3.35^{d}	3.71^{B}	2.41^{cd}	2.28^{ef}	1.98^{g}	1.75^{h}	2.11^{C}
Cd_{25}	3.58^{cd}	2.65^{e}	2.48^{e}	2.39^{e}	2.76^{C}	1.98^{g}	1.06^{i}	0.88^{j}	0.73^{k}	1.16^{D}
Mean	4.05^{A}	3.69^{B}	3.47^{C}	3.30^{C}		2.45^{A}	2.09^{B}	1.87^{C}	1.70^{D}	
LSD($p\leq0.05$)	Pb	0.188				Pb	0.412			
	Cd	0.188				Cd	0.412			
	Pb×Cd	NS				Pb×Cd	0.083			
Toona ciliata										
3 months						6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	5.70^{a}	4.48^{b}	4.27^{bc}	3.97^{def}	4.60^{A}	4.47^{a}	4.17^{b}	3.96^{c}	3.25^{f}	3.96^{A}
Cd_5	4.35^{b}	4.21^{bcd}	4.01^{cde}	3.83^{efg}	4.10^{B}	4.04^{bc}	3.90^{cd}	3.74^{d}	3.52^{e}	3.80^{B}
Cd_{15}	4.04^{cde}	3.98^{def}	3.81^{efg}	3.58^{g}	3.85^{C}	3.93^{cd}	3.84^{cd}	3.30^{f}	3.27^{f}	3.59^{C}
Cd_{25}	3.84^{efg}	3.76^{efg}	3.69^{fg}	3.56^{g}	3.71^{D}	3.504^{e}	2.57^{g}	2.40^{gh}	2.25^{h}	2.68^{D}
Mean	4.48^{A}	4.11^{B}	3.94^{C}	3.735^{D}		3.99^{A}	3.62^{B}	3.35^{C}	3.07^{D}	
LSD($p\leq0.05$)	Pb	0.088				Pb	0.063			
	Cd	0.088				Cd	0.063			
	Pb×Cd	0.177				Pb×Cd	0.126			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)
(NS: Non significant)

High concentration of Pb and Cd extremely reduce the pigment (chlorophyll and carotenoids) concentration in *Salix* and *Toona* leaves through different mechanisms. The present results are in agreement with Zhang *et al* (2020); they reported that Cd stress inhibits the chlorophyll biosynthesis in tobacco leaves and down-regulatates the expressions of photosynthesis-related proteins. The pronounced Cd phytotoxicity is due to its primary sites of action on the photosynthetic apparatus that significantly affect the chlorophyll and carotenoid synthesis (Rafiq *et al* 2014). Iron (Fe2+), which is necessary to increase chlorophyll content and produce other pigments, is deficient in heavy metal-stressed plants due to Cd induced inhibition of iron (Fe3+) reductase, which also contributes to the lower pigment concentration (Hassan *et al* 2019). Pb disrupts the chlorophyll biosynthesis and photosynthesis either by preventing the translocation of vital photosynthetic pigment elements viz., Mg, K, Ca and Fe or by replacing divalent cations with trivalent cations (Gopal and Rizvi 2008). Further, it has been also reported that presence of Cd in soil reduces the availability of manganese (Mn) to plants that results into Mn^{2+}deficiency in plant tissues which could also be considered as the key factor for limiting photosynthesis and chloroplast development (Shi *et al* 2015). Under Pb stress, the enhanced production of ROS in plants cause peroxidation of chloroplast membranes which may results in reduced pigment accumulation and declined photosynthetic rate (Srinivasan *et al* 2014).

Among both plant species, *Salix* plants showed more reduction in chlorophyll content with higher heavy metal concentrations ($Pb_{300}Cd_{25}$) than *Toona* at three and six months. Thus, higher reduction in chlorophyll results in formation of chlorosis and necrosis spots in *Salix* as shown in Plate II.The heavy metal toxicity symptoms such as chlorosis, necrotic spots, leaf curling and leaf tip burning were recorded only in *Salix* plants in response to Pb and Cd concentrations, whereas in case of *Toona*, no such toxicity symptoms were recorded.

Visual symptoms such as stunting, chlorosis, necrosis and desiccation in foliage are also the peculiar features promoted by Cd toxicity (Khan *et al* 2021). Chlorosis and necrosis in most of their leaves treated with higher concentration of Cd and Pb were observed during the development of plant that is mainly because of the Cd accumulation that impairs the mesophyll tissue between the veins and further affects the synthesis and translocation of photosynthates via phloem tissues (Yadav 2010). These toxicity symptoms were decreased with long term exposure to heavy metal stress which reflects that *Salix* tolerance for heavy metal stress increased with aging. These results in accordance with Yan *et al* (2020), they reported that plant tolerance to abiotic stress increases with plant age, resulted in decreased toxicity symptoms at old age.

b. Carotenoid content (mg/gFW)

The data presented in Table 4.9 reveals the decrease in carotenoid content of *Salix* and *Toona* with different concentrations of Pb, Cd and their combinations.

In *Salix*, with increasing Pb and Cd concentrations the non-significant change in carotenoid content was recorded after three months, whereas after six months the significant decrease in carotenoid content was observed. Thus, after six months, maximum mean carotenoid content was recorded in control ($Pb_{0,}$ 0.115 mg/g FW) which decreased significantly with increase in Pb concentration and recorded to be minimum at highest Pb concentration i.e. Pb_{300} (0.07 mg/g FW). Similarly for Cd concentrations, maximum mean carotenoid content was observed in control (Cd_0, 0.118 mg/g FW) which decreased significantly with increase in Cd concentration from Cd_5 (0.104 mg/g FW) to Cd_{25} (0.067 mg/g FW). Among combinations, minimum carotenoid content was recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (0.049 mg/g FW).

Toona plants also showed non-significant change in carotenoid content after three months, which was recorded to be significant after six months in response to different Pb, Cd and their combination concentrations. After six months, among Pb concentrations maximum mean carotenoid content was recorded in control (Pb_0, 1.53 mg/g FW) which was statistically equivalent with Pb_{100} (1.52 mg/g FW), Pb_{200} (1.51 mg/g FW) and minimum with highest Pb concentrations i.e. Pb_{300} (1.49 mg/g FW). Similar trend was observed in Cd that maximum mean carotenoid content was recorded in control (Cd_0, 1.54 mg/g FW), Cd_5 (1.52 mg/g FW), Cd_{15} (1.51 mg/g FW) and recorded to be minimum with highest Cd concentration i.e. Cd_{25} (1.49 mg/g FW). Among combinations (Pb+Cd), minimum carotenoid content was observed in plants grown on highest concentration i.e. $Pb_{300}Cd_{25}$ (1.47 mg/g FW).

Thus, the negative effect of Pb and Cd concentrations on carotenoid content was recorded in both species (*Salix* and *Toona*), but the significant decrease in carotenoid content was recorded after only six months. The carotenoid content of both species significantly decreased with higher heavy metal concentrations (i.e. Pb_{300}, Cd_{25}) individually as well as in combinations; however, the maximum reduction was recorded with combination (Pb+Cd) than individual Cd and Pb.

These results are in concordance with Pilipović *et al* (2019), who recorded significant negative effects of heavy metals (Pb, Cd and Zn) on pigments concentration in poplar and willow. Goh *et al* (2014) reported that plant photosynthesis mainly depends on the content of photosynthetic pigments such as chlorophyll and carotenoids. Further, the decrease in pigment concentration directly affects the plant growth by affecting their biomass (Manzoor *et al* 2021)

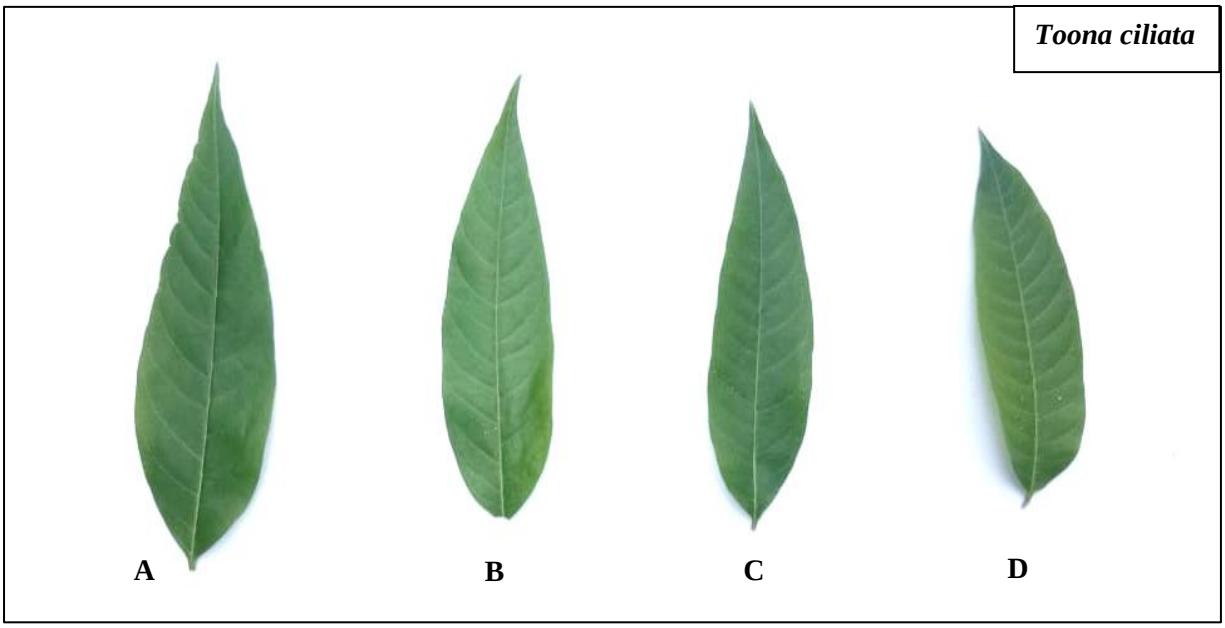


PLATE II: Appearance of toxic symptoms on leaves of *Salix alba* and *Toona ciliata* due to Pb and Cd treatments
(A: control plant leaves; B, C, D: treated plant leaves)

Table 4.9: Effect of heavy metals on carotenoid content of *Salix alba* and *Toona ciliata*

Carotenoids (mg/g FW)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	1.11^{a}	1.05^{a}	1.05^{ab}	1.02^{ab}	1.06^{A}	0.133^{a}	0.128^{a}	0.117^{bc}	0.097^{e}	0.118^{A}
Cd_5	1.05^{a}	1.03^{ab}	1.02^{ab}	1.01^{ab}	1.03^{AB}	0.125^{ab}	0.109^{c}	0.098^{de}	0.085^{f}	0.104^{B}
Cd_{15}	1.02^{ab}	1.03^{ab}	1.01^{ab}	0.98^{ab}	1.01^{AB}	0.114^{c}	0.108^{cd}	0.087^{ef}	0.048^{h}	0.089^{C}
Cd_{25}	1.03^{ab}	0.98^{ab}	0.96^{bc}	0.95^{c}	0.98^{B}	0.089^{ef}	0.074^{g}	0.058^{h}	0.049^{h}	0.067^{D}
Mean	1.05^{A}	1.02^{AB}	1.01^{BC}	0.99^{C}		0.115^{A}	0.105^{B}	0.090^{C}	0.070^{D}	
LSD($p \leq 0.05$)	Pb NS Cd NS Pb×Cd NS					Pb 0.004 Cd 0.004 Pb×Cd 0.008				
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	2.34^{a}	2.28^{ab}	2.28^{a}	2.25^{bcde}	2.29^{A}	1.55^{a}	1.55^{a}	1.54^{a}	1.52^{bc}	1.54^{A}
Cd_5	2.28^{abc}	2.26^{abcd}	2.25^{abcd}	2.24^{de}	2.26^{A}	1.54^{a}	1.53^{ab}	1.52^{ab}	1.50^{a}	1.52^{A}
Cd_{15}	2.25^{abcd}	2.26^{cde}	2.24^{ef}	2.21^{efg}	2.24^{B}	1.53^{a}	1.53^{a}	1.51^{a}	1.47^{ab}	1.51^{A}
Cd_{25}	2.26^{efg}	2.21^{efg}	2.18^{fg}	2.18^{g}	2.21^{B}	1.51^{ab}	1.49^{ab}	1.48^{b}	1.47^{c}	1.49^{B}
Mean	2.28^{A}	2.25^{A}	2.24^{B}	2.22^{C}		1.53^{A}	1.52^{A}	1.51^{A}	1.49^{B}	
LSD($p \leq 0.05$)	Pb NS Cd NS Pb×Cd NS					Pb 0.030 Cd 0.030 Pb×Cd NS				

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)
(NS: Non significant)

c. Total soluble sugars (mg/g DW)

An appraisal of Table 4.10 showed significant differences in total soluble sugar content of *Salix* and *Toona* grown on different Pb and Cd concentrations, meanwhile the Pb x Cd interaction showed non-significant differences in *Toona* after three and six months.

In *Salix*, the significant increase in total soluble sugar content was recorded in response to different Pb and Cd concentrations. After three months, among Pb concentrations, minimum mean total soluble sugar content was observed in control (Pb_0, 12.9 mg/g DW) which increased significantly with increase in Pb concentration from Pb_{100} (14.2 mg/g DW) to Pb_{300} (17.4 mg/g DW). Similar trend was observed with Cd concentrations that minimum mean total soluble sugar content was observed in control (Cd_0, 11.6 mg/g DW) which increased significantly with increase in Cd concentration and maximum with Cd_{25} concentration (19.3 mg/g DW). Similarly, among combinations maximum total soluble sugar content was observed with highest concentration i.e. $Pb_{300}Cd_{25}$ (21.2 mg/g DW).

Similar trend was observed after six months that significant increase in total soluble sugar content with increase in Pb and Cd concentrations. The minimum total soluble sugar content was recorded in control (9.65 mg/g DW) and maximum with highest concentration i.e. $Pb_{300}Cd_{25}$ (21.2 mg/g DW).

Toona plants also showed significant variation in total soluble sugar content in response to different Pb and Cd concentrations, but the Pb x Cd interactions was recorded non-significant after three and six months. After three months, among Pb concentrations the minimum mean total soluble sugar content was observed in control (Pb_0, 12.6 mg/g DW) which was at par with Pb_{100} (12.9 mg/g DW) and increased significantly with increase in Pb concentrations, thus recorded to be maximum with highest concentration i.e. Pb_{300} (13.6 mg/g DW). Similar trend was followed for Cd concentrations that minimum mean total soluble sugar content was observed in control (Cd_0, 12.1 mg/g DW) and maximum with highest concentration i.e. Cd_{25} (14.3 mg/g DW). Among combinations, minimum total soluble sugar content observed in control (11.3 mg/g DW) which increased significantly with increase in metal concentration, thus maximum total soluble sugar content were recorded with highest concentrations of Pb and Cd i.e. ($Pb_{300}Cd_{25}$, 14.7 mg/g DW).

Similar trend was observed after six months that minimum total soluble sugar content was observed in control (15.6 mg/g DW) and maximum in plants grown on highest heavy metal concentration i.e. $Pb_{300}Cd_{25}$ (19.9 mg/g DW).

When subjected to challenging conditions, a shift in plant metabolism is to be expected; in these cases, plants allocate their resources (including energy use) to the adaptive response mechanism for stress tolerance while retaining a balanced oxidation-reduction status.

Table 4.10: Effect of heavy metals on total soluble sugar content of *Salix alba* and *Toona ciliata*

Total soluble sugars (mg/g DW)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	9.65^{i}	10.9^{h}	11.2^{h}	14.8^{ef}	11.6^{D}	12.4^{h}	14.6^{gh}	18.6^{efg}	25.4^{cd}	17.7^{D}
Cd_5	10.9^{h}	11.1^{h}	13.4^{g}	15.6^{e}	12.7^{C}	16.4^{fgh}	18.8^{efg}	19.8^{ef}	22.6^{de}	19.4^{C}
Cd_{15}	14.3^{fg}	15.6^{e}	16.7^{d}	18.2^{c}	16.2^{B}	20.6^{ef}	25.8^{cd}	26.7^{bcd}	29.4^{abc}	25.6^{B}
Cd_{25}	17.1^{d}	19.2^{bc}	19.8^{b}	21.2^{a}	19.3^{A}	28.6^{abc}	30.6^{ab}	31.7^{a}	32.0^{a}	30.7^{A}
Mean	12.9^{D}	14.2^{C}	15.3^{B}	17.4^{A}		19.5^{D}	22.4^{C}	24.2^{B}	27.4^{A}	
LSD($p \leq 0.05$)	Pb 0.036 Cd 0.036 Pb×Cd 0.073					Pb 1.37 Cd 1.37 Pb×Cd 2.75				
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	11.3^{i}	11.9^{hi}	12.2^{fghi}	12.9^{defgh}	12.1^{D}	15.7^{f}	16.6^{ef}	17.3^{de}	17.9^{cde}	16.9^{D}
Cd_5	12.1^{ghi}	12.4^{efgh}	12.9^{defgh}	13.3^{cdef}	12.7^{C}	17.3^{de}	17.9^{cde}	18.1^{bcde}	18.5^{abcd}	17.9^{C}
Cd_{15}	13.1^{cdefg}	13.4^{cde}	13.5^{bcde}	13.7^{abcd}	13.4^{B}	18.3^{bcd}	18.5^{abcd}	18.8^{abcd}	18.9^{abcd}	18.6^{B}
Cd_{25}	13.9^{abcd}	14.2^{abc}	14.5^{ab}	14.7^{a}	14.3^{A}	19.1^{abc}	19.3^{abc}	19.7^{ab}	19.9^{a}	19.5^{A}
Mean	12.6^{C}	12.9^{BC}	13.3^{AB}	13.7^{A}		17.6^{C}	18.1^{BC}	18.5^{AB}	18.8^{A}	
LSD($p \leq 0.05$)	Pb 0.334 Cd 0.034 Pb×Cd NS					Pb 0.459 Cd 0.459 Pb×Cd NS				

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)
(NS: Non-significant)

Our results indicate total soluble sugar content increased in response to Pb and Cd concentrations after both three and six month intervals in *Salix* and *Toona*. Along with this, enhanced total soluble sugars accumulation was recorded from three month to six month interval. Under stress conditions, the enhanced accumulation of total soluble sugars might be considered as the adaptive mechanism of plants to maintain optimum osmotic potential in cell and provide sufficient carbohydrate supply to support plant metabolism (Verma and Dubey 2001). In plants growing in Cd-contaminated soil, the accumulation of soluble sugars may serve as an adaptation mechanism for ensuring a favourable osmotic potential in plant cells. In addition, total soluble sugars enhance the plant's ability to store enough carbohydrates to maintain basal metabolism in a challenging environment (Zhao *et al* 2021).

d. Total soluble proteins (mg/g DW)

The data presented in Table 4.11 shows the significant variation in total soluble proteins in response to different concentrations of Pb, Cd and their combinations.

In *Salix*, the significant increase in total soluble proteins was recorded after three and six months in response to Pb and Cd concentrations. After three months, among Pb concentrations, minimum mean total soluble proteins recorded in control (1.13 mg/g DW), which increased significantly with increase in Pb concentration in following order Pb_{100} (1.22 mg/g DW), Pb_{200} (1.27 mg/g DW) and Pb_{300} (1.32 mg/g DW). Similar trend was observed for Cd that minimum mean total soluble protein content recorded in control (Cd_0, 0.971 mg/g DW) which significantly increased with increase in Cd concentrations i.e. Cd_5 (1.09 mg/g DW), Cd_{15} (1.30 mg/g DW) and Cd_{25} (1.59 mg/g DW). Similarly, among combinations, control plants (Pb_0Cd_0) showed minimum total soluble protein content (0.84 mg/g DW), which also increased significantly with increase in Pb and Cd concentration, thus recorded to be maximum with highest concentration i.e. $Pb_{300}Cd_{25}$ (1.65 mg/g DW).

Similar trend was observed after six months that minimum total soluble protein content was observed in control (Pb_0Cd_0, 1.97 mg/g DW) which increased with increase in heavy metal concentrations and recorded to be maximum with highest concentration i.e. $Pb_{300}Cd_{25}$ (3.98 mg/g DW).

Toona plants also showed significant increase in total soluble proteins in response to different concentrations of Pb, Cd and their combinations as compared to control. After three months, among Pb concentrations, maximum mean total soluble proteins recorded in Pb_0 (2.32 mg/g DW) which increased significantly with increase in Pb concentration, thus maximum with highest Pb concentration i.e. Pb_{300} (2.84 mg/g DW). Similarly, among Cd concentrations, minimum mean total soluble proteins was recorded in control (Cd_0, 2.68 mg/g DW) which increased significantly with increase in Cd concentration and recorded be maximum with Cd_{25} (3.23 mg/g DW). Among combinations, minimum total soluble proteins was recorded in control (Pb_0Cd_0, 1.69 mg/g DW) and maximum in plants grown on highest concentration i.e. $Pb_{300}Cd_{25}$ (3.42 mg/g DW).

Table 4.11: Effect of heavy metals on total soluble proteins of *Salix alba* and *Toona ciliata*

Total soluble proteins (mg/g DW)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.845^{h}	0.921^{gh}	1.01^{g}	1.11^{f}	0.971^{D}	1.97^{i}	2.15^{hi}	2.23^{hi}	2.69^{fg}	2.26^{D}
Cd_5	0.95^{g}	1.10^{f}	1.14^{ef}	1.16^{ef}	1.09^{C}	2.38^{gh}	2.44^{gh}	2.45^{gh}	2.58^{g}	2.46^{C}
Cd_{15}	1.22^{de}	1.29^{cd}	1.32^{c}	1.38^{c}	1.30^{B}	2.96^{ef}	3.20^{de}	3.56^{bc}	3.76^{ab}	3.37^{B}
Cd_{25}	1.52^{b}	1.58^{ab}	1.61^{ab}	1.65^{a}	1.59^{A}	3.40^{cd}	3.62^{bc}	3.77^{ab}	3.98^{a}	3.69^{A}
Mean	1.13^{D}	1.22^{C}	1.27^{B}	1.32^{A}		2.68^{D}	2.85^{C}	3.00^{B}	3.25^{A}	
LSD(p≤0.05)	Pb	0.032				Pb	0.101			
	Cd	0.032				Cd	0.101			
	Pb×Cd	0.063				Pb×Cd	0.203			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	1.69^{i}	1.97^{h}	2.15^{fgh}	2.46^{de}	2.68^{D}	2.35^{g}	3.23^{f}	3.87^{e}	4.25^{d}	3.46^{C}
Cd_5	2.09^{gh}	2.16^{fgh}	2.17^{fgh}	2.25^{fg}	2.17^{C}	3.78^{e}	4.26^{cd}	4.45^{bcd}	4.65^{ab}	4.29^{B}
Cd_{15}	2.35^{ef}	2.57^{d}	2.92^{c}	3.25^{ab}	2.77^{B}	4.57^{abc}	4.62^{ab}	4.68^{ab}	4.72^{ab}	4.65^{A}
Cd_{25}	3.14^{b}	3.12^{b}	3.23^{ab}	3.42^{a}	3.23^{A}	4.70^{ab}	4.74^{ab}	4.79^{ab}	4.82^{a}	4.76^{A}
Mean	2.32^{D}	2.46^{C}	2.62^{B}	2.84^{A}		3.85^{D}	4.21^{C}	4.45^{B}	4.61^{A}	
LSD(p≤0.05)	Pb	0.063				Pb	0.062			
	Cd	0.063				Cd	0.062			
	Pb×Cd	0.126				Pb×Cd	0.124			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference (p≤0.05)

In *Toona*, similar trend was observed after six months that minimum total soluble proteins recorded in control (Pb_0Cd_0, 2.35 mg/g DW) which increased significantly with increase in heavy metal concentration, thus recorded to be maximum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (4.82 mg/g DW). Hence, Pb, Cd and their combination concentrations cause accumulation of total soluble proteins in both species. Under heavy metal stress, the accumulation of proteins might be due to the upregulation of defense related proteins for maintenance of cell redox status.

Extreme environmental conditions such as heavy metal stress further alter the gene expression that contributes to variable protein diversity in plants. At molecular level, a change in protein abundance considered as biomarkers for the manifestation of plant responses to stress (Nanjo *et al* 2011). In the cytosol, Cd, Pb and other toxic metal ions interacts with proteins and reduces the protein pool in most of the plant species, but in some cases protein content increases in response to heavy metal stress (Mishra *et al* 2006). This accumulation is is mostly triggered by the production of defence related proteins involved in the maintenance of the cell redox status (ascorbate), metal sequestration, or detoxification (methalothionines, glutathione and phytochelatins) (Rasool *et al* 2020). In this context, Taamalli *et al* (2015) reported the upregulation of protein production in plant cell implicated in Cd chelation and compartmentalization processes in Cd-tolerant halophyte.

e. **Proline (µg/g DW)**

Data presented in Table 4.12 vividly shows the increase in proline content of *Salix* and *Toona* in response to Pb and Cd concentrations after three and six months.

In *Salix*, significant increase in proline content was recorded with different Pb, Cd and their combination concentrations after three and six months. Among Pb concentrations, minimum mean proline content was recorded in control (Pb_0, 0.469 µg/g DW) which increased significantly with increase in Pb concentrations in following order Pb_{100} (0.543 µg/g DW), Pb_{200} (0.585 µg/g DW) and Pb_{300} (0.643 µg/g DW). Similar trend was observed for Cd that control plants recorded with minimum proline content (0.349 µg/g DW) which increased significantly with increase in Cd concentrations i.e. Cd_5 (0.485 µg/g DW), Cd_{15} (0.637 µg/g DW) and Cd_{25} (0.767 µg/g DW). Among Pb and Cd combinations, control (Pb_0Cd_0) plants recorded with minimum proline content (0.195 µg/g DW) and maximum proline accumulation was recorded in highest concentration i.e. $Pb_{300}Cd_{25}$ (0.88 µg/g DW). Similar trend was observed after six months that proline accumulation increased with increase in Pb and Cd concentration, thus maximum proline content recorded with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (3.22 µg/g DW).

Table 4.12: Effect of heavy metals on proline content of *Salix alba* and *Toona ciliata*

Proline (µg/g FW)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.195^{i}	0.344^{h}	0.411^{g}	0.451^{f}	0.349^{D}	0.289^{i}	1.34^{h}	1.56^{g}	1.62^{g}	1.20^{D}
Cd_5	0.361^{h}	0.471^{f}	0.552^{e}	0.563^{e}	0.485^{C}	1.45^{gh}	1.59^{g}	1.82^{f}	1.91^{f}	1.69^{C}
Cd_{15}	0.612^{d}	0.633^{d}	0.633^{d}	0.683^{c}	0.637^{B}	1.94^{f}	2.13^{e}	2.42^{d}	2.58^{d}	2.27^{B}
Cd_{25}	0.712^{bc}	0.732^{b}	0.754^{b}	0.881^{a}	0.767^{A}	2.89^{c}	2.99^{bc}	3.14^{ab}	3.22^{a}	3.06^{A}
Mean	0.469^{D}	0.543^{C}	0.585^{B}	0.643^{A}		1.64^{D}	2.01^{C}	2.23^{B}	2.33^{A}	
LSD(p≤0.05)	Pb	0.013				Pb	0.061			
	Cd	0.013				Cd	0.061			
	Pb×Cd	0.027				Pb×Cd	0.121			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	1.17^{j}	1.32^{i}	1.39^{hi}	1.43^{ghi}	1.33^{D}	1.53^{h}	2.31^{g}	2.87^{f}	3.89^{cd}	2.65^{D}
Cd_5	1.34^{i}	1.45^{fgh}	1.53^{efg}	1.54^{ef}	1.47^{C}	2.45^{g}	2.89^{f}	2.94^{f}	3.92^{cd}	3.05^{C}
Cd_{15}	1.59^{de}	1.61^{cde}	1.61^{cde}	1.66^{bcd}	1.62^{B}	3.34^{e}	3.78^{d}	3.98^{cd}	4.08^{c}	3.79^{B}
Cd_{25}	1.69^{bcd}	1.71^{bc}	1.73^{b}	1.86^{a}	1.75^{A}	4.32^{b}	4.37^{ab}	4.43^{ab}	4.57^{a}	4.42^{A}
Mean	1.45^{D}	1.53^{C}	1.57^{B}	1.63^{A}		2.91^{D}	3.34^{C}	3.56^{B}	4.11^{A}	
LSD(p≤0.05)	Pb	0.034				Pb	0.072			
	Cd	0.034				Cd	0.072			
	Pb×Cd	0.068				Pb×Cd	0.145			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference (p≤0.05)
(NS: Non significant)

Similarly in case of *Toona*, significant increase in proline accumulation was recorded in response to Pb, Cd and their combination concentrations. After three months, among Pb concentrations, the minimum mean proline content was recorded in control (Pb_0, 1.45 µg/g DW) which increased significantly with increase in Pb concentration, thus recorded to be maximum with highest concentration i.e. Pb_{300} (1.63 µg/g DW). Similarly for Cd concentrations, control plants (Cd_0) showed minimum proline accumulation (1.33 µg/g DW) which increased significantly with increase in concentration from Cd_5 (1.47 µg/g DW) to Cd_{25} (1.75 µg/g DW). Among Pb, Cd and their combinations, minimum proline content was recorded in control (Pb_0Cd_0, 1.17 µg/g DW) which increased significantly with increase in heavy metal concentration, thus maximum proline content was recorded with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (1.86 µg/g DW).

Similar trend was followed after six months that proline accumulation increased with increase in Pb and Cd concentration, hence minimum proline content was observed in control (Pb_0Cd_0, 1.53 µg/g DW) and maximum in plants grown on highest concentration i.e. $Pb_{300}Cd_{25}$ (4.57 µg/g DW).

Thus, both species (*Salix* and *Toona*) showed enhanced accumulation of proline in response to Pb and Cd concentrations. Along with this, proline accumulation was also increased from three month to six months. Under stress conditions, the enhanced proline production might be considered as plant tolerance mechanism to protect the plant cell from osmotic and oxidative damage. The effect of Cd concentrations on proline content was recorded more than Pb concentrations, but the effect of their combination concentrations was more pronounced as in following order Pb+Cd >Cd > Pb.

Proline, a multifunctional amino acid, is widely known for its crucial role in adaption mechanism toward abiotic stress (Chun *et al* 2018, Liang *et al* 2013). In the present study, maximum proline content was recorded in highest treated plants ($Pb_{300}Cd_{25}$), and these results are in agreement with Islam *et al* (2009), who reported that Cd toxicity enhance the proline level that further reduces its inhibitory effect on cell growth and its expansion in tobacco.

f. Total antioxidants (µg/g DW)

The data presented in Table 4.13 shows the significant differences in total antioxidants in *Salix* and *Toona* plants grown on different Pb, Cd and their combination concentrations after three and six months.

In *Salix*, the significant increase in total antioxidant content was recorded in response to different Pb and Cd concentrations. After three months, among Pb concentrations, minimum mean antioxidants were recorded in control (Pb_0, 5.87 µg/g DW) which increased significantly with increase in Pb concentration from Pb_{100} (6.34 µg/g DW) to Pb_{300} (6.53 µg/g DW).

Table 4.13: Effect of heavy metals on total antioxidants in *Salix alba* and *Toona ciliata*

Total antioxidant content (µg/g DW)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	4.63^{i}	5.74^{fgh}	5.86^{efgh}	5.62^{gh}	5.46^{D}	9.45^{l}	10.3^{l}	12.2^{j}	15.5^{gh}	11.8^{D}
Cd_5	5.55^{h}	5.81^{efgh}	6.05^{efg}	6.16^{def}	5.89^{C}	11.3^{k}	13.9^{i}	14.9^{h}	16.4^{fg}	14.1^{C}
Cd_{15}	6.24^{cde}	6.53^{cd}	6.52^{cd}	6.68^{c}	6.49^{B}	15.5^{gh}	16.9^{ef}	17.3^{de}	17.9^{d}	16.9^{B}
Cd_{25}	7.09^{b}	7.28^{ab}	7.48^{ab}	7.62^{a}	7.37^{A}	19.2^{c}	20.3^{b}	20.9^{b}	22.4^{a}	20.7^{A}
Mean	5.87^{C}	6.34^{B}	6.47^{AB}	6.53^{A}		13.9^{D}	15.4^{C}	16.4^{B}	18.0^{A}	
LSD(p≤0.05)	Pb	0.141				Pb	0.299			
	Cd	0.141				Cd	0.299			
	Pb×Cd	0.282				Pb×Cd	0.599			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	2.63^{i}	3.74^{gh}	3.86^{fg}	3.62^{gh}	3.46^{D}	3.68^{h}	3.73^{h}	3.78^{h}	3.84^{h}	3.76^{D}
Cd_5	3.55^{h}	3.81^{g}	4.05^{ef}	4.19^{e}	3.89^{C}	3.80^{h}	3.84^{h}	3.93^{h}	4.25^{g}	3.95^{C}
Cd_{15}	4.24^{e}	4.53^{d}	4.52^{d}	4.69^{d}	4.49^{B}	4.32^{g}	4.56^{fg}	4.75^{ef}	5.22^{cd}	4.71^{B}
Cd_{25}	5.09^{c}	5.29^{bc}	5.48^{ab}	5.62^{a}	5.38^{A}	4.98^{de}	5.45^{c}	5.83^{b}	6.21^{a}	5.62^{A}
Mean	3.87^{C}	4.34^{B}	4.48^{A}	4.53^{A}		4.19^{D}	4.39^{C}	4.57^{B}	4.88^{A}	
LSD(p≤0.05)	Pb	0.077				Pb	0.108			
	Cd	0.077				Cd	0.108			
	Pb×Cd	0.154				Pb×Cd	0.217			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference (p≤0.05)

Under Cd concentrations, minimum mean antioxidants observed in control (Cd_0, 5.46 µg/g DW) which increased significantly with increase in cadmium concentration, thus recorded to be maximum with Cd_{25} concentration (7.37 µg/g DW). Similarly, among combinations, maximum antioxidants were recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (7.62 µg/g DW).

Similar trend was observed after six months that antioxidants increased significantly with increase in Pb and Cd concentrations. The minimum antioxidant content was recorded in control (Pb_0Cd_0, 9.45 µg/g DW) and maximum with highest concentration i.e. $Pb_{300}Cd_{25}$ (22.4 (µg/g DW).

Toona plants also showed significant variation in total antioxidants in response to different Pb and Cd concentrations. After three months, among Pb concentrations, the minimum mean antioxidants in control (Pb_0, 3.87 µg/g DW) which increased significantly with increase in Pb concentrations and recorded to be maximum with highest concentration i.e. Pb_{300} (4.48 µg/g DW). Similar trend was observed with Cd concentrations that minimum antioxidants were recorded in control (Cd_0, 3.46 µg/g DW) and maximum with highest concentration i.e. Cd_{25} (5.37 µg/g DW). Among Pb and Cd combinations, minimum antioxidants were recorded in control (2.63 µg/g DW) which increased significantly with increase in metal concentration, thus maximum antioxidants were recorded with highest concentrations of Pb and Cd i.e. $Pb_{300}Cd_{25}$ (5.62 µg/g DW).

Similar trend was observed after six months that minimum antioxidants were observed in control (Pb_0Cd_0, 3.68 µg/g DW) and maximum in plants grown on highest heavy metal concentration i.e. $Pb_{300}Cd_{25}$ (6.21 µg/g DW).

Thus, results showed that total antioxidants increased in response to Pb and Cd concentrations in both *Salix* and *Toona*. Along with this, total antioxidants also increased from three month to six month interval and the increasing trend was more prominent in *Salix* than *Toona*. Under stress conditions, the increased antioxidant concentrations reveals that plants had efficient antioxidant defense mechanism to tolerate adverse stress conditions.

g. Peroxidase activity (units/min/protein)

An appraisal of Table 4.14 shows the significant differences in peroxidase enzyme activity of *Salix* and *Toona* plants grown under different Pb, Cd and their combination concentrations after three and six months.

In *Salix*, the significant increase in peroxidase activity was recorded in response to different Pb and Cd concentrations. After three months, among Pb concentrations, minimum mean peroxidase activity were recorded in control (Pb_0, 1.62 units/min/protein) which increased significantly with increase in Pb concentration from Pb_{100} (1.79 units/min/protein) to Pb_{300} (1.97 units/min/protein) after three months.

Table 4.14: Effect of heavy metals on peroxidase activity of *Salix alba* and *Toona ciliata*

Peroxidase activity (units/min/protein)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	1.26^{j}	1.46^{i}	1.52^{hi}	1.58^{ghi}	1.45^{D}	2.45^{j}	3.76^{i}	5.62^{fg}	7.65^{abc}	4.87^{D}
Cd_5	1.56^{ghi}	1.62^{fgh}	1.64^{fgh}	1.66^{fg}	1.62^{C}	4.55^{hi}	5.12^{gh}	5.80^{fg}	6.15^{ef}	5.40^{C}
Cd_{15}	1.72^{ef}	1.74^{ef}	1.83^{de}	1.85^{de}	1.78^{B}	6.45^{def}	6.84^{cde}	7.36^{bcd}	7.88^{ab}	7.13^{B}
Cd_{25}	1.95^{d}	2.34^{c}	2.56^{b}	2.78^{a}	2.41^{A}	7.95^{ab}	8.26^{ab}	8.51^{a}	8.67^{a}	8.35^{A}
Mean	1.62^{D}	1.79^{C}	1.89^{B}	1.97^{A}		5.35^{D}	5.99^{C}	6.82^{B}	7.59^{A}	
LSD(p≤0.05)	Pb	0.041				Pb	0.31			
	Cd	0.041				Cd	0.31			
	Pb×Cd	0.083				Pb×Cd	0.62			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	2.56^{j}	2.88^{i}	3.41^{h}	4.22^{def}	3.27^{D}	3.67^{h}	4.23^{g}	5.49^{f}	6.44^{cd}	4.96^{D}
Cd_5	2.96^{i}	3.48^{h}	3.94^{fg}	4.45^{bcd}	3.71^{C}	4.38^{g}	4.58^{g}	5.52^{f}	6.78^{abc}	5.31^{C}
Cd_{15}	3.69^{gh}	3.99^{efg}	4.27^{cdef}	4.56^{bcd}	4.13^{B}	5.88^{ef}	5.92^{ef}	6.25^{de}	6.67^{bc}	6.18^{B}
Cd_{25}	4.32^{cde}	4.62^{bc}	4.78^{ab}	4.99^{a}	4.68^{A}	6.94^{ab}	7.08^{ab}	7.13^{a}	7.22^{a}	7.09^{A}
Mean	3.38^{D}	3.74^{C}	4.10^{B}	4.55^{A}		5.22^{D}	5.45^{C}	6.09^{B}	6.78^{A}	
LSD(p≤0.05)	Pb	0.110				Pb	0.137			
	Cd	0.110				Cd	0.137			
	Pb×Cd	0.220				Pb×Cd	0.274			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference (p≤0.05)

Among Cd concentrations, minimum mean peroxidase activity recorded in control (Cd_0, 1.45 units/min/protein) which increased significantly with increase in Cd concentration and recorded to be maximum with Cd_{25} concentration (2.41 units/min/protein). Similarly, among combinations, maximum peroxidase activity was recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (2.78 units/min/protein). Similar trend was observed after six months that peroxidase activity significantly increased with increase in Pb and Cd concentrations. The minimum peroxidase activity was recorded in control (2.45 units/min/protein) and maximum with highest concentration i.e. $Pb_{300}Cd_{25}$ (8.67 units/min/protein).

Toona plants also showed significant variation in peroxidase activity in response to different Pb and Cd concentrations. After three months, among Pb concentrations the minimum mean peroxidase activity were recorded in control (Pb_0, 3.38 units/min/protein) which increased significantly with increase in Pb concentrations and recorded to be maximum with highest concentration i.e. Pb_{300} (4.55 units/min/protein). Similar trend was observed with Cd concentrations that minimum mean peroxidase activity were recorded in control (Cd_0, 3.27 units/min/protein) and maximum with highest concentration i.e. Cd_{25} (4.68 units/min/protein). Among Pb and Cd combinations, minimum peroxidase activity recorded in control (2.56 units/min/protein) which increased significantly with increase in metal concentration, thus maximum peroxidase activity was recorded with highest concentrations of Pb and Cd i.e. $Pb_{300}Cd_{25}$ (4.99 units/min/protein).

Similar trend was observed after six months that minimum peroxidase activity was observed in control (Pb_0Cd_0, 3.67 units/min/protein) and maximum in plants grown on highest heavy metal concentration i.e. $Pb_{300}Cd_{25}$ (7.22 units/min/protein).

Thus, results showed that peroxidase activity increased in response to Pb and Cd concentrations at both three and six month intervals in *Salix* and *Toona*. In these plant species, peroxidase activity also increased from three month to six month interval.The increased peroxidase activity under Pb and Cd stress showed that plants had efficient antioxidant defense mechanism to tolerate stress conditions. These results further indicates that heavy metal stress do not impose any kind of oxidative stress to plant due to higher antioxidant enzyme activities that are also responsible for balancing plant biomass.

h. Catalase activity (units/min/protein)

The data presented in Table 4.15 shows the significant variation in catalase activity in response to different concentrations of Pb, Cd and their combinations. In *Salix*, the significant increase in catalase activity was recorded after three and six months in response to Pb and Cd concentrations. After three months, among Pb concentrations, minimum mean catalase activity recorded in control (4.75 units/min/protein), which increased significantly with increase in Pb concentration in following order Pb_{100} (5.01 units/min/protein), Pb_{200} (5.50 units/min/protein) and Pb_{300} (5.95 units/min/protein).

Table 4.15: Effect of heavy metals on catalase activity of *Salix alba* and *Toona ciliata*

Catalase activity (units/min/protein)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	3.21^{j}	3.68^{i}	4.55^{g}	5.34^{ef}	4.19^{D}	4.77^{o}	5.26^{n}	7.56^{k}	9.12^{h}	6.67^{D}
Cd_5	3.89^{i}	4.22^{h}	4.56^{g}	4.79^{g}	4.36^{C}	6.32^{m}	7.11^{l}	7.87^{j}	8.25^{i}	7.39^{C}
Cd_{15}	5.25^{f}	5.36^{ef}	5.67^{de}	5.78^{d}	5.52^{B}	9.45^{g}	9.97^{f}	10.2^{e}	11.0^{d}	10.2^{B}
Cd_{25}	6.66^{c}	6.78^{c}	7.24^{b}	7.88^{a}	7.14^{A}	11.3^{c}	11.5^{c}	12.1^{b}	12.8^{a}	11.9^{A}
Mean	4.75^{D}	5.01^{C}	5.50^{B}	5.95^{A}		7.97^{D}	8.46^{C}	9.44^{B}	10.3^{A}	
LSD($p\leq0.05$)	Pb	0.111				Pb	0.072			
	Cd	0.111				Cd	0.072			
	Pb×Cd	0.222				Pb×Cd	0.145			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	4.03^{g}	4.68^{f}	4.72^{f}	5.21^{e}	4.66^{D}	6.89^{g}	6.91^{g}	7.28^{fg}	8.46^{c}	7.38^{C}
Cd_5	4.58^{f}	4.69^{f}	5.37^{de}	5.41^{de}	5.01^{C}	6.95^{g}	7.12^{fg}	7.34^{efg}	7.67^{ef}	7.27^{C}
Cd_{15}	5.68^{cd}	5.78^{c}	5.82^{c}	5.99^{bc}	5.82^{B}	7.39^{efg}	7.75^{ef}	7.68^{ef}	7.89^{de}	7.63^{B}
Cd_{25}	6.21^{ab}	6.31^{ab}	6.42^{a}	6.52^{a}	6.36^{A}	8.26^{ab}	8.43^{ab}	8.95^{b}	9.47^{a}	8.78^{A}
Mean	5.13^{D}	5.36^{B}	5.58^{C}	5.78^{D}		7.37^{C}	7.51^{C}	7.81^{B}	8.37^{A}	
LSD($p\leq0.05$)	Pb	0.113				Pb	0.030			
	Cd	0.113				Cd	0.030			
	Pb×Cd	0.226				Pb×Cd	NS			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)
(NS: Non significant)

Similarly, under Cd concentrations, that minimum mean catalase activity recorded in control (Cd_0, 4.19 units/min/protein) which significantly increased with increase in Cd concentrations i.e. Cd_5 (4.36 units/min/protein), Cd_{15} (5.51 units/min/protein) and Cd_{25} (7.14 units/min/protein). Similarly, among Pb and Cd combinations, control plants (Pb_0Cd_0) showed minimum catalase activity (3.21 units/min/protein), which also increased significantly with increase in Pb and Cd concentration, thus recorded to be maximum with highest concentration i.e. $Pb_{300}Cd_{25}$ (7.88 units/min/protein). Similar trend was observed after six months that minimum catalase activity was recorded in control (Pb_0Cd_0, 4.77 units/min/protein) which increased with increase in heavy metal concentrations and recorded to be maximum with highest concentration i.e. $Pb_{300}Cd_{25}$ (12.8 units/min/protein).

Toona plants also showed significant increase in catalase activity in response to different concentrations of Pb, Cd and their combinations as compared to control. After three months, among Pb concentrations, maximum mean catalase activity recorded in Pb_0 (5.13 units/min/protein) which increased significantly with increase in Pb concentration, thus maximum with highest Pb concentration i.e. Pb_{300} (5.78 units/min/protein). Similarly, among Cd concentrations, minimum mean catalase activity was recorded in control (Cd_0, 4.66 units/min/protein) which increased significantly with increase in Cd concentration and recorded be maximum with Cd_{25} (6.36 units/min/protein). Among, Pb and Cd combinations, minimum catalase activity was recorded in control (Pb_0Cd_0, 4.03 units/min/protein) and maximum in plants grown on highest concentration i.e. $Pb_{300}Cd_{25}$ (6.52 units/min/protein).

Similar trend was observed after six months that minimum catalase activity recorded in control (Pb_0Cd_0, 6.89 units/min/protein) which increased significantly with increase in heavy metal concentration, thus recorded to be maximum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (9.47 units/min/protein).

Thus, both *Salix* and *Toona* plants also showed significant increase in catalase activity in response to different concentrations of Pb, Cd and their combinations as compared to control. In both species, catalase activity also increased from three month to six month interval. The results indicate that Pb and Cd stress in plants generate the synthesis of antioxidant enzymes such as peroxidase and catalase to scavenge ROS and to protect the plants from oxidative damage.

i. Superoxide dismutase (SOD) activity (units/min/protein)

The data presented in Table 4.16 shows the significant differences in superoxide dismutase (SOD) enzyme activity of *Salix* and *Toona* plants grown on different Pb, Cd and their combination concentrations after three and six months.

Table 4.16: Effect of heavy metals on superoxide dismutase activity of *Salix alba* and *Toona ciliata*

Superoxide dismutase activity (units/min/protein)										
Salix alba										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	4.65^{h}	5.66^{fg}	5.78^{fg}	5.92^{f}	5.50^{D}	6.06^{j}	7.63^{i}	8.10^{hi}	9.95^{ef}	7.93^{D}
Cd_5	5.52^{g}	5.64^{fg}	5.65^{fg}	5.89^{f}	5.67^{C}	8.87^{gh}	9.23^{fg}	9.43^{efg}	10.2^{e}	9.44^{C}
Cd_{15}	5.95^{f}	6.33^{e}	6.35^{de}	6.58^{cde}	6.30^{B}	11.6^{d}	11.6^{d}	12.3^{cd}	12.9^{bc}	12.1^{B}
Cd_{25}	6.65^{cd}	6.78^{c}	7.20^{b}	7.53^{a}	7.04^{A}	13.2^{b}	13.4^{b}	13.8^{ab}	14.5^{a}	13.7^{A}
Mean	5.69^{C}	6.10^{B}	6.24^{AB}	6.48^{A}		9.96^{D}	10.5^{C}	10.9^{B}	11.9^{A}	
LSD(p≤0.05)	Pb	0.101				Pb	0.273			
	Cd	0.101				Cd	0.273			
	Pb×Cd	0.202				Pb×Cd	0.546			
Toona ciliata										
	3 months					6 months				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	6.61^{j}	7.26^{i}	7.84^{gh}	8.87^{cd}	7.64^{D}	8.07^{i}	8.35^{i}	9.18^{h}	9.83^{efg}	8.86^{C}
Cd_5	7.48^{hi}	7.96^{fgh}	8.43^{def}	9.25^{bc}	8.28^{C}	9.24^{h}	9.50^{fgh}	9.96^{ef}	11.1^{ab}	9.96^{B}
Cd_{15}	7.91^{fgh}	8.29^{efg}	8.81^{cde}	9.54^{ab}	8.64^{B}	9.29^{gh}	9.84^{efg}	10.5^{cd}	10.8^{bc}	10.1^{B}
Cd_{25}	8.89^{efg}	8.74^{cde}	9.16^{bc}	9.96^{a}	9.19^{A}	10.1^{de}	10.0^{ef}	10.6^{bcd}	11.3^{a}	10.5^{A}
Mean	7.72^{D}	8.06^{C}	8.56^{B}	9.40^{A}		9.18^{D}	9.42^{C}	10.1^{B}	10.8^{A}	
LSD(p≤0.05)	Pb	0.179				Pb	0.178			
	Cd	0.179				Cd	0.178			
	Pb×Cd	0.359				Pb×Cd	0.356			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference (p≤0.05)

In *Salix*, the significant increase in SOD activity was recorded in response to different Pb and Cd concentrations. After three months, among Pb concentrations, minimum mean SOD activity were recorded in control (Pb_0, 5.69 units/min/protein) which increased significantly with increase in Pb concentration from Pb_{100} (6.10 units/min/protein) to Pb_{300} (6.48 units/min/protein). Similar trend was observed for Cd concentrations that minimum mean SOD activity recorded in control (Cd_0, 5.50 units/min/protein) which increased significantly with increase in cadmium concentration and recorded to be maximum with Cd_{25} concentration (7.04 units/min/protein). Similarly, among combinations, maximum SOD activity was recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (7.53 units/min/protein).

Similar trend was observed after six months that SOD activity significant increased with increase in Pb and Cd concentrations. The minimum SOD activity was recorded in control (Pb_0Cd_0, 6.06 units/min/protein) and maximum with highest concentration i.e. $Pb_{300}Cd_{25}$ (14.5 units/min/protein).

Toona plants also showed significant variation in SOD activity in response to different Pb and Cd concentrations. After three months, among Pb concentrations the minimum mean SOD activity were recorded in control (Pb_0, 7.72 units/min/protein) which increased significantly with increase in Pb concentrations and recorded to be maximum with highest concentration i.e. Pb_{300} (9.40 units/min/protein). Similar trend was observed with Cd concentrations that minimum mean SOD activity were recorded in control (Cd_0, 7.64 units/min/protein) and maximum with highest concentration i.e. Cd_{25} (9.19 units/min/protein). Among Pb and Cd combinations, minimum SOD activity recorded in control (6.61 units/min/protein) which increased significantly with increase in metal concentration, thus maximum SOD activity were recorded with highest concentrations of Pb and Cd i.e. $Pb_{300}Cd_{25}$ (9.96 units/min/protein).

Similar trend was observed after six months that minimum SOD activity was observed in control (Pb_0Cd_0, 8.07 units/min/protein) and maximum in plants grown on highest heavy metal concentration i.e. $Pb_{300}Cd_{25}$ (11.3 units/min/protein).

Thus, results showed that SOD activity increased in response to Pb and Cd concentrations in *Salix* and *Toona*. In these plant species, SOD activity also increased from three month to six month interval. Under stress conditions, plants upregulates the synthesis of antioxidant enzyme such as peroxidase, catalase and superoxide dismutase to convert the reactive oxygen species to less toxic form and to protect the plant from oxidative damage.

According to Ehsan *et al* (2014), heavy metals induced imbalance between the formation of oxygen radicals (ROS) and their scavenging through the antioxidant defence mechanism is the main cause of the development of oxidative stress in plants exposed to heavy metals. The antioxidant defence system in plants provides efficient criteria for detoxification and scavenging of the toxic oxygen species through an adaptive mechanism

involving upregulation of anti-oxidative enzymes such as SOD, CAT, POD, APX and GR. Along with this, the enhanced accumulation of cellular antioxidants including ascorbate glutathione cycle down-regulates the conversion of the super oxide ions to the most reactive and toxic hydroxyl (OH) ions (Ashraf *et al* 2017). Similarly, increased activity of antioxidant enzymes in *Salix* and *Toona* with increase in Pb and Cd concentration showed that plants had efficient antioxidant defense mechanism to tolerate stress conditions. The results further indicates that heavy metal stress do not impose oxidative stress to plant due to higher antioxidant enzyme activities that are responsible for further reduction in biomass traits by stabilizing tolerance index.

4.1.3 Anatomical studies

The morpho-physiological parameters of both *Salix* and *Toona* revealed negative effect with highest concentration of Pb and Cd combination i.e. $Pb_{300}Cd_{25}$. Hence, this concentration ($Pb_{300}Cd_{25}$) was selected for anatomical studies to study the Pb and Cd induced ultra-structural changes in plants as compared to control by using light microscopy and field emission scanning electron microscopy (FESEM). Along with this, Pb and Cd accumulation sites are predicted in different tissues of root and leaves of both species (*Salix* and *Toona*) with energy dispersive x-ray spectroscopy (EDS).

4.1.3.1 Anatomical changes induced due Pb and Cd toxicity

a. **Leaf anatomy**

The leaf anatomy of both *Salix* and *Toona* were performed to assess the Pb and Cd induced ultra-structural changes in plants through light microscopy and field emission scanning electron microscopy (FESEM).

In *Salix*, light microscopic images (Plate III, A,B) illustrated that stomatal pore size decreased with $Pb_{300}Cd_{25}$ concentration (13.3µm) as compared to control (18.0µm) (Table 4.17). In contrary, the stomatal density decreased with $Pb_{300}Cd_{25}$ concentration (100.2 no./mm^2) as compared to control (133.7 no./mm^2). Similarly, the stomatal index was also observed to be higher in control (10.0%) which decreased significantly with $Pb_{300}Cd_{25}$ treatment (6.99%). Trichome density on leaves decreased sharply with Pb and Cd concentration (74.33 no./mm^2) as compared to control (118.7 no./mm^2) (Plate IV). Thus, data speculated that stomatal attributes (pore size, density and stomatal index) and trichome density decreased due to response of plants to heavy metal stress.

In *Toona*, light microscopic measurements (Plate V, AB) conferred that the stomatal pore size decreased with $Pb_{300}Cd_{25}$ (18.24 µm) concentration as compared to control (21.86µm) as shown in Table 4.17. However, the reverse trend was recorded in stomatal density that number of stomata increased with $Pb_{300}Cd_{25}$ concentrations (165.4 no./mm^2) as compared to control (142.8 no./mm^2) plants. Similarly, the higher stomatal index was observed in $Pb_{300}Cd_{25}$ (18.1%) treated plants as compared to control (13.8%).

Table 4.17: Anatomical parameters of *Salix alba* and *Toona ciliata* as affected by heavy metal treatments

S. No.	Parameters	*Salix alba*		*Toona ciliata*	
		Control (Pb_0Cd_0)	Treated ($Pb_{300}Cd_{25}$)	Control (Pb_0Cd_0)	Treated ($Pb_{300}Cd_{25}$)
1.	Stomatal pore size (µm)	18.0	13.3	21.9	18.2
2.	Stomatal density (no./mm^2)	134	100	143	165
3.	Stomatal index (%)	10.1	6.99	13.8	18.1
4.	Trichome density (no./mm^2)	118	74.3	85.2	109
5.	Total root area (mm^2)	0.91	0.72	1.24	1.18
6.	Root vascular area(mm^2)	0.34	0.29	0.68	0.65

Trichome density on adaxial surface of *Toona* leaves were calculated and observed increase in total number of trichomes in response to $Pb_{300}Cd_{25}$ concentration (109.5 no./mm^2) as compared to control (85.2 no./mm^2). Hence, the recorded data showed that stomatal frequency, stomatal index and trichome density increased in response to heavy metal concentrations, whereas the stomatal pore size was decreased in *Toona* as compared to control.

Field emission scanning electron microscopy (FESEM) technique was used to study the structural alterations in plants with $Pb_{300}Cd_{25}$ concentrations as compared to control. In *Salix*, the scanning electron micrographs clearly indicate the alterations such as reduced stomatal number and reduced pore size (Plate III, D) along with ruptured guard cells due to Pb and Cd accumulation in leaves. Pb and Cd toxicity reduced the stomatal density by disorganizing structure of stomata as its guard cells becomes flaccid and ruptured at high concentration of heavy metals i.e. $Pb_{300}Cd_{25}$ (Plate III, F). Similarly, the distorted structure of trichomes was also observed due to Pb and Cd accumulation; at higher concentrations trichome cells undergo programmed cell death (PCD) that leads to reduction in number of trichomes on leaf surface (Plate IV).

FESEM of *Toona* confirmed the increased stomatal number with reduced pore size in $Pb_{300}Cd_{25}$ treated plants as compared to control (Pb_0Cd_0) plants (Plate VI CD). The stomata of *Toona* leaves showed reduced stomatal pore with no change in guard cells in response to Pb and Cd toxicity. Higher trichome number due to Pb and Cd accumulation in $Pb_{300}Cd_0$ treated plants as compared to control (Pb_0Cd_0) was observed as shown in Plate VI. In the plant leaves, trichomes are known to accumulate higher heavy metal toxic ions than other cells which prevent the interference of heavy metal in metabolism, thus the increased number of trichomes due to heavy metal toxicity considered as activation of plant tolerance mechanism

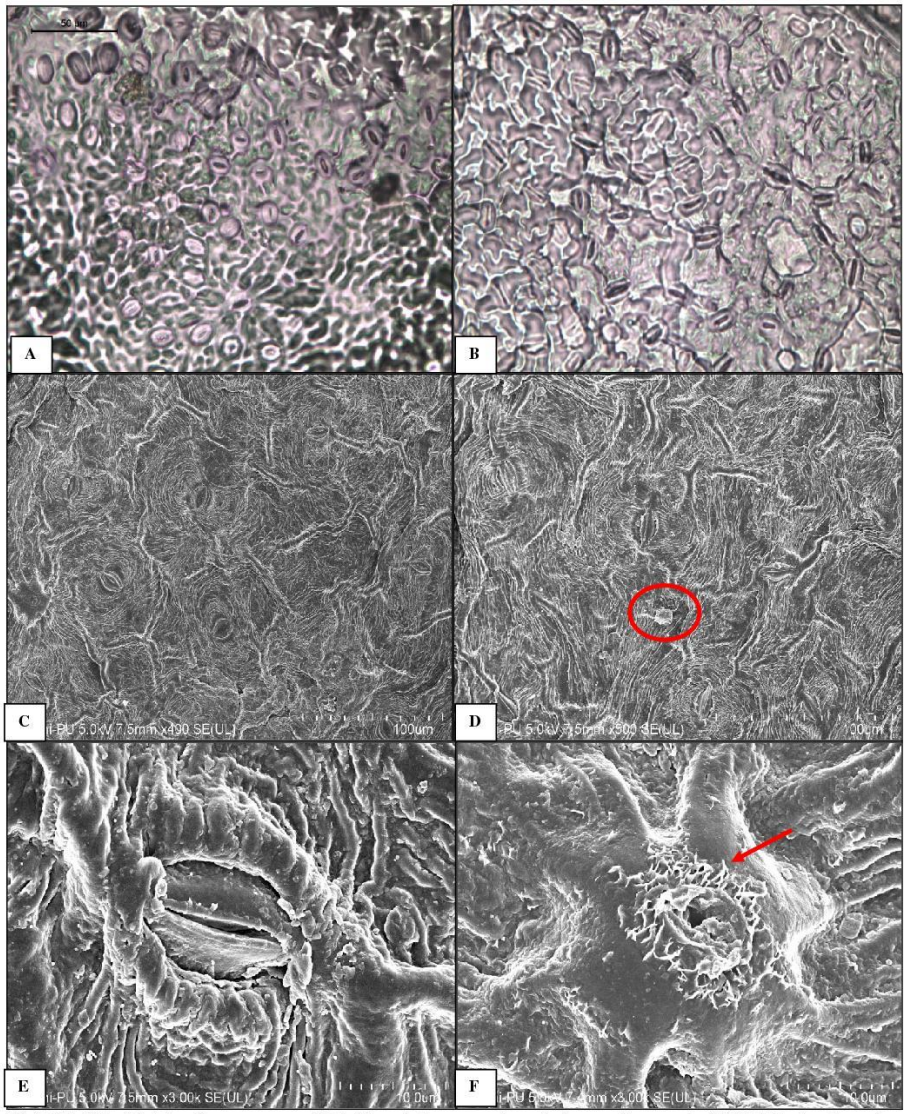


PLATE III: Light microscopy and Field emission scanning electron micrograph (FESEM) showing stomatal alterations in leaves of *Salix alba* induced due to Pb and Cd toxicity

(A, C, E control Pb_0Cd_0; B, D, F heavy metal treated $Pb_{300}Cd_{25}$; Red marks indicates distorted structures)

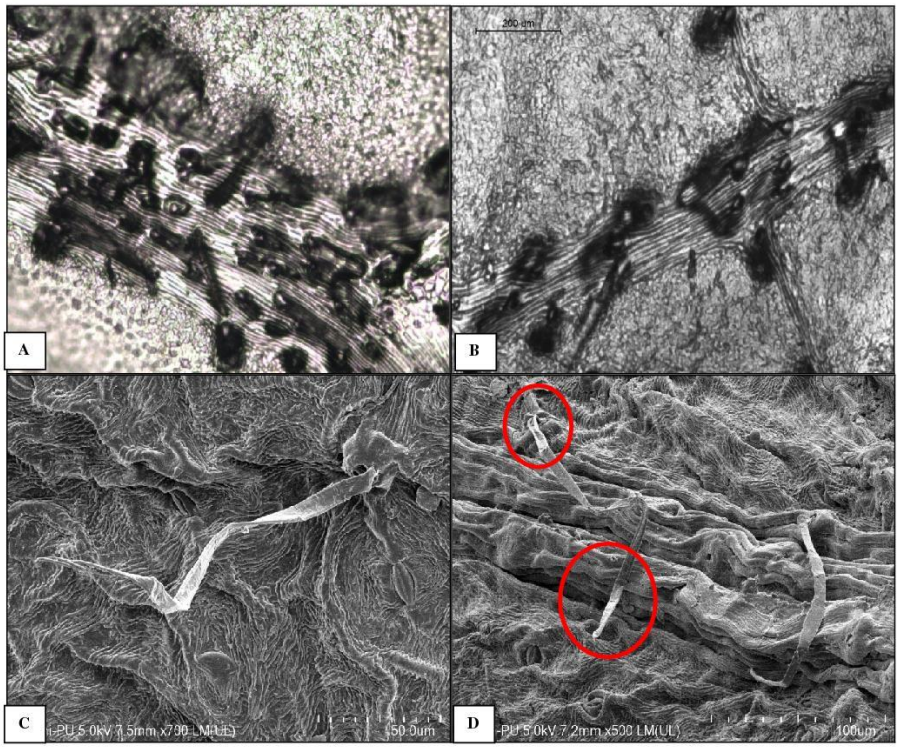


PLATE IV: Field emission scanning electron micrographs (FESEM) showing structural alterations in trichomes of *Salix alba* leaves induced due to Pb and Cd toxicity

(A, C: control Pb_0Cd_0; B, D: heavy metal treated $Pb_{300}Cd_{25}$; Red marks indicates distorted structures)

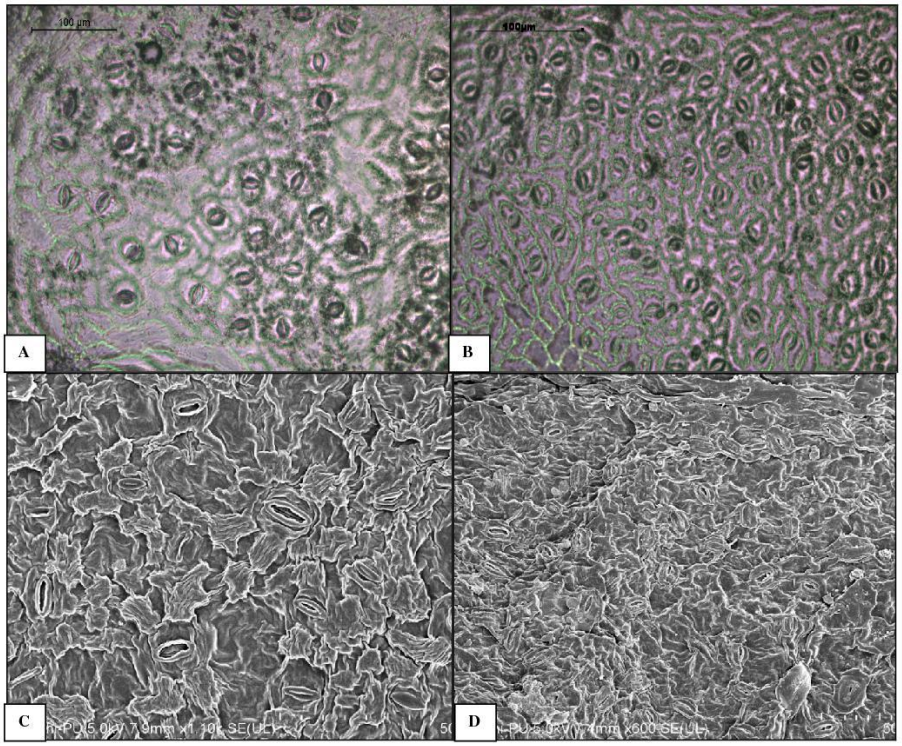


PLATE V: Light microscopy and Field emission scanning electron micrograph (FESEM) showing stomatal alterations in leaves of *Toona ciliata* induced due to Pb and Cd toxicity

(A, C control (Pb_0Cd_0); B, D heavy metal treated $Pb_{300}Cd_{25}$)

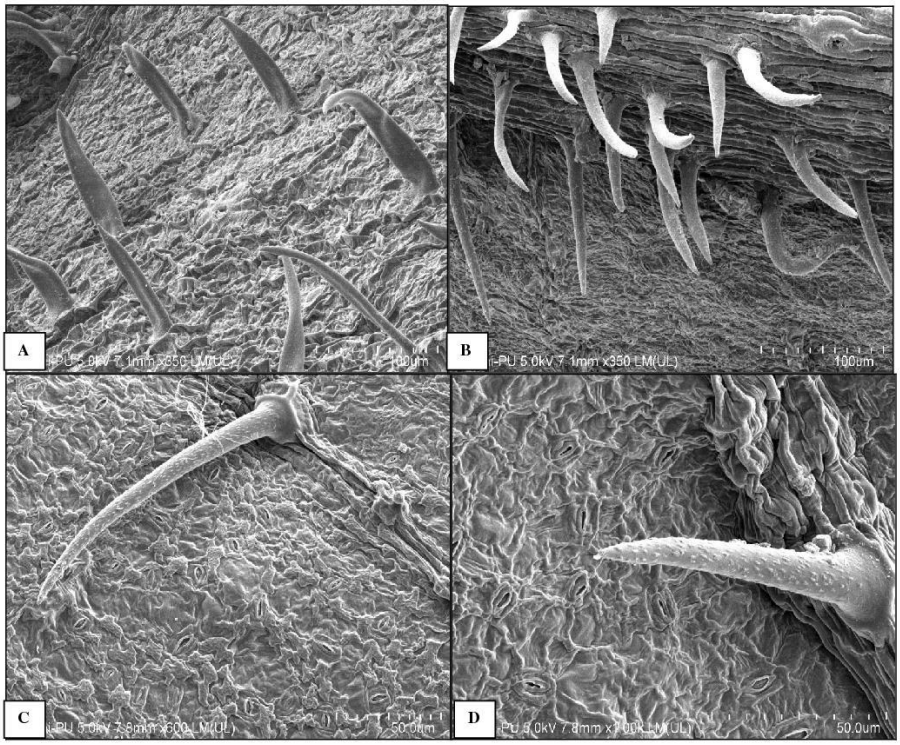


PLATE VI: Field emission scanning electron micrographs (FESEM) showing structural alterations in trichomes in leaves of *Toona ciliata* induced due to Pb and Cd toxicity

(A, C control Pb_0Cd_0; B, D heavy metal treated $Pb_{300}Cd_{25}$)

against stress.

Hence, both tree species *Salix* and *Toona* showed different behavior to cope up with heavy meta1 stress. A marked decrease in stomatal pore size, stomatal density and trichome density were found in heavy metal treated *Salix* plant ($Pb_{300}Cd_{25}$) as compared to control, whereas *Toona* plants showed increased stomatal density and trichome density along with decreased stomatal pore size with $Pb_{300}Cd_{25}$ concentrations. Our findings are in support with Hermle *et al* (2007), who reported that Pb and Cd accumulation in *Populus tremula* leaves lowers the turgor of the subsidiary cells, which further results into reduced stomatal opening. This may be the consequences of rapidly and preferentially absorbing metals subsidiary cells, which is followed by alteration in membrane permeability that reduces cell turgor and impairs the structural integrity of stomata. Reduced stomatal pore and stomatal closure have been linked to decreased photosynthetic rate along with reduced transpiration (Liu *et al* 2010). Evidence from current investigation indicate that Pb and Cd at higher concentration causes ultra-structural changes in stomata such as distortion of guard cells, which would be responsible for altered stomatal functioning of *Salix*. In addition, Cd induce reduction in stomatal density, total number of open stomata and CO_2 conductance (Li *et al* 2019), which would decline net photosynthetic rate and hence reduced biomass.

In case of *Toona*, higher number of stomata with reduced stomatal pore size was observed in response to Pb and Cd concentrations as compared to control. Rucińska-Sobkowiak (2016) reported that water balance disturbance is an early stress-induced event; *Arabidopsis thaliana* had efficient adaptive mechanism to survive under heavy metal stress conditions by increased stomatal density to maintain sufficient CO_2 flow without affecting photosynthesis and by reduced stomatal pore size to reduce excess water loss through transpiration.

In *Salix*, trichome density was reduced in response to Pb and Cd stress, whereas *Toona* plants observed with increased trichome density in plants treated with $Pb_{300}Cd_{25}$ concentrations as compared to control. Based on the bibliographic data (Weryszko-Chmielewska and Chwil 2005), it is considered that higher trichome density is one of the adaptative mechanisms of plant growing on heavy metal contaminated soils, that reduces the quantity of toxic metal in internal leaf tissues, as high concentration of metals accumulate in trichomes, from where they can be easily eliminated without interfering plant metabolism. Similarly, Guo *et al* (2022) reported that trichomes served as Cd^{2+} accumulation site in *Arabidopsis thaliana*, thereby it helps the plant to cope with heavy metal stress and also plays an important role in soil detoxification. Thus, the trichomes are believed to be chief plant defenses against biotic and abiotic stress. But, *Salix* results are not in agreement with these studies, because Pb and Cd accumulation in leaves possess toxicity by inducing inhibitory signal that negatively affects the trichome development and further reduce the total trichome

number on leaf epidermal surface. The decrease in trichome density might be due to chlorosis and necrotic spots that disrupts the normal development and functioning of epidermal layer in *Salix*, however, as such no toxicity symptom was observed in leaves of *Toona* under Pb and Cd stress.

b. **Root anatomy**

The root system is the first organ exposed to polluted soil and is known to modify its anatomical characteristics in order to confer the plant adaptation under heavy metal stress. The cross-section of *Salix* roots of control plants contain intact tissue structures organized in circular form (Plate VIII). The cortex is filled with mostly intact parenchyma tissue of almost uniform in size. The vascular region (stele) is intact and consisted of pericycle, phloem and xylem tissues; separated from the cortex by endodermis at the outer part. The cross-section of heavy metal treated roots (Plate VII B) showed that root was not completely circular in shape as some parenchymatous cells of cortical region was damaged at some places that lead to reduced size of cortical region and ultimately resulted into reduced total root area (0.72 mm^2) as compared to control (0.91 mm^2). In addition, parenchymal tissues possess cells that are not uniform as in the cortex of control plant and damaged xylem vessels in the vascular region a1ong with collapsed intercellular spaces were observed in heavy metal treated roots as compared to control (Plate VII F). The root vascular bundle area also decreased with $Pb_{300}Cd_{25}$ treatment (0.29 mm^2) as compared to control plants (0.34mm^2).

The cross-section of *Toona* roots is circular in shape in control plants, whereas the heavy metal treated roots showed wavy outline due to destruction of some epidermal and cortical cells under Pb and Cd induced toxicity (Plate VIII A, B). This destruction is further responsible for reduced cortical region and reduced total root area (1.18 mm^2) as compared to control (1.24 mm^2). The vascular region consisted of pericycle, phloem and xylem tissues and outer endodermis. Due to Pb and Cd induced toxicity, structural changes in xylem vessels which includes enlarged or collapsed intercellular spaces in plants treated with $Pb_{300}Cd_{25}$ as compared to control (Plate VIII D,F). Further, the reduced root vascular bundle area was observed in $Pb_{300}Cd_{25}$ treated plants (0.65 mm^2) as compared to control plants (0.68mm^2).

Roots in Pb and Cd-treated plants undergo visible alterations including reduced root number, root length and biomass of both *Salix* and *Toona*. Similar Cd induced modifications were also reported by Vaculík *et al* (2012) in *Salix caprea.* Lux *et al* (2011) analysed the Cd-induced variations in shape and size of root cells which showed modification in structure of root tissues such as rhizodermis, cortex and vascular bundles compared to control plants. These modifications demonstrate the decreased ability of roots to absorb water and minerals, which disturbs the primary physiological processes viz., photosynthesis and transpiration (Huang *et al* 2019). It is evidenced from current investigations that heavy metal accumulation in plant tissues stimulates the suberization and lignification of the root cells that further

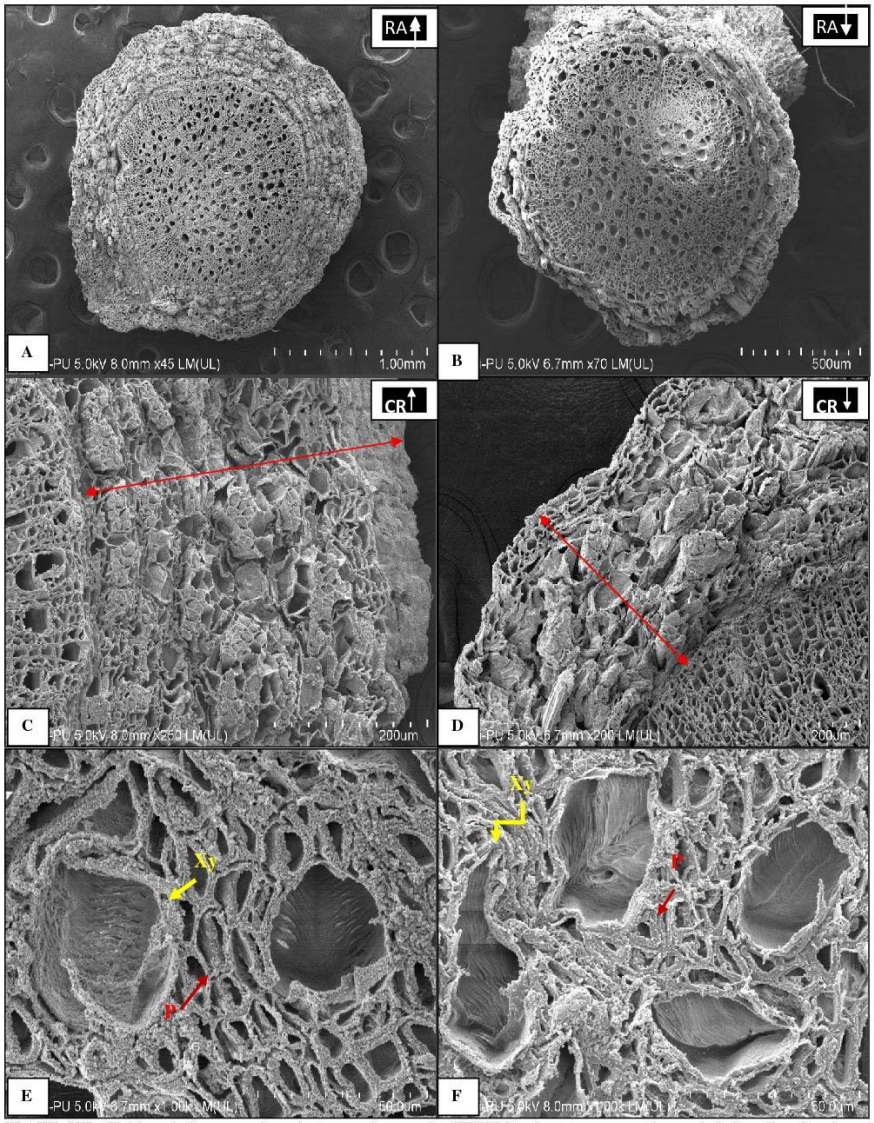


PLATE VII: Field emission scanning electron micrograph (FESEM) of root cross-sections of *Salix alba* showing structural alterations induced due to Pb and Cd toxicity (A, C, E Control Pb_0Cd_0; B, D, F Heavy metal treated $Pb_{300}Cd_{25}$)
(RA= Root area, CR=Cortical region, Xy=xylem vessels, P = Pith cells)

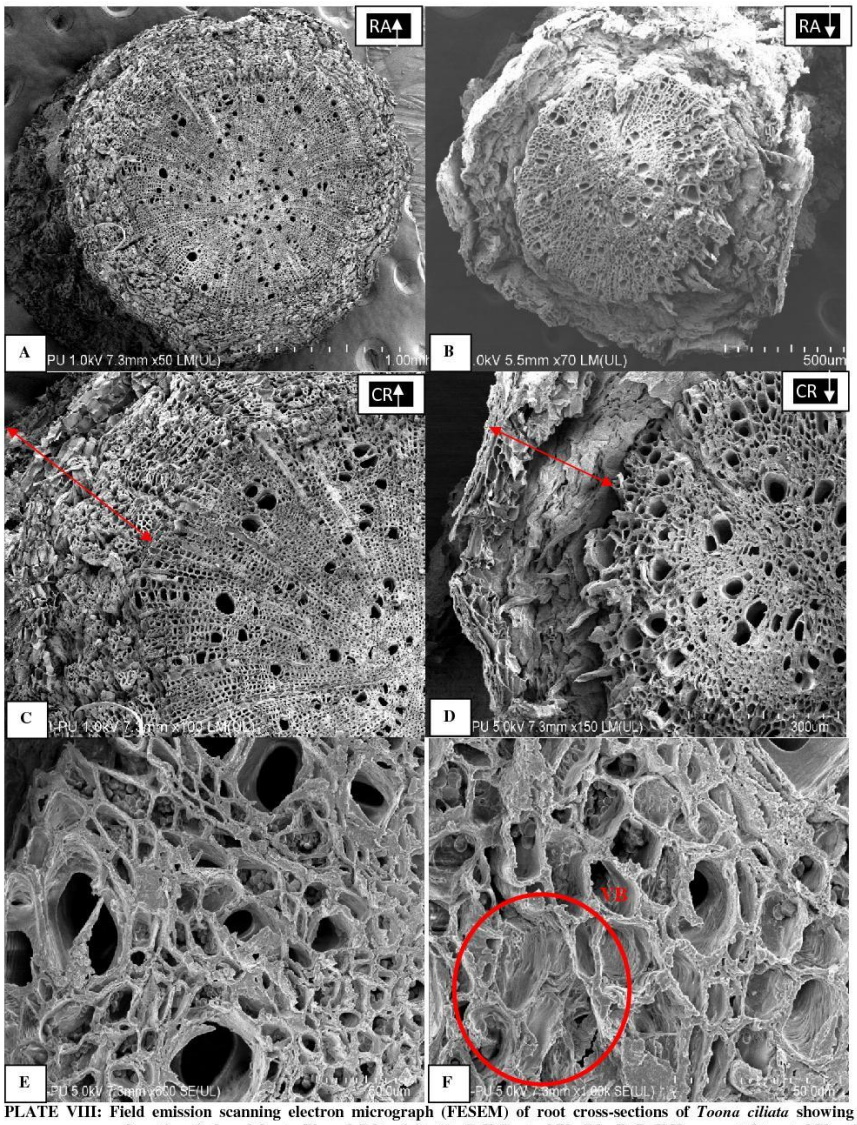


PLATE VIII: Field emission scanning electron micrograph (FESEM) of root cross-sections of *Toona ciliata* showing structural alterations induced due to Pb and Cd toxicity (A, C, E Control Pb_0Cd_0; B, D, F Heavy metal treated $Pb_{300}Cd_{25}$)

(RA= Root area, CR=Cortical region, Xy=xylem vessels, P = Pith cells)

increase the cell thickness and collapse intercellular spaces, thus prevents the toxic ion movement inside plant tissues in both *Salix* and *Toona*. The accumulation of toxic heavy metals increased the resistance to flowing water and nutrients from roots to aerial plant parts by reducing vascular tissue translocation efficiency. Hamim *et al* (2018) reported that decreased root area and vascular region results in diminished conductive potential of the phloem and xylem tissues as inductive response to heavy metal stress, which is also in favour with present study.

4.1.3.2 Lead and cadmium accumulation sites in plant tissues

The energy dispersive x-ray spectroscopy (EDS) used to predict the lead (Pb) and cadmium (Cd) accumulation sites in different tissue of root and leaves of both *Salix* and *Toona*.

a. Energy dispersive x-ray spectroscopy (EDS) in *Salix alba*

Pb and Cd accumulation in *Salix* roots of $Pb_{300}Cd_{25}$ treated plant was recorded and results presented in Plate IX indicates that higher concentration of Pb (0.5 wt.%) than Cd (0.1 wt.%) in outer region (epidermal and cortical tissues), whereas Cd accumulation (0.37 wt.%) was higher than Pb concentration (0.25 wt.%) in vascular region.

Energy dispersive spectra (EDS) in leaves of $Pb_{300}Cd_{25}$treated plant denoted that among Pb and Cd concentrations, Cd accumulation was more than the Pb concentration (Plate X). EDS values of outer (epidermal) surface of leaves showed that higher accumulation of Cd (1.17 wt. %) over Pb (0.01 wt. %). Similarly, the inner (mesophyll cells) region of $Pb_{300}Cd_{25}$ treated leaves showed the more concentration of Cd (0.28 wt. %) over Pb (0.25 wt. %).

Hence, EDS results of *Salix* roots concluded that Pb accumulation was higher in outer (epidermal and cortex) region, whereas Cd accumulation was highest in vascular region that is due to the mobile nature of Cd as it is easily transported through endodermal barrier. Since, the Cd loading in root vascular channel was higher than Pb, the EDS values of leaves showed the maximum distribution of Cd over Pb in whole leaf tissues. Further, EDS values are in favor with BCF and TF values for both Pb and Cd; illustrated that high accumulation of Pb was confined to root region only, whereas due to high TF values of Cd than Pb indicated its higher translocation and accumulation in aerial plant parts (Fig. 4.9).

b. Energy dispersive x-ray spectroscopy (EDS) in *Toona ciliata*

EDS performed to expound the Pb and Cd accumulation sites in *Toona* root and leaves. The EDS results of *Toona* roots of $Pb_{300}Cd_{25}$ treated plant is shown in Plate XI indicates that higher concentration of Pb (0.93 wt.%) than Cd (0.13 wt.%) in outer (epidermal and cortical) tissues, similarly in vascular region the higher Pb accumulation (0.80 wt.%) over Cd (0.60 wt.%) was recorded.

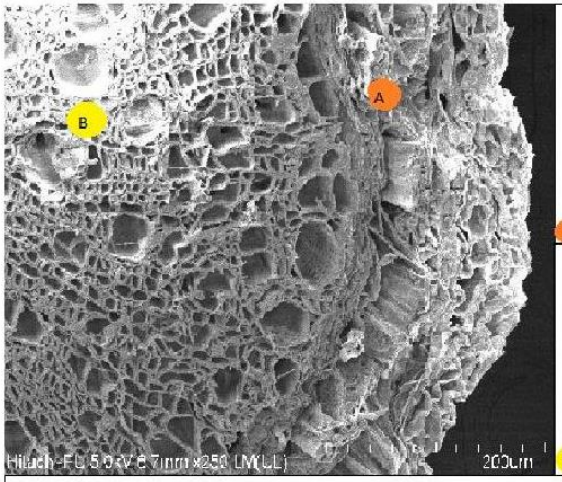
EDS values in leaves of $Pb_{300}Cd_{25}$ treated plants denoted that among Pb and Cd concentrations, Pb accumulation was comparatively higher than the Cd (Plate XII). EDS

values of epidermal surface of leaves showed that higher accumulation of Pb (4.10 wt.%) over Cd (1.52 wt.%). Similarly, the cortical and vascular region of $Pb_{300}Cd_{25}$ treated leaves showed the higher concentration of Pb (2.39 wt.%) over Cd (1.22 wt. %).

Thus, EDS results of *Toona* conferred higher Pb accumulation than Cd in epidermal, cortex and vascular region of roots, similarly higher Pb accumulation over Cd was recorded in all leaf tissues. Among different plant parts, higher heavy metal accumulation was recorded in aerial plant parts (leaves) than roots and these results are in favor with BCF and TF values (Fig. 4.10).

In *Salix*, results conferred that comparatively higher concentrations of both Pb and Cd level in root tissues than leaves of heavy metal treated plants, indicating that the translocation of toxic ion into the vascular bundles may be slows down by endodermal barriers. Additionally, the casparian strip exerts negative effect on Pb and Cd transport through endodermis and into the vascular bundles by restricting apoplastic transport (Bhatia *et al* 2004). The results revealed lignification and suberization in the root cells under Pb and Cd stress restricts the transport of toxic ions that prevent its interference to the plant metabolism. Increased thickening of cell walls and collapsed intercellular spaces in root cells due to Pb and Cd toxicity affect their transport to aerial plant tissues. Additionally, suberin like compounds, contain a number of ligands, have the capacity to adsorb heavy metal ions, leading to the regional enrichment of heavy metal in roots (Palliyath and Puthur 2018). The xylem can load and transport heavy metals to the aerial plant parts and accumulate heavy metals in leaf vacuoles. High affinity for particular metal (Pb or Cd) in the vascular region (xylem) is considered as crucial detoxification mechanism in leaves (Rucinska-Sobkowiak 2016). The regional enrichment of Pb > Cd in the root cortical tissues of *Salix* might be due to the storage functioning of vacuoles in cortical cells, suggesting that the *Salix* roots had better detoxification and sequestration mechanism for Pb storage than Cd. Thus, the Cd toxicity effect was more pronounced than Pb on morpho-physiological traits. Meanwhile, the EDS results of *Toona* confirmed that aerial plant parts (leaves) had more Pb > Cd enrichment ability than roots which suggests that plant efficient translocation and detoxification mechanism.

Hence, The FESEM and EDS analysis confirmed the uptake and accumulation of Pb and Cd ions in different tissues of $Pb_{300}Cd_{25}$treated plants are responsible for all morpho-physiological and ultra-structural alterations under heavy metal stress.



A

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	63.31	63.31	69.80	40.22
Nitrogen	K-series	2.39	2.39	2.26	11.17
Oxygen	K-series	33.70	33.70	27.90	32.71
Lead	M-series	0.50	0.50	0.03	0.53
Cadmium	L-series	0.10	0.10	0.01	0.27
	Total:	100.00	100.00	100.00	

B

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	65.17	65.17	71.63	37.52
Nitrogen	K-series	0.77	0.77	0.72	5.31
Oxygen	K-series	33.44	33.44	27.59	28.88
Cadmium	L-series	0.37	0.37	0.04	0.41
Lead	M-series	0.25	0.25	0.02	0.36
	Total:	100.00	100.00	100.00	

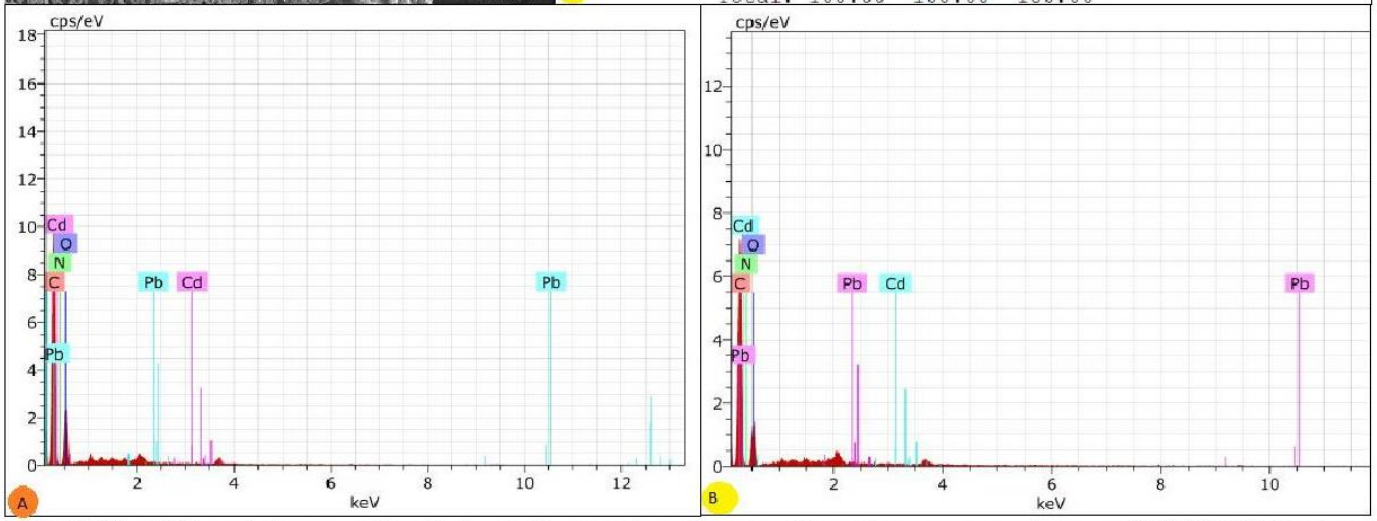
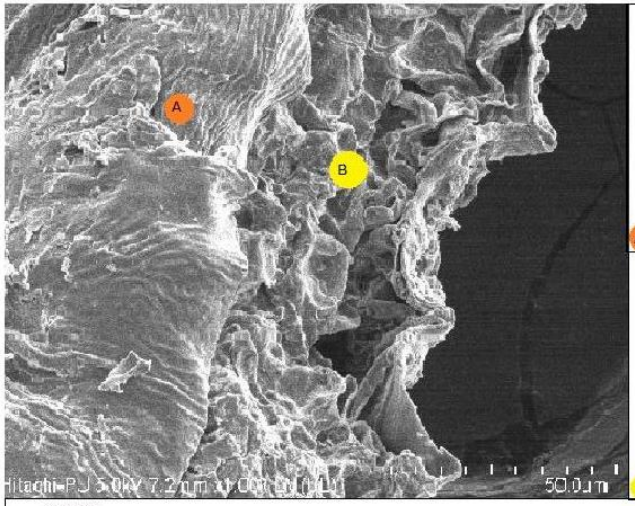


PLATE IX: Field emission scanning electron micrograph and energy dispersive x-ray spectroscopy (FESEM-EDS) of root cross-section of *Salix alba* ($Pb_{300}Cd_{25}$) in different tissues

(A. Epidermal and cortical region, B. Vascular region)

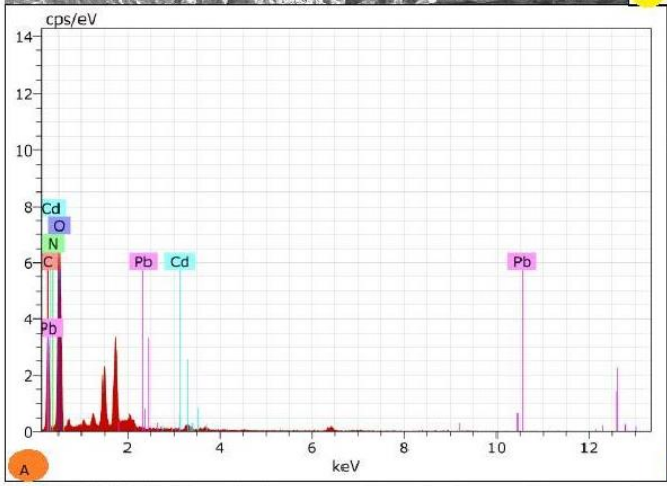


Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	29.96	29.96	36.49	14.01
Nitrogen	K-series	2.99	2.99	3.13	3.49
Oxygen	K-series	65.87	65.87	60.23	27.24
Cadmium	L-series	1.17	1.17	0.15	0.35
Lead	M-series	0.01	0.01	0.00	0.10
Total:		100.00	100.00	100.00	

A

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	47.52	47.52	54.57	17.87
Nitrogen	K-series	4.92	4.92	4.84	3.94
Oxygen	K-series	47.03	47.03	40.54	18.66
Lead	M-series	0.25	0.25	0.02	0.15
Cadmium	L-series	0.28	0.28	0.03	0.15
Total:		100.00	100.00	100.00	

B



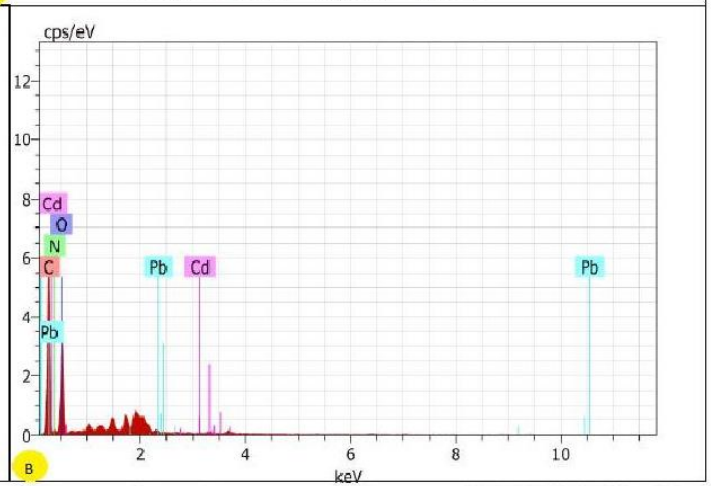


PLATE X: Field emission scanning electron micrograph and energy dispersive x-ray spectroscopy (FESEM-EDS) of leaf cross-section of *Salix alba* ($Pb_{300}Cd_{25}$) in different tissues

(A. Epidermal and cortical region, B. Vascular region)

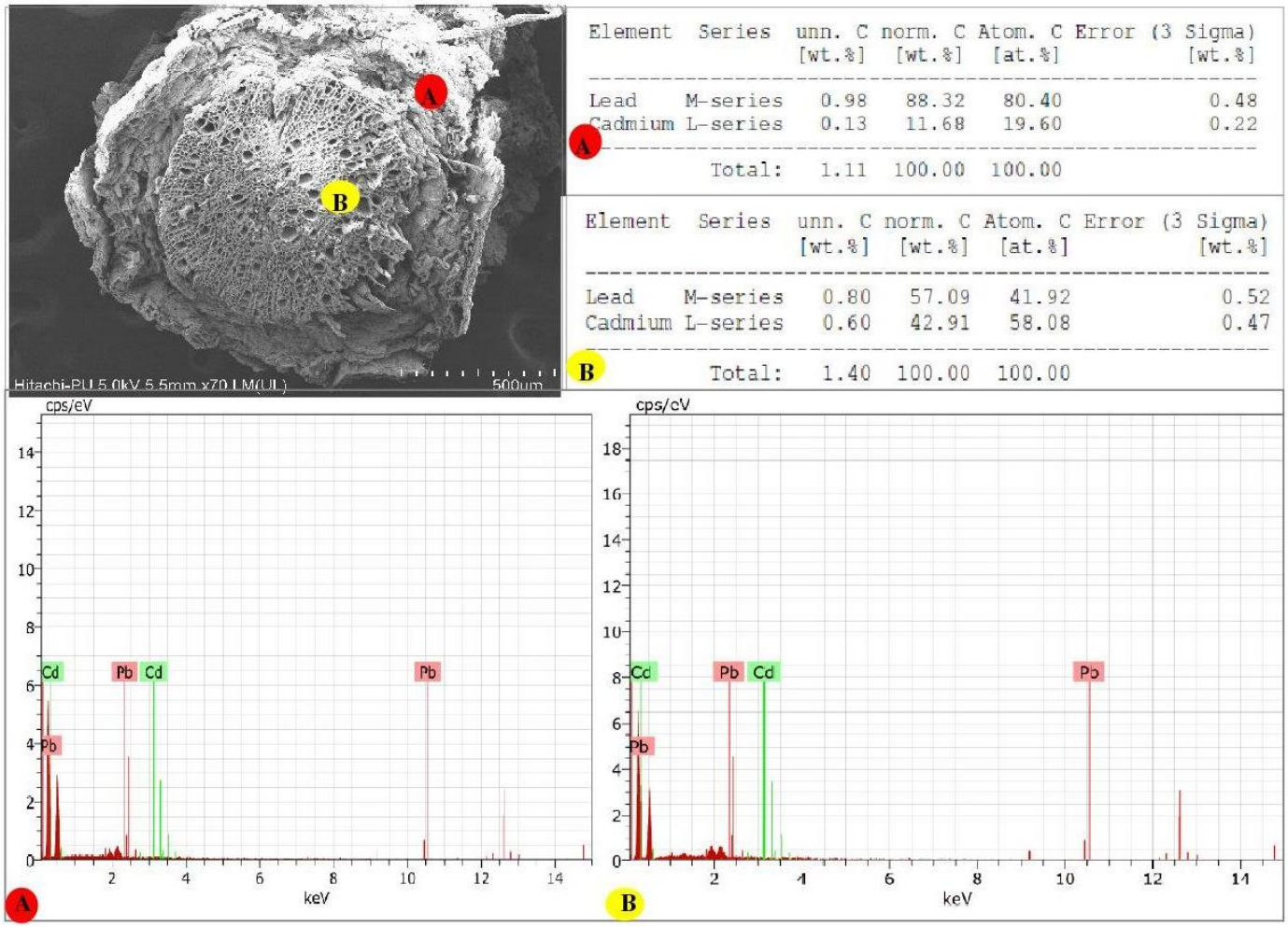


PLATE XI: Field emission scanning electron micrograph and energy dispersive x-ray spectroscopy (FESEM-EDS) of root cross-section of *Toona ciliata* ($Pb_{300}Cd_{25}$) in different tissues

(A. Epidermal and cortical region, B. Vascular region)

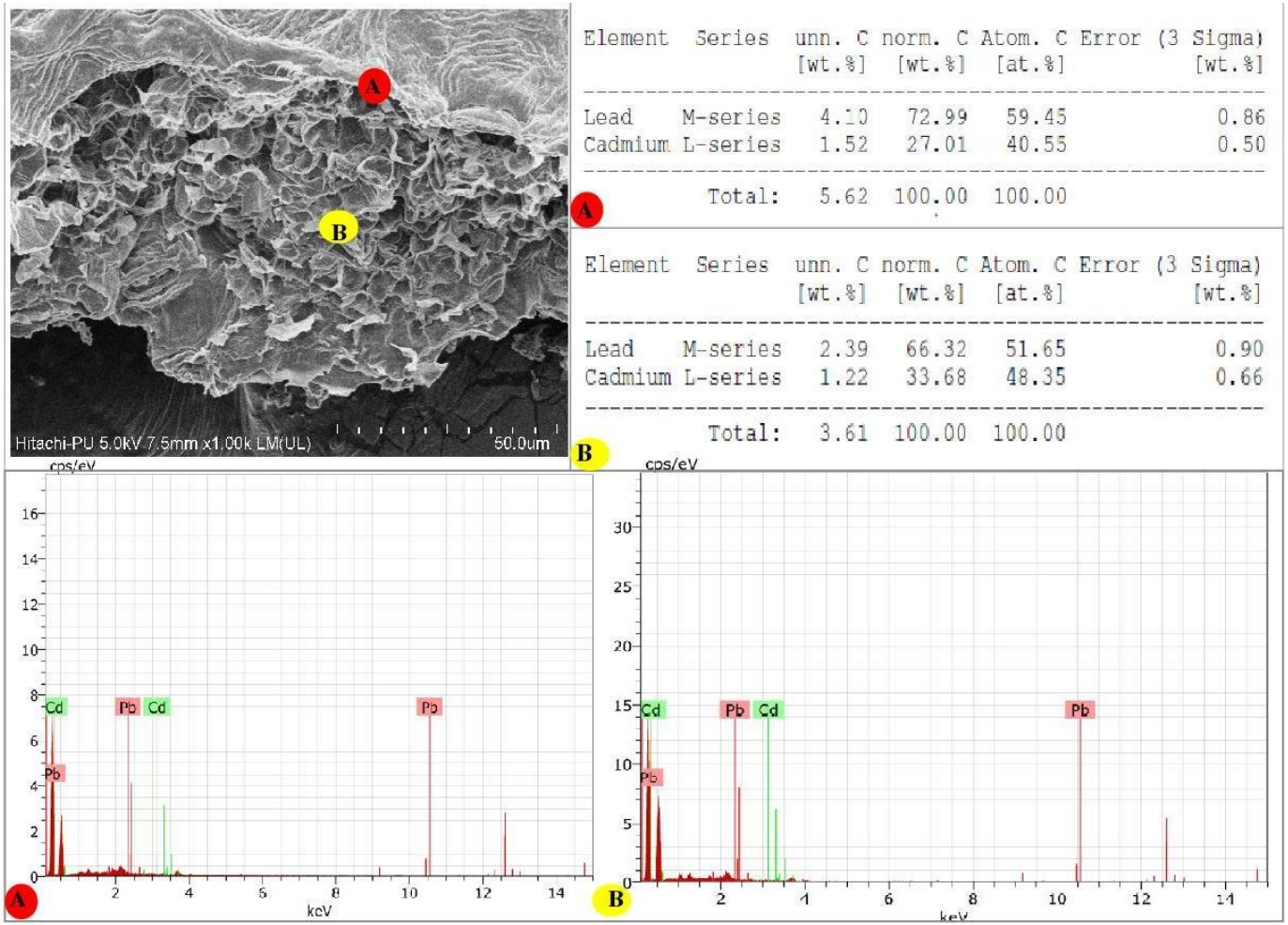


PLATE XII: Field emission scanning electron micrograph and energy dispersive x-ray spectroscopy (FESEM-EDS) of leaf cross-section of *Toona ciliata* ($Pb_{300}Cd_{25}$) in different tissues
(A. Epidermal and cortical region, B. Vascular region)

Experiment No. 2

4.2 Effect of heavy metals on uptake, translocation and metal accumulation potential of tree species

4.2.1 Heavy metal (Pb and Cd) accumulation in plant parts (root, stem and leaves)

After six months, Pb and Cd concentration was estimated from different plant parts (i.e. root, stem and leaves) of *Salix* and *Toona* through inductively coupled plasma mass spectroscopy (ICP-MS).

a. Lead (Pb) accumulation in plant parts of *Salix* and *Toona*

The data presented in Table 4.18 exhibits that lead (Pb) accumulation in *Salix* and *Toona* plant parts (root, stem and leaves) was increased with increasing concentrations of Pb (individual as well as in combination with Cd).

In *Salix*, with increase in Pb concentration, significant increase in Pb accumulation was recorded in all plant parts. The mean values for Pb accumulation was minimum in control plants i.e. Pb_0 (6.79 mg/kg in roots, 2.23 mg/kg in stem and 0.508 mg/kg in leaves) which increased significantly with increase in Pb concentration, thus maximum accumulation was recorded with highest Pb concentration i.e. Pb_{300} (210.3 mg/kg in roots, 16.5 mg/kg in stem and 6.00 mg/kg in leaves). Comparing the plant parts, the accumulation was maximum in roots followed by stem and leaves.

Pb accumulation in *Salix* was higher with Pb_{100}, Pb_{200} and Pb_{300} as compared to their combinations with Cd (Pb+Cd). Thus, among combinations, maximum Pb accumulation was observed with highest Pb concentration i.e. $Pb_{300}Cd_0$ (224.8 mg/kg in root, 20.8 mg/kg in stem, 8.45 mg/kg in leaves) followed by $Pb_{300}Cd_5$ (215.7 mg/kg in root, 18.0 mg/kg in stem and 6.5 mg/kg in leaves), $Pb_{300}Cd_{15}$ (209.7 mg/kg in root, 14.8 mg/kg in stem, 5.01 mg/kg in leaves) and $Pb_{300}Cd_{25}$(190.9 mg/kg in root, 12.4 mg/kg in stem, 4.05 mg/kg in leaves).

In case of *Toona*, the significant increase in Pb accumulation was recorded with increase in Pb concentration. The minimum mean Pb accumulation was recorded in control plant parts i.e. Pb_0 (3.77 mg/kg in roots, 4.22 mg/kg in stem and 5.82 mg/kg in leaves) which increased significantly with increase in Pb concentration and maximum accumulation was recorded with highest Pb concentration i.e. Pb_{300} (81.7 mg/kg in roots, 86.0 mg/kg in stem and 131.7 mg/kg in leaves).

Pb accumulation in *Toona* was higher with sole Pb concentrations (Pb_{100}, Pb_{200} and Pb_{300}) as compared to their combination with Cd (Pb+Cd). Hence, maximum Pb accumulation was recorded with highest Pb concentration i.e. $Pb_{300}Cd_0$ (90.5 mg/kg of root, 92.1 mg/kg of stem, 139.8 mg/kg of leaves) followed by $Pb_{300}Cd_5$ (84.5 mg/kg of root, 90.2 mg/kg of stem and 132.5mg/kg of leaves), $Pb_{300}Cd_{15}$ (79.3 mg/kg of root, 84.7 mg/kg of stem, 128.9 mg/kg of leaves) and $Pb_{300}Cd_{25}$ (72.3 mg/kg of root, 76.9 mg/kg of stem, 125.7 mg/kg of leaves), thus the order followed is leaves > stem > roots (Table 4.18).

Table 4.18: Lead accumulation in different plant parts of *Salix alba* and *Toona ciliata*

Salix alba															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	7.80^{m}	90.4^{i}	175.6^{e}	224.8^{a}	124.6^{A}	2.80^{j}	11.1^{fg}	16.0^{bc}	20.8^{a}	12.7^{A}	0.801^{ef}	4.56^{bdc}	6.54^{ab}	8.45^{a}	5.09^{B}
Cd_5	6.95^{m}	85.9^{j}	149.8^{f}	215.7^{b}	114.6^{B}	2.41^{j}	10.1^{gh}	13.6^{de}	18.0^{b}	11.0^{AB}	0.531^{ef}	3.82^{dc}	4.98^{bdc}	6.52^{bac}	3.95^{AB}
Cd_{15}	6.45^{m}	74.7^{k}	137.9^{g}	209.7^{c}	107.2^{B}	2.20^{j}	8.56^{h}	12.1^{efg}	14.8^{cd}	9.42^{BC}	0.392^{f}	3.18^{de}	4.33^{bdc}	5.01^{bdc}	3.23^{A}
Cd_{25}	5.95^{m}	68.5^{l}	135.6^{h}	190.9^{d}	100.3^{D}	1.50^{j}	7.55^{i}	11.3^{fg}	12.4^{ef}	8.19^{C}	0.310^{f}	2.71^{def}	3.81^{cdc}	4.05^{bcd}	2.71^{A}
Mean	6.79^{D}	79.9^{C}	149.7^{B}	210.3^{A}		2.23^{D}	9.33^{C}	13.3^{B}	16.5^{A}		0.508^{C}	3.568^{BC}	4.905^{B}	6.00^{A}	
LSD ($p\leq0.05$)	Pb	1.48				Pb	1.44				Pb	1.48			
	Cd	1.48				Cd	1.44				Cd	1.48			
	Pb×Cd	2.97				Pb×Cd	2.88				Pb×Cd	2.96			
Toona ciliata															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	3.55^{l}	38.7^{h}	56.7^{e}	90.5^{a}	47.4^{A}	3.67^{j}	40.8^{g}	59.8^{d}	92.1^{a}	49.1^{A}	5.34^{j}	62.9^{g}	89.4^{d}	139.8^{a}	74.4^{A}
Cd_5	3.67^{l}	33.5^{i}	50.4^{f}	84.5^{b}	43.0^{B}	4.11^{j}	39.7^{g}	54.3^{e}	90.2^{a}	47.1^{B}	6.82^{j}	58.4^{g}	85.4^{de}	132.5^{b}	70.8^{B}
Cd_{15}	3.63^{l}	29.8^{j}	45.6^{g}	79.3^{c}	39.6^{C}	4.21^{j}	32.9^{h}	50.6^{e}	84.7^{b}	43.1^{C}	5.89^{j}	52.5^{h}	82.5^{ef}	128.9^{bc}	67.4^{C}
Cd_{25}	4.20^{l}	22.9^{k}	40.2^{h}	72.3^{d}	34.9^{D}	4.89^{j}	27.7^{i}	46.3^{f}	76.9^{c}	38.9^{D}	5.22^{j}	46.4^{i}	79.6^{f}	125.7^{c}	64.2^{D}
Mean	3.77^{D}	31.2^{C}	48.2^{B}	81.7^{A}		4.22^{D}	35.3^{C}	52.8^{B}	86.0^{A}		5.82^{D}	55.1^{C}	84.2^{B}	131.7^{A}	
LSD ($p\leq0.05$)	Pb	1.24				Pb	1.44				Pb	1.76			
	Cd	1.24				Cd	1.44				Cd	1.76			
	Pb×Cd	2.47				Pb×Cd	2.89				Pb×Cd	3.53			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Hence, with increase in Pb concentration (individual as well as combination) the significant increase in Pb accumulation was recorded in both *Salix* and *Toona* species. Similar trend recorded in both species that Pb accumulation was recorded higher with sole Pb concentrations as compared to combinations (Pb+Cd). This indicates that sole application of Pb increases the Pb accumulation in root, stem and leaves, while in combinations the antagonistic effect of Cd on Pb was being observed.

However, among plant parts, the differential Pb accumulation behavior was observed in both species that *Salix* accumulates higher Pb in roots than stem and leaves in following order: root> stem> leaves, whereas *Toona* showed maximum Pb accumulation in leaves than stem and roots in following order: leaves > stem > roots. Overall, the Pb accumulation was quantitatively higher in *Salix* (5.95 to 225 mg/kg) than *Toona* (3.5 to 140mg/kg), which is also responsible for visible toxicity symptoms observed in *Salix* (Plate II).

b. Cadmium (Cd) accumulation in plant parts of *Salix* and *Toona*

The data presented in Table 4.19 reveals the increasing concentrations of Cd in soil (individual as well as in combination with Pb) facilitates the corresponding increased accumulation in all plant parts i.e. root, stem and leaves.

In *Salix*, increasing Cd concentration in soil resulted in significant increase in Cd accumulation in all plant parts. The mean value for Cd accumulation was minimum in control (Cd_0, 0.029 to 0.012 mg/kg) which increased significantly with increase in Cd concentration, thus maximum accumulation was recorded with highest Cd concentration i.e. Cd_{25} (16.6 mg/kg in roots, 13.2 mg/kg in stem and 8.58 mg/kg in leaves).

Cd accumulation in *Salix* was recorded higher with sole Cd application i.e. Cd_5, Cd_{15} and Cd_{25} as compared to their combinations (Pb+Cd). Hence, the maximum Cd accumulation was recorded with highest Cd concentration Pb_0Cd_{25} (18.4 mg/kg in root, 14.6 mg/kg in stem, 11.2 mg/kg in leaves) followed by $Pb_{100}Cd_{25}$ (17.5 mg/kg in root, 14.1 mg/kg in stem and 8.80 mg/kg in leaves), $Pb_{200}Cd_{25}$ (16.1 mg/kg in root, 12.8 mg/kg in stem, 7.81 mg/kg in leaves) and $Pb_{300}Cd_{25}$ (14.2 mg/kg in root, 11.3 mg/kg in stem, 6.51 mg/kg in leaves) in an order as root > stem > leaves.

In case of *Toona*, with increase in Cd concentration, the significant increase in Cd accumulation was recorded in all plant parts. The minimum mean Cd accumulation was observed in control i.e. Cd_0 (0.02 to 0.03 mg/kg) which increased significantly with increase in Cd concentration, thus maximum accumulation was with highest Cd concentration i.e. Cd_{25} (12.1 mg/kg in roots, 12.8 mg/kg in stem and 16.9 mg/kg in leaves).

Table 4.19: Cadmium accumulation in different plant parts of *Salix alba* and *Toona ciliata*

Salix alba															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.02^{l}	0.025^{l}	0.03^{l}	0.04^{l}	0.029^{D}	0.01^{j}	0.02^{j}	0.025^{j}	0.036^{j}	0.023^{D}	0.009^{h}	0.01^{h}	0.02^{h}	0.01^{h}	0.012^{D}
Cd_5	5.08^{i}	4.80^{ij}	4.45^{jk}	4.04^{k}	4.59^{C}	2.84^{h}	2.65^{hi}	2.40^{hi}	2.26^{i}	2.54^{C}	2.03^{g}	1.91^{g}	1.72^{g}	1.59^{g}	1.81^{C}
Cd_{15}	13.5^{e}	12.9^{f}	11.6^{g}	9.32^{h}	11.9^{B}	7.82^{e}	5.94^{f}	5.14^{g}	4.8^{g}	5.92^{B}	6.08^{d}	4.60^{e}	3.89^{f}	3.45^{f}	4.50^{B}
Cd_{25}	18.4^{a}	17.5^{b}	16.1^{c}	14.2^{d}	16.6^{A}	14.7^{a}	14.1^{b}	12.8^{c}	11.3^{d}	13.2^{A}	11.2^{a}	8.80^{b}	7.81^{c}	6.51^{d}	8.58^{A}
Mean	9.26^{A}	8.84^{B}	8.06^{C}	6.91^{D}		6.33^{A}	5.64^{B}	5.09^{C}	4.59^{D}		4.83^{A}	3.83^{B}	3.36^{C}	2.89^{D}	
LSD ($p\leq0.05$)	Pb	0.158				Pb	0.158				Pb	0.158			
	Cd	0.158				Cd	0.158				Cd	0.158			
	Pb×Cd	0.316				Pb×Cd	0.316				Pb×Cd	0.316			
Toona ciliata															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.01^{h}	0.01^{h}	0.02^{h}	0.02^{h}	0.02^{D}	0.01^{f}	0.02^{f}	0.025^{f}	0.036^{f}	0.023^{D}	0.02^{i}	0.025^{i}	0.029^{i}	0.03^{i}	0.03^{D}
Cd_5	4.89^{g}	4.74^{g}	4.68^{g}	4.62^{g}	4.73^{C}	5.32^{e}	4.93^{e}	4.82^{e}	4.75^{e}	4.96^{C}	5.89^{g}	5.42^{h}	5.23^{h}	4.99^{h}	5.38^{C}
Cd_{15}	10.3^{d}	9.68^{e}	9.21^{ef}	8.79^{f}	9.50^{B}	11.2^{c}	9.84^{d}	9.58^{d}	9.25^{d}	9.92^{B}	14.1^{d}	13.7^{e}	12.8^{f}	12.8^{f}	13.4^{B}
Cd_{25}	12.6^{a}	12.3^{ab}	11.9^{bc}	11.4^{c}	12.1^{A}	13.2^{a}	13.5^{a}	12.7^{ab}	12.3^{b}	12.8^{A}	18.3^{a}	17.6^{b}	15.9^{c}	15.6^{c}	16.9^{A}
Mean	6.95^{A}	6.68^{B}	6.45^{C}	6.21^{D}		7.38^{A}	6.95^{B}	6.78^{C}	6.58^{D}		9.58^{A}	9.19^{B}	8.49^{C}	8.36^{D}	
LSD ($p\leq0.05$)	Pb	0.192				Pb	0.213				Pb	0.166			
	Cd	0.192				Cd	0.213				Cd	0.166			
	Pb×Cd	0.385				Pb×Cd	0.425				Pb×Cd	0.332			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Cd accumulation in *Toona* was higher with sole Cd application (Cd_5, Cd_{15} and Cd_{25}) as compared to their combination with Pb concentrations (Pb+Cd). Hence, maximum Cd accumulation was recorded in plants treated with highest Cd concentration i.e. Pb_0Cd_{25} (12.6 mg/kg of root, 13.2 mg/kg of stem, 18.3 mg/kg of leaves) followed by $Pb_{100}Cd_{25}$ (12.3 mg/kg of root, 13.5 mg/kg of stem and 17.6 mg/kg of leaves), $Pb_{200}Cd_{25}$ (11.9 mg/kg of root, 12.7 mg/kg of stem, 15.9 mg/kg of leaves) and $Pb_{300}Cd_{25}$ (11.4 mg/kg of root, 12.3 mg/kg of stem, 15.6 mg/kg of leaves) in following order as leaves > stem > roots. The decreasing Cd accumulation in combination with Pb again indicates the antagonistic effect of Pb and Cd.

Hence, with increase in Cd concentration (individual as well as combination) the significant increase in Pb accumulation was recorded in both *Salix* and *Toona* species. Similar trend recorded in both species that Cd accumulation was recorded higher with individual Cd concentrations as compared to combinations (Pb+Cd). However, among plant parts, the differential Cd accumulation behavior was observed in both species that *Salix* accumulates higher Cd in roots than stem and leaves in following order: root > stem > leaves, whereas *Toona* plants showed maximum Pb accumulation in leaves than stem and roots in following order: leaves > stem > roots. Overall, the quantitative Cd accumulation was almost similar in range (0.01 to18.5 mg/kg) in both *Salix* and *Toona*.

4.2.2 Phytoremediation efficiency evaluation factors

Bioconcentration factor (BCF) and translocation factor (TF) are the two important parameters that provides an insight towards the phytoremediation potential of a plant. BCF defines the relationship between the concentration of element in plant parts and the substrate, thus reflects the elemental accumulation (Yoon *et al* 2006). TF determines the efficiency of plants to translocate any eleent or metal from the root to the aerial plant parts. A plant is considered as efficient in metal translocation if TF is higher than one indicating an efficient metal transport system (Gajic *et al* 2018).

Bioconcentration factors were evaluated on the basis of availability of heavy metals in soil (Appendix i and ii) and its accumulation in plant parts of *Salix* and *Toona* (Table 4.18 and 4.19). The metal removal efficiency of plants also calculated on the basis of soil heavy metal concentration estimated before plantation and after the uprooting of plants (Appendix i & ii). The tolerance index (TI %) calculated on the basis of plant biomass as affected by different concentration of heavy metals (Table 4.6 and 4.7).

A. Phytoremediation efficiency of *Salix alba*

The phytoremediation efficiency evaluation factors such as bioconcentration factor (BCF), translocation factor (TF) and metal removal efficiency (MRE) of *Salix* is presented in Fig. 4.9. With increasing Pb and Cd concentration, corresponding increase in BCF and TF values were observed. Moreover, both BCF and TF values decreased with combinations (Pb+Cd) as compared to their individual element concentrations (Fig. 4.9).

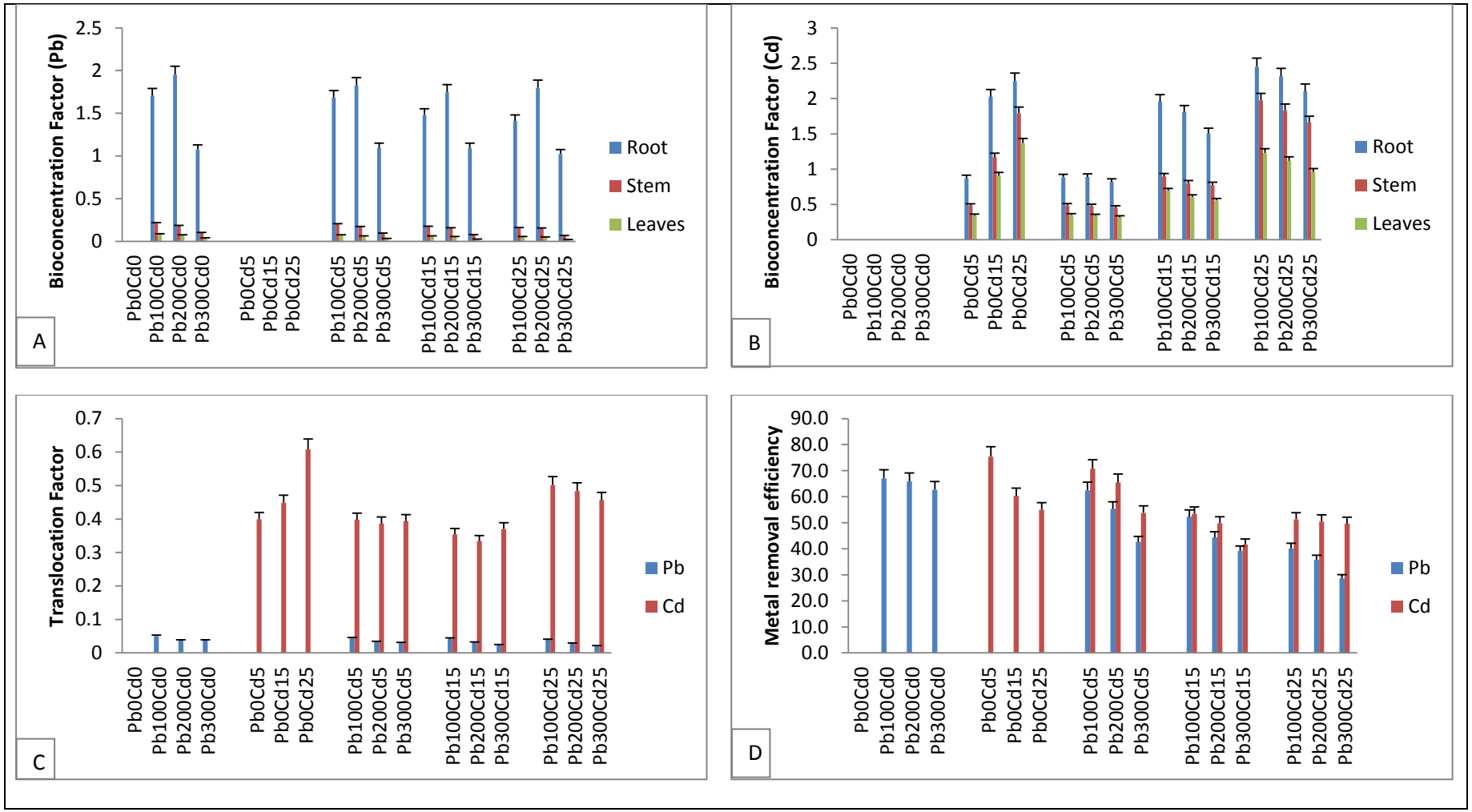


Fig 4.9: Phytoremediation efficiency evaluation factors in *Salix alba*

A: Bioconcentration factor (BCF) of Pb
B: Bioconcentration factor (BCF) of Cd
C: Translocation factor (TF)
D: Metal removal efficiency (MRE)

a. Bioconcentration factor (BCF)

Pb and Cd bioconcentration factor (BCF) values represents the metal accumulation ability of plant depends upon the heavy metal availability in soil (Fig. 4.9). BCF for Pb was maximum with single Pb treatment, but these values were decreased with combinations (Pb+Cd). Among all plant parts, BCF range was maximum in roots (1.03 to 1.85) followed by stem (0.07 to 0.21) and leaves (0.02 to 0.09) in decreasing order as: roots > stem > leaves.

Similarly, the BCF values for Cd were also fluctuated with increase in Cd concentration. Among all plant parts, BCF recorded maximum in roots (0.57 to 2.25) followed by stem (0.52 to 1.96) and leaves (0.52 to 1.0) and follows an order i.e. roots > stem > leaves. *Salix* showed more BCF for Cd compared to Pb indicating that their roots had more Cd enrichment ability.

b. Translocation factor (TF)

The effect of heavy metals on translocation presented as translocation factor (TF) from root to shoots in *Salix* is depicted in Fig. 4.9. The TF values showed drastic decrease with Pb and Cd combination treatments as compared to their individual element concentration.

The TF values for Pb concentrations showed poor translocation (< 0.15), whereas TF values for Cd indicates the medium translocation (0.45 to 0.65) from root to shoot. Cd translocation was more in comparison to Pb, which might be due to the greater mobility of Cd ions. Overall, Pb and Cd translocation factors are less than one (< 1) indicating the poor translocation ability of *Salix* from roots to shoots.

Pb and Cd bioconcentration values are higher in roots (BCF > 1) than stem and leaves (BCF < 1) with poor translocation (TF < 1) which represents that *Salix* roots had more Cd enrichment ability than Pb.

c. **Metal removal efficiency (MRE)**

The metal removal efficiency (MRE) of *Salix* was calculated on the basis of total soil heavy metal concentration before plantation and after the uprooting of plants and is expressed in Fig. 4.9. The MRE values for Pb concentrations were in range of 60 to70% that decreased with application of Pb and Cd in combinations.

Although the MRE values fluctuated with different heavy metal concentrations, overall the highest MRE values was evaluated with sole Pb and Cd concentrations as compared to their combinations in an order as Cd > Pb > Pb+Cd.

B. Phytoremediation efficiency of *Toona ciliata*

The phytoremediation efficiency evaluation factors (BCF, TF and MRE) of *Toona* are expressed in Fig. 4.10. With increasing Pb and Cd concentration, corresponding fluctuations in BCF and TF values were observed. Moreover, both BCF and TF values decreased with combinations (Pb+Cd) as compared to their individual element concentrations.

a. Bioconcentration factor (BCF)

Pb and Cd bioconcentration factor (BCF) values increased with increase in heavy metal concentration in soil (Fig. 4.9). BCF values for Pb was maximum with highest Pb treatment i.e. Pb_{300} (1.52 roots, 2.48 stem and 2.63 leaves), but these values were decreased with combinations (Pb+Cd) as compared to individual Pb concentrations. Among all plant parts, BCF range of Pb was minimum in roots (0.96 to 1.52) and maximum in leaves (1.87 to 2.63) which was equivalent with stem (1.49 to 2.48) followed the order as roots < stem ≤ leaves.

Similarly, the BCF for Cd was also increased with increase in Cd concentration. The maximum BCF values were recorded with highest Cd concentration i.e. Cd_{25} (1.77 roots, 1.69 stem and 2.54 leaves), but these values were decreased with combinations (Pb+Cd) as compared to the sole Cd concentrations. Among all plant parts, BCF values recorded maximum in leaves (1.04 to 2.54) and minimum in roots (0.979 – 1.78) equivalent with stem (0.90 to 1.69), follows an order i.e. leaves > stem ≥ roots.

For both Pb and Cd, *Toona* showed BCF values more than one indicating that their aerial plant parts had more metal accumulation capability than roots.

b. Translocation factor (TF)

The effect of heavy metals on translocation factors (TF) from root to shoots of *Toona* are expressed in Fig. 4.10. The TF values showed drastic decrease with Pb and Cd combination treatments as compared to their individual element concentration.

The TF values for both Pb (1.55 -2.03) and Cd (1.07 – 1.46) concentrations are more than one (TF >1), which indicates that *Toona* plants had efficient translocation mechanism for both Pb and Cd.

c. **Metal removal efficiency (MRE)**

The Pb and Cd metal removal efficiency (MRE) of *Toona* was calculated and is expressed in Fig. 4.10. The MRE values for pure Pb concentrations was in range of 60% to70% which decreased with combinations, thus minimum MRE values were recorded with highest combination i.e. $Pb_{300}Cd_{25}$ (53.9 %). Similar trend was recorded for Cd that maximum MRE values were obtained with individual Cd concentrations (65-85%) and minimum MRE values with $Pb_{300}Cd_{25}$ (57.6%).

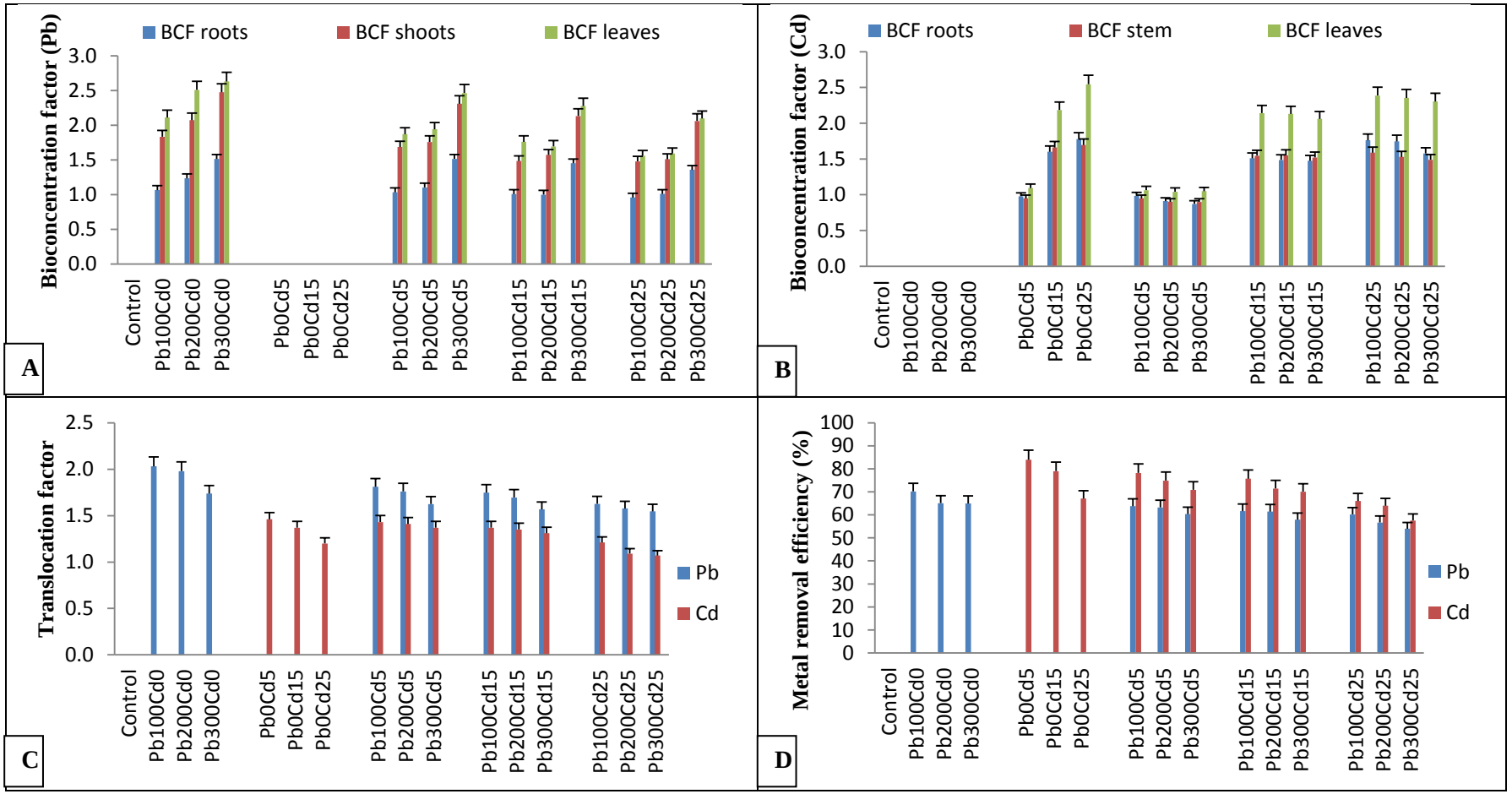


Fig 4.10: Phytoremediation efficiency evaluation factors in *Toona ciliata*

A: Bioconcentration factor (BCF) of Pb

B: Bioconcentration factor (BCF) of Cd

C: Translocation factor (TF)

D: Metal removal efficiency (MRE %)

The values of MRE of *Toona* were fluctuated with different heavy metal concentrations, overall the highest MRE values was evaluated with sole Pb and Cd concentrations as compared to their combinations in an order as Cd > Pb > Pb+Cd.

C. Tolerance index of *Salix alba* and *Toona ciliata*

On the basis of biomass, the tolerance index of *Salix* and *Toona* was calculated in response to heavy metal concentrations (Fig. 4.11). With increasing Pb and Cd concentrations, the decrease in tolerance was recorded in both species. However, the decreased tolerance was more pronounced with combinations (Pb+Cd) as compared to their individual element concentrations in an order Pb > Cd > Pb+Cd.

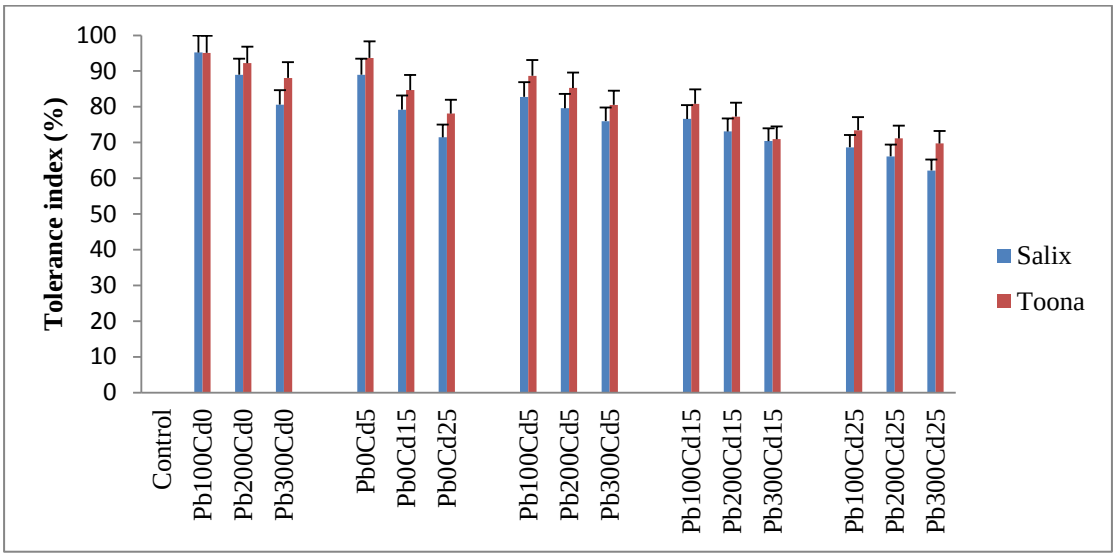


Fig. 4.11: Tolerance index of *Salix alba* and *Toona ciliata* against heavy metal stress

In *Salix*, higher tolerance index was recorded under individual Pb and Cd concentrations (75 to 95%) as compared to their combinations (60 to 70%). Similarly, *Toona* plants also showed higher tolerance index (80 to 95%) with pure Pb and Cd concentrations than combinations (70 to 80%). Although both *Salix* and *Toona* showed strong tolerance (> 65%) against heavy metal stress, but the tolerance was comparatively higher in *Toona* than *Salix*.

The determination of bioconcentration factor (BCF) and translocation factor (TF) are crucial for developing a better understanding of the tolerance mechanism and survival strategy of plants on degraded sites. BCF and TF values exhibited more reduction during combined heavy metal pollution in comparison to the single element contamination, as the heavy metals present in soil as well as plant tissues interfere with each other as well as other micronutrients for bioavailability and translocation either through xylem or phloem tissues using transporter proteins. Similar findings of Zhang *et al* (2019) in ryegrass suggested that Pb, Cd and Zn combination concentrations in soil adversely affects the metal translocation in plants.

Among the two selected heavy metal elements, phytoremediation evaluation factors i.e. BCF, TF and MRE signifies that the both *Salix* and *Toona* has stronger enrichment ability for Cd than Pb, however, their partitioning and accumulation behaviour were different. The higher translocation and accumulation of Cd in plant tissues is due to its polarized nature and soft cationic character that justifies its high translocation potential to form stable complex with soft ligands like amino and sulfhydryl groups (Dalton *et al* 2005), which is also confirmed by FTIR analysis (Table 4.20 and 4.21). Owing to its elemental characteristics and mode of uptake, Cd is readily bioavailable and quickly translocated from roots to various regions of the plants. Cd tends to enter the plant through the essential elements (such as Ca, Fe and Zn) absorption pathway, as evidenced that Ca^{2+} channels in the guard cells are permeable to Cd^{2+} (Kumar *et al* 2017). Pourrut *et al* (2011) and Kushwaha *et al* (2018) reported that the negative charged components in the cell wall, such as pectin, either trap Pb ions during transit or held them in the endodermis with casparian-strips, or follow the symplastic transport to excrete the majority of the isolated Pb ions out of the plant tissues, which is also confirmed by FTIR analysis (Table 4.20 and 4.21).

In the current study, phytoremediation evaluation factors of *Salix* and *Toona* reveals that both species showed differential metal accumulation behaviour. Overall, BCF and TF values were recorded higher in *Toona* (>1) as compared to *Salix* (<1) which conferred that *Salix* showed maximum Pb and Cd accumulation in roots, whereas *Toona* plants showed maximum accumulation in their aerial plant parts.

According to Baker (1981), plants with BCF > 1 and TF >1 as well as BCF >1 and TF < 1 are considered to possess phytostabilization potential. The results in present study indicate *Salix* showed BCF >1 (for roots only) and TF < 1, thus can be categorized as excluders, as they maintain high uptake of soil-root with small root to shoot translocation. Along with this, *Salix* shows sufficient metal removal efficiency (65%) up to Pb_{200} mg/kg and Cd_{15} mg/kg (individual as well as in combination), further increase in Pb and Cd concentration affect their uptake, translocation and accumulation capabilities as well as survival percentage as expressed in Fig.4.9 and Fig. 4.1.

Toona ciliata showed BCF >1 and TF >1 which is suitable for phytoextraction and can be categorized as accumulators/hyper accumulators as per the criteria described by Gajic *et al* (2018). *Toona* plants show sufficient metal removal efficiency (> 65%) as well as better survival percentage with higher Pb and Cd concentrations ($Pb_{300}Cd_{25}$ mg/kg) as expressed in Fig. 4.10 and 4.1.

4.2.3 Plant nutrient analysis

In the present study, the effect of heavy metals on macro nutrient (N, P, K, Ca, Mg, S) and micro nutrient profile (Mn, Zn, Cu, Fe) of *Salix* and *Toona* were investigated. These macro and micro nutrients were estimated from different plant parts (i.e. root, stem and

leaves) of *Salix* and *Toona* after six months and are discussed as follows:

a. Nitrogen (N)

The data presented in Table 4.20 shows the effect of different concentrations of Pb, Cd and their combinations on nitrogen (N) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, the significant decrease in N content of both *Salix* and *Toona* was recorded.

In *Salix*, significant decrease in nitrogen content of all plant parts was observed in response to Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for N was maximum in control i.e. Pb_0 (2.53% in roots, 2.68% in stem and 3.49% in leaves) which decreased significantly with increase in Pb concentration, thus minimum N content was recorded with highest Pb concentration i.e. Pb_{300} (1.57% in roots, 2.26% in stem and 2.38% in leaves). Similar trend was observed for Cd concentrations where maximum mean N content was in control (Cd_0, 2.40% in roots, 2.93% in stem and 3.72% in leaves) which decreased significantly with increase in concentration from Cd_5 (2.04% in roots, 2.80% in stem and 3.43% in leaves) to Cd_{25} (1.59% in roots, 1.87% in stem and 2.44% in leaves). Among combinations, maximum N content was observed in control Pb_0Cd_0 (3.60% in root, 3.15% in stem, 4.54% in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (1.38% in root, 1.77% in stem, 2.27% in leaves).

In case of *Toona*, the significant decrease in nitrogen content of all plant parts was observed because of Pb, Cd and their combination concentrations (Table 4.20). Among Pb concentrations, the mean value for N content was maximum in control plants i.e. Pb_0 (3.80% in roots, 2.94% in stem and 3.99% in leaves) which decreased significantly with increase in Pb concentration, thus minimum N content was recorded with highest Pb concentration i.e. Pb_{300} (2.94% in roots, 2.62% in stem and 2.96% in leaves). Similarly, among Cd concentrations, maximum mean N content was observed in control (Cd_0, 3.68% in roots, 3.22% in stem and 4.21% in leaves) which decreased significantly with increase in concentration from Cd_5 (3.38% in roots, 3.12% in stem and 3.94% in leaves) to Cd_{25} (2.95% in roots, 2.16% in stem and 3.02% in leaves.

Among combinations, maximum N content was observed in control Pb_0Cd_0 (4.63% in root, 3.34% in stem, 4.87% in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (2.72% in root, 2.05% in stem, 2.92% in leaves).

Table 4.20: Effect of heavy metals on nitrogen content in different plant parts of *Salix alba* and *Toona ciliata*

Nitrogen (%)															
Salix alba															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	3.60^{a}	2.19^{c}	2.03^{d}	1.78^{ef}	2.40^{A}	3.15^{a}	3.12^{a}	2.89^{bc}	2.54^{ef}	2.93^{A}	4.54^{a}	4.04^{b}	3.87^{cd}	2.43^{h}	3.72^{A}
Cd_5	2.70^{b}	2.05^{cd}	1.73^{ef}	1.66^{fg}	2.04^{B}	3.04^{ab}	2.79^{cd}	2.72^{d}	2.65^{de}	2.80^{B}	4.00^{bc}	3.77^{d}	3.56^{e}	2.41^{h}	3.43^{B}
Cd_{15}	2.08^{cd}	1.84^{e}	1.69^{efg}	1.47^{h}	1.77^{C}	2.55^{ef}	2.43^{f}	2.25^{g}	2.09^{h}	2.33^{C}	2.86^{f}	2.65^{g}	2.43^{h}	2.40^{h}	2.59^{C}
Cd_{25}	1.73^{ef}	1.66^{fg}	1.56^{gh}	1.38^{i}	1.59^{D}	1.99^{hi}	1.86^{i}	1.85^{i}	1.77^{j}	1.87^{D}	2.55^{gh}	2.51^{gh}	2.43^{h}	2.27^{i}	2.44^{D}
Mean	2.53^{A}	1.94^{B}	1.75^{C}	1.57^{D}		2.68^{A}	2.55^{B}	2.43^{C}	2.26^{D}		3.49^{A}	3.24^{B}	3.07^{C}	2.38^{D}	
LSD ($p\leq0.05$)	Pb	0.049				Pb	0.055				Pb	0.057			
	Cd	0.049				Cd	0.055				Cd	0.057			
	Pb×Cd	0.098				Pb×Cd	0.110				Pb×Cd	0.115			
Toona ciliata															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	4.63^{a}	3.50^{c}	3.42^{c}	3.18^{de}	3.68^{A}	3.34^{a}	3.32^{b}	3.26^{abc}	2.95^{def}	3.22^{A}	4.87^{a}	4.48^{b}	4.46^{b}	3.02^{ef}	4.21^{A}
Cd_5	3.93^{b}	3.45^{c}	3.16^{de}	2.97^{def}	3.38^{B}	3.28^{ab}	3.09^{bcd}	3.03^{de}	3.06^{cde}	3.12^{B}	4.55^{b}	4.16^{c}	4.11^{c}	2.94^{f}	3.94^{B}
Cd_{15}	3.51^{c}	3.21^{d}	3.07^{def}	2.88^{fg}	3.17^{C}	2.87^{de}	2.80^{ef}	2.57^{g}	2.41^{gh}	2.66^{C}	3.44^{d}	3.28^{de}	2.94^{f}	2.96^{ef}	3.16^{C}
Cd_{25}	3.15^{de}	3.03^{def}	2.90^{efg}	2.72^{g}	2.95^{D}	2.26^{hi}	2.15^{i}	2.19^{i}	2.05^{i}	2.16^{D}	3.11^{ef}	3.07^{ef}	2.98^{ef}	2.92^{f}	3.02^{D}
Mean	3.80^{A}	3.30^{B}	3.14^{C}	2.94^{D}		2.94^{A}	2.84^{B}	2.76^{C}	2.62^{D}		3.99^{A}	3.75^{B}	3.62^{C}	2.96^{D}	
LSD ($p\leq0.05$)	Pb	0.069				Pb	0.066				Pb	0.098			
	Cd	0.069				Cd	0.066				Cd	0.098			
	Pb×Cd	0.139				Pb×Cd	0.132				Pb×Cd	0.196			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Thus, Pb, Cd and their combination concentrations negatively affect the N content of both *Salix* and *Toona* plant parts. For both the species, similar trend was observed that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd ≥ Pb. Overall, among different plant parts, the higher N content was observed in leaves than stem and roots in an order as leaves > stem ≥ roots.

b. Phosphorous (P)

The data presented in Table 4.21 reveals the negative effect of different concentrations of Pb, Cd and their combinations on phosphorous (P) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, significant decrease in P content of both *Salix* and *Toona* was recorded.

In *Salix*, significant decrease in phosphorous (P) content of all plant parts was observed in response to Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for P was maximum in control i.e. Pb_0 (0.489% in roots, 0.396% in stem and 0.708% in leaves) which decreased significantly with increase in concentration from Pb_{100} (0.457% in roots, 0.361% in stem and 0.629% in leaves) to Pb_{300} (0.318% in roots, 0.257% in stem and 0.418% in leaves). Similar trend was observed for Cd concentrations where maximum mean P content was observed in control (Cd_0, 0.586% in roots, 0.489% in stem and 0.777% in leaves) which decreased significantly with increase in concentration from Cd_5 (0.548% in roots, 0.402% in stem and 0.698% in leaves) to Cd_{25} (0.164% in roots, 0.183% in stem and 0.256% in leaves). Among combinations, maximum P content was observed in control Pb_0Cd_0 (0.673% in root, 0.566% in stem, 0.954% in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (0.085% in root, 0.144% in stem and 0.132% in leaves).

In case of *Toona*, the significant decrease in phosphorous content of all plant parts was observed with Pb, Cd and their combination concentrations (Table 4.21). Among Pb concentrations, the mean value for P content was maximum in control plants i.e. Pb_0 (0.506% in roots, 0.453% in stem and 0.693% in leaves) which decreased significantly with increase in Pb concentration, thus minimum P content was recorded with highest Pb concentration i.e. Pb_{300} (0.412% in roots, 0.310% in stem and 0.433% in leaves). Similarly, among Cd concentrations, maximum mean P content was observed in control (Cd_0, 0.573% in roots, 0.481% in stem and 0.731% in leaves) which decreased significantly with increase in concentration from Cd_5 (0.531% in roots, 0.430% in stem and 0.632% in leaves) to Cd_{25} (0.260% in roots, 0.274% in stem and 0.340% in leaves. Among combinations, maximum P content was observed in control Pb_0Cd_0 (0.609% in root, 0.563% in stem, 0.899% in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (0.196% in root, 0.214% in stem, 0.228% in leaves).

Table 4.21: Effect of heavy metals on phosphorous content in different plant parts of *Salix alba* and *Toona ciliata*

Phosphorous (%)															
Salix alba															
	Root					Stem					Leaves				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.673^{a}	0.621^{b}	0.583^{cd}	0.467^{e}	0.586^{A}	0.566^{a}	0.532^{b}	0.453^{d}	0.405^{f}	0.489^{A}	0.954^{a}	0.894^{b}	0.684^{d}	0.575^{fg}	0.777^{A}
Cd_5	0.603^{bc}	0.593^{c}	0.563^{d}	0.432^{f}	0.548^{B}	0.503^{c}	0.431^{e}	0.389^{f}	0.285^{h}	0.402^{B}	0.863^{b}	0.732^{c}	0.653^{d}	0.545^{g}	0.698^{B}
Cd_{15}	0.464^{e}	0.421^{f}	0.289^{g}	0.289^{g}	0.392^{C}	0.309^{g}	0.284^{h}	0.252^{i}	0.195^{i}	0.260^{C}	0.638^{de}	0.603^{ef}	0.453^{h}	0.421^{h}	0.529^{C}
Cd_{25}	0.216^{h}	0.192^{i}	0.164^{j}	0.085^{k}	0.164^{D}	0.204^{i}	0.195^{i}	0.189^{i}	0.144^{j}	0.183^{D}	0.375^{h}	0.287^{i}	0.231^{j}	0.132^{k}	0.256^{D}
Mean	0.489^{A}	0.457^{B}	0.426^{C}	0.318^{D}		0.396^{A}	0.361^{B}	0.321^{C}	0.257^{D}		0.708^{A}	0.629^{B}	0.505^{C}	0.418^{D}	
LSD ($p\leq0.05$)	Pb	0.008				Pb	0.007				Pb	0.016			
	Cd	0.008				Cd	0.007				Cd	0.016			
	Pb×Cd	0.016				Pb×Cd	0.014				Pb×Cd	0.032			
Toona ciliata															
	Root					Stem					Leaves				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.609^{a}	0.564^{b}	0.562^{b}	0.557^{b}	0.573^{A}	0.563^{a}	0.483^{b}	0.482^{b}	0.394^{e}	0.481^{A}	0.899^{a}	0.762^{c}	0.675^{d}	0.587^{f}	0.731^{A}
Cd_5	0.566^{b}	0.558^{b}	0.523^{c}	0.473^{d}	0.531^{B}	0.472^{b}	0.468^{bc}	0.443^{cd}	0.336^{f}	0.430^{B}	0.795^{b}	0.625^{e}	0.584^{f}	0.523^{g}	0.632^{B}
Cd_{15}	0.521^{c}	0.493^{cd}	0.475^{d}	0.423^{e}	0.491^{C}	0.429^{d}	0.392^{e}	0.353^{f}	0.295^{g}	0.367^{C}	0.583^{f}	0.498^{g}	0.424^{h}	0.395^{i}	0.475^{C}
Cd_{25}	0.329^{f}	0.293^{g}	0.221^{h}	0.196^{h}	0.260^{D}	0.349^{f}	0.284^{g}	0.248^{h}	0.214^{i}	0.274^{D}	0.495^{g}	0.341^{j}	0.294^{k}	0.228^{l}	0.340^{D}
Mean	0.506^{A}	0.477^{B}	0.445^{C}	0.412^{D}		0.453^{A}	0.407^{B}	0.382^{C}	0.310^{D}		0.693^{A}	0.557^{B}	0.494^{C}	0.433^{D}	
LSD ($p\leq0.05$)	Pb	0.010				Pb	0.008				Pb	0.010			
	Cd	0.010				Cd	0.008				Cd	0.010			
	Pb×Cd	0.021				Pb×Cd	0.017				Pb×Cd	0.020			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Hence, the P content of both *Salix* and *Toona* plant parts (root, stem and leaves) was negatively affected in response to Pb, Cd and their combination concentrations. However, similar trend was observed for both *Salix* and *Toona* that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd ≥ Pb. Overall, among different plant parts, the higher P content was observed in leaves than roots and stem in an order as leaves > roots > stem.

c. Potassium (K)

The data pertaining to Table 4.22 depicts the effect of different concentrations of Pb, Cd and their combinations on potassium (K) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, significant decrease in K content of both *Salix* and *Toona* was recorded.

In *Salix*, significant decrease in potassium content of all plant parts was observed in response to Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for K was maximum in control i.e. Pb_0 (4.24% in roots, 3.37% in stem and 2.76% in leaves) which decreased significantly with increase in Pb concentration from Pb_{100} (3.89% in roots, 3.32% in stem and 2.49% in leaves) to Pb_{300} (2.43% in roots, 2.59% in stem and 1.74% in leaves). Similarly, the significant decrease in K content was also observed with increasing Cd concentrations, thus maximum mean K content was in control (Cd_0, 4.70 in roots, 4.14% in stem and 3.0% in leaves) and minimum K content was recorded with highest Cd concentration i.e. Cd_{25} (1.98% in roots, 2.10% in stem and 1.77% in leaves). Among combinations, maximum K content was observed in control Pb_0Cd_0 (5.69% in root, 4.86% in stem, 3.88% in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (1.73% in root, 1.89% in stem, 1.50% in leaves).

In *Toona*, the significant decrease in potassium content of all plant parts was also observed with increasing concentrations of Pb, Cd and their combinations (Table 4.22). Among Pb concentrations, the mean value for K content was maximum in control plants i.e. Pb_0 (4.85% in roots, 3.84% in stem and 2.95% in leaves) which decreased significantly with increase in Pb concentration, thus minimum K content was recorded with highest Pb concentration i.e. Pb_{300} (3.32% in roots, 2.75% in stem and 1.88% in leaves). Similarly, among Cd concentrations, maximum mean K content was observed in control (Cd_0, 5.09% in roots, 4.22% in stem and 3.15% in leaves) which decreased significantly with increase in concentration from Cd_5 (4.68% in roots, 3.42% in stem and 2.59% in leaves) to Cd_{25} (2.69% in roots, 2.21% in stem and 1.89% in leaves). Among combinations, maximum K content was observed in control Pb_0Cd_0 (6.29% in root, 4.89% in stem, 4.03% in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (2.48% in root, 1.97% in stem, 1.63% in leaves).

Table 4.22: Effect of heavy metals on potassium content in different plant parts of *Salix alba* and *Toona ciliata*

Potassium (%)															
Salix alba															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	5.69^{a}	5.35^{b}	4.87^{d}	2.90^{g}	4.70^{A}	4.86^{a}	4.32^{b}	3.84^{c}	3.55^{d}	4.14^{A}	3.88^{a}	3.53^{b}	2.60^{d}	1.98^{f}	3.00^{A}
Cd_5	5.08^{c}	4.93^{cd}	3.87^{e}	2.57^{h}	4.12^{B}	4.09^{bc}	3.54^{d}	3.08^{e}	2.58^{f}	3.32^{B}	2.90^{c}	2.58^{d}	2.29^{e}	1.98^{f}	2.44^{B}
Cd_{15}	3.89^{e}	3.29^{f}	2.79^{g}	2.53^{h}	3.13^{C}	3.82^{c}	3.21^{e}	2.57^{f}	2.33^{fg}	2.98^{C}	2.24^{e}	1.99^{f}	1.86^{g}	1.49^{i}	1.89^{C}
Cd_{25}	2.28^{i}	1.98^{j}	1.93^{j}	1.73^{k}	1.98^{D}	2.32^{fg}	2.20^{gh}	1.99^{hi}	1.89^{i}	2.10^{D}	2.04^{f}	1.86^{g}	1.70^{h}	1.50^{i}	1.77^{D}
Mean	4.24^{A}	3.89^{B}	3.37^{C}	2.43^{D}		3.77^{A}	3.32^{B}	2.87^{C}	2.59^{D}		2.76^{A}	2.49^{B}	2.11^{C}	1.74^{D}	
LSD ($p\leq0.05$)	Pb	0.062				Pb	0.087				Pb	0.037			
	Cd	0.062				Cd	0.087				Cd	0.037			
	Pb×Cd	0.124				Pb×Cd	0.175				Pb×Cd	0.075			
Toona ciliata															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	6.29^{a}	5.37^{c}	4.89^{d}	3.82^{fg}	5.09^{A}	4.89^{a}	4.21^{b}	4.07^{bc}	3.71^{d}	4.22^{A}	4.03^{a}	3.73^{b}	2.72^{d}	2.13^{fg}	3.15^{A}
Cd_5	5.68^{b}	5.19^{c}	4.25^{e}	3.61^{g}	4.68^{B}	4.04^{bc}	3.55^{de}	3.33^{f}	2.77^{g}	3.42^{B}	3.09^{c}	2.74^{e}	2.36^{e}	2.17^{f}	2.59^{B}
Cd_{15}	4.50^{e}	4.37^{e}	3.74^{f}	3.38^{h}	4.00^{C}	3.95^{c}	3.47^{ef}	2.83^{g}	2.54^{h}	3.20^{C}	2.48^{e}	2.12^{fg}	2.00^{gh}	1.60^{j}	2.05^{C}
Cd_{25}	2.94^{i}	2.79^{i}	2.54^{j}	2.48^{j}	2.69^{D}	2.47^{hi}	2.28^{ij}	2.15^{jk}	1.97^{k}	2.21^{D}	2.19^{f}	1.93^{hi}	1.81^{i}	1.63^{j}	1.89^{D}
Mean	4.85^{A}	4.43^{B}	3.85^{C}	3.32^{D}		3.84^{A}	3.38^{B}	3.09^{C}	2.75^{D}		2.95^{A}	2.63^{B}	2.22^{C}	1.88^{D}	
LSD ($p\leq0.05$)	Pb	0.074				Pb	0.069				Pb	0.056			
	Cd	0.074				Cd	0.069				Cd	0.056			
	Pb×Cd	0.149				Pb×Cd	0.138				Pb×Cd	0.112			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Thus, Pb, Cd and their combination concentrations negatively affect the K content of both *Salix* and *Toona* plant parts. For both the species, similar trend was observed that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd > Pb. Overall, among different plant parts of *Salix* and *Toona*, the higher K content was observed in roots than stem and leaves in an order as roots > stem> leaves > stem.

d. Calcium (Ca)

The data presented in Table 4.23 reveals the negative effect of different concentrations of Pb, Cd and their combinations on calcium (Ca) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, significant decrease in Ca content in both *Salix* and *Toona* was recorded.

In *Salix*, significant decrease in calcium (Ca) content of all plant parts was observed in response to Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for Ca was maximum in control i.e. Pb_0 (2059 mg/kg in roots, 2945 mg/kg in stem and 3207 mg/kg in leaves) which decreased significantly with increase in concentration from Pb_{100} (1922 mg/kg in roots, 2575 mg/kg in stem and 2632 mg/kg in leaves) to Pb_{300} (1552 mg/kg in roots, 1907 mg/kg in stem and 1689 mg/kg in leaves). Similar trend was observed for Cd concentrations where maximum mean Ca content was observed in control (Cd_0, 2324 mg/kg in roots, 3247 mg/kg in stem and 3337 mg/kg in leaves) which showed drastic decrease with increase in concentration from Cd_5 (2080 mg/kg in roots, 2755 mg/kg in stem and 2981 mg/kg in leaves) to Cd_{25} (957 mg/kg in roots, 1303 mg/kg in stem and 1381 mg/kg in leaves). Among combinations, maximum Ca content was observed in control Pb_0Cd_0 (2657 mg/kg in root, 3633 mg/kg in stem, 4356 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (683 mg/kg in root, 674 mg/kg in stem, 987 mg/kg in leaves).

In case of *Toona*, the significant decrease in calcium content of all plant parts was observed with Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for Ca was maximum in control plants i.e. Pb_0 (2582 mg/kg in roots, 3102 mg/kg in stem and 3577 mg/kg in leaves) which decreased significantly with increase in Pb concentration, thus minimum Ca content was recorded with highest Pb concentration i.e. Pb_{300} (1801 mg/kg in roots, 2192 mg/kg in stem and 2273 mg/kg in leaves). Similarly, among Cd concentrations, maximum mean Ca content was observed in control (Cd_0, 2801 mg/kg in roots, 3412 mg/kg in stem and 3632 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (2306 mg/kg in roots, 3020 mg/kg in stem and 3170 mg/kg in leaves) to Cd_{25} (1457 mg/kg in roots, 1540 mg/kg in stem and 2058 mg/kg in leaves).

Table 4.23: Effect of heavy metals on calcium content in different plant parts of *Salix alba* and *Toona ciliata*

Calcium (mg/kg)															
Salix alba															
	Root					**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	2657^{a}	2467^{b}	2214^{cd}	1957^{ef}	2324^{A}	3633^{a}	3432^{b}	3093^{c}	2830^{d}	3247^{A}	4356^{a}	3642^{c}	3210^{d}	2140^{f}	3337^{A}
Cd_5	2267^{c}	2145^{d}	1983^{ef}	1923^{f}	2080^{B}	3278^{b}	2956^{cd}	2540^{e}	2245^{f}	2755^{B}	3892^{b}	3213^{d}	2820^{e}	1998^{f}	2981^{B}
Cd_{15}	2045^{e}	1983^{g}	1843^{h}	1645^{hi}	1879^{C}	2921^{cd}	2543^{g}	1986^{gh}	1877^{gh}	2332^{C}	2693^{e}	2242^{g}	1932^{gh}	1632^{ghi}	2125^{C}
Cd_{25}	1268^{hi}	1094^{i}	783.2^{j}	683.4^{k}	957.4^{D}	1946^{ghi}	1367^{hi}	1225^{i}	674.2^{j}	1303^{D}	1885^{ghi}	1432^{hij}	1221^{ij}	987^{j}	1381^{D}
Mean	2059^{A}	1922^{B}	1706^{C}	1552^{D}		2945^{A}	2575^{B}	2211^{C}	1907^{D}		3207^{A}	2632^{B}	2296^{C}	1689^{D}	
LSD ($p\leq0.05$)	Pb	34.4				Pb	60.7				Pb	67.8			
	Cd	34.4				Cd	60.7				Cd	67.8			
	Pb×Cd	68.7				Pb×Cd	121				Pb×Cd	135			
Toona ciliata															
	Root					**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	3692^{a}	2985^{b}	2419^{d}	2108^{f}	2801^{A}	3777^{a}	3572^{b}	3274^{c}	3025^{d}	3412^{A}	4438^{a}	3947^{b}	3279^{c}	2864^{e}	3632^{A}
Cd_5	2694^{c}	2287^{de}	2194^{ef}	2048^{fg}	2306^{B}	3458^{b}	3280^{c}	2774^{e}	2569^{f}	3020^{B}	3974^{b}	3184^{cd}	2982^{de}	2541^{f}	3170^{B}
Cd_{15}	2295^{de}	2058^{fg}	1946^{gh}	1844^{h}	2036^{C}	3071^{d}	2684^{ef}	2103^{g}	2080^{g}	2485^{C}	3297^{c}	3204^{cd}	2833^{e}	2094^{g}	2857^{C}
Cd_{25}	1647^{i}	1492^{j}	1484^{j}	1205^{k}	1457^{D}	2103^{g}	1560^{h}	1402^{i}	1094^{j}	1540^{D}	2597^{f}	2185^{g}	1857^{h}	1592^{i}	2058^{D}
Mean	2582^{A}	2206^{B}	2011^{C}	1801^{D}		3102^{A}	2774^{B}	2388^{C}	2192^{D}		3577^{A}	3130^{B}	2738^{C}	2273^{D}	
LSD ($p\leq0.05$)	Pb	49.7				Pb	50.6				Pb	73.4			
	Cd	49.7				Cd	50.6				Cd	73.4			
	Pb×Cd	99.5				Pb×Cd	101				Pb×Cd	146			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Among combinations, maximum Ca content in *Toona* was observed in control Pb_0Cd_0 (3692 mg/kg in root, 3777 mg/kg in stem, 4438 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (1205 mg/kg in root, 1094 mg/kg in stem, 1592 mg/kg in leaves).

Hence, the Ca content of both *Salix* and *Toona* plant parts (root, stem and leaves) was negatively affected in response to Pb, Cd and their combination concentrations. However, similar trend was observed for both *Salix* and *Toona* that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd > Pb. Overall, among different plant parts, the higher Ca content was observed in leaves than stem and leaves in an order as leaves > stem > roots.

e. Magnesium (Mg)

The data pertaining to Table 4.24 reveals the effect of different concentrations of Pb, Cd and their combinations on magnesium (Mg) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, significant decrease in Mg content of both *Salix* and *Toona* was recorded.

In *Salix*, significant decrease in magnesium content of all plant parts was observed in response to Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for Mg was maximum in control i.e. Pb_0 (1325 mg/kg in roots, 1637 mg/kg in stem and 1636 mg/kg in leaves) which decreased significantly with increase in concentration from Pb_{100} (1194 mg/kg in roots, 1432 mg/kg in stem and 1636 mg/kg in leaves) to Pb_{300} (780.3 mg/kg in roots, 948.3 mg/kg in stem and 1047 mg/kg in leaves). Similar trend was observed for Cd concentrations where maximum mean Mg content was observed in control (Cd_0, 1405 mg/kg in roots, 1784 mg/kg in stem and 1972 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (1253 mg/kg in roots, 1639 mg/kg in stem and 1357 mg/kg in leaves) to Cd_{25} (619 mg/kg in roots, 758 mg/kg in stem and 834 mg/kg in leaves). Among combinations, maximum Mg content was observed in control Pb_0Cd_0 (1855 mg/kg in root, 2263 mg/kg in stem, 2820 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (406 mg/kg in root, 516 mg/kg in stem, 700 mg/kg in leaves).

In case of *Toona*, the significant decrease in magnesium content of all plant parts was observed with Pb, Cd and their combination concentrations (Table 4.24). Among Pb concentrations, the mean value for Mg content was maximum in control plants i.e. Pb_0 (1654 mg/kg in roots, 1799 mg/kg in stem and 2212 mg/kg in leaves) which decreased significantly with increase in Pb concentration, thus minimum Mg content was recorded with highest Pb concentration i.e. Pb_{300} (1338 mg/kg in roots, 1538 mg/kg in stem and 1556 mg/kg in leaves).

Table 4.24: **Effect of heavy metals on magnesium content in different plant parts of *Salix alba* and *Toona ciliata***

Magnesium (mg/kg)															
Salix alba															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	1855^{a}	1573^{b}	1207^{d}	984^{f}	1405^{A}	2263^{a}	1968^{c}	1698^{e}	1205^{h}	1784^{A}	2820^{a}	2168^{b}	1656^{c}	1243^{f}	1972^{A}
Cd_5	1504^{bc}	1489^{c}	1103^{e}	916^{fg}	1253^{B}	2089^{b}	1876^{d}	1508^{f}	1084^{i}	1639^{B}	1486^{d}	1378^{e}	1308^{ef}	1256^{f}	1357^{B}
Cd_{15}	1134^{e}	958^{f}	855^{gh}	815^{hi}	940.5^{C}	1289^{g}	1045^{ij}	1022^{ij}	988.2^{j}	1086^{C}	1298^{ef}	1105^{g}	1058^{gh}	990^{hi}	1113^{C}
Cd_{25}	806.2^{hi}	755.1^{i}	509.2^{j}	406.5^{k}	619.9^{D}	906.2^{k}	840.4^{l}	769.1^{m}	516.4^{n}	758.8^{D}	940.4^{ij}	870.2^{jk}	825.2^{k}	700^{l}	833.8^{D}
Mean	1325^{A}	1194^{B}	918.5^{C}	780.3^{D}		1637^{A}	1432^{B}	1249^{C}	948.3^{D}		1636^{A}	1380^{B}	1212^{C}	1047^{D}	
LSD ($p\leq0.05$)	Pb	24.5				Pb	21.2				Pb	29.2			
	Cd	24.5				Cd	21.2				Cd	29.2			
	Pb×Cd	49.0				Pb×Cd	42.4				Pb×Cd	58.4			
Toona ciliata															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	1966^{a}	1832^{b}	1693^{cde}	1566^{fg}	1764^{A}	2089^{a}	1937^{b}	1815^{c}	1638^{de}	1870^{A}	2756^{a}	2389^{b}	2196^{cd}	1855^{f}	2299^{A}
Cd_5	1783^{bc}	1736^{bcd}	1662^{def}	1628^{ef}	1702^{B}	1973^{b}	1824^{c}	1805^{c}	1693^{d}	1824^{B}	2347^{b}	2274^{bc}	2082^{de}	1906^{f}	2152^{B}
Cd_{15}	1593^{ef}	1493^{g}	1364^{h}	1104^{jk}	1389^{C}	1708^{d}	1697^{d}	1628^{de}	1535^{e}	1642^{C}	2073^{de}	1958^{ef}	1599^{gh}	1477^{h}	1777^{C}
Cd_{25}	1273^{i}	1158^{j}	1095^{jk}	1053^{k}	1145^{D}	1426^{f}	1388^{fg}	1304^{g}	1285^{g}	1351^{D}	1673^{g}	1593^{gh}	1254^{i}	984.4^{j}	1376^{D}
Mean	1654^{A}	1555^{B}	1454^{C}	1338^{D}		1799^{A}	1712^{B}	1638^{C}	1538^{D}		2212^{A}	2054^{B}	1783^{C}	1556^{D}	
LSD ($p\leq0.05$)	Pb	31.8				Pb	34.0				Pb	41.3			
	Cd	31.8				Cd	34.0				Cd	41.3			
	Pb×Cd	63.7				Pb×Cd	68.1				Pb×Cd	82.5			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Similarly, among Cd concentrations, maximum mean Mg content was observed in control (Cd_0, 1764 mg/kg in roots, 1870 mg/kg in stem, 2299 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (1702 mg/kg in roots, 1824 mg/kg in stem and 2154 mg/kg in leaves) to Cd_{25} (1145 mg/kg in roots, 1351 mg/kg in stem and 1376 mg/kg in leaves). Among combinations, maximum Mg content was observed in control Pb_0Cd_0 (1966 mg/kg in root, 2089 mg/kg in stem, 2756 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (1053 mg/kg in root, 1285 mg/kg in stem, 984 mg/kg in leaves).

Hence, the Mg content of both *Salix* and *Toona* plant parts (root, stem and leaves) was negatively affected in response to Pb, Cd and their combination concentrations. However, similar trend was observed for both *Salix* and *Toona* that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd > Pb. Overall, among different plant parts, the higher Mg content was observed in roots than stem and leaves in an order as roots > stem > leaves.

f. Sulphur (S)

The data presented in Table 4.25 exhibits the effect of different concentrations of Pb, Cd and their combinations on sulphur (S) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, significant decrease in S content of both *Salix* and *Toona* was recorded.

In *Salix*, significant decrease in sulphur content of all plant parts was observed in response to Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for S was maximum in control i.e. Pb_0 (2076 mg/kg in roots, 1049 mg/kg in stem and 1895 mg/kg in leaves) which decreased significantly with increase in Pb concentration and recorded to minimum with highest Pb concentration i.e. Pb_{300} (1399 mg/kg in roots, 944 mg/kg in stem and 1333 mg/kg in leaves). Similarly, the significant decrease in S content was also observed with increasing Cd concentrations, thus maximum mean S content was in control (Cd_0, 2076 mg/kg in roots, 1049 mg/kg in stem and 1895 mg/kg in leaves) and minimum S content was recorded with highest Cd concentration i.e. Cd_{25} (1063 mg/kg in roots, 930 mg/kg in stem and 1037 mg/kg in leaves). Among combinations, maximum S content was observed in control Pb_0Cd_0 (2903 mg/kg in root, 1160 mg/kg in stem, 2341 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (980 mg/kg in root, 905 mg/kg in stem, 722 mg/kg in leaves).

Table 4.25: Effect of heavy metals on sulphur content in different plant parts of *Salix alba* and *Toona ciliata*

Sulphur (mg/kg)															
Salix alba															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	2903^{a}	2246^{b}	1905^{de}	1589^{fg}	2161^{A}	1160^{a}	1066^{b}	986^{def}	944^{efg}	1039^{A}	2341^{a}	2194^{b}	1971^{c}	1714^{de}	2055^{A}
Cd_5	2506^{ab}	2045^{c}	1983^{cd}	1832^{e}	2092^{B}	1089^{efg}	1054^{bc}	1021^{bcd}	995^{cdef}	1040^{B}	2083^{b}	1965^{c}	1683^{de}	1600^{ef}	1833^{B}
Cd_{15}	1693^{f}	1490^{g}	1322^{h}	1195^{i}	1425^{C}	998.2^{cde}	992^{cdef}	974^{def}	932^{efg}	974^{C}	1782^{d}	1544^{f}	1305^{g}	1294^{g}	1481^{C}
Cd_{25}	1200^{i}	1074^{i}	997.2^{j}	980.1^{j}	1063^{D}	950.1^{efg}	939^{efg}	925^{fg}	905^{g}	930^{D}	1373^{g}	1067^{g}	985^{h}	722^{i}	1037^{D}
Mean	2076^{A}	1714^{B}	1552^{C}	1399^{D}		1049^{A}	1013^{A}	977^{B}	944^{C}		1895^{A}	1693^{B}	1486^{C}	1333^{D}	
LSD ($p\leq0.05$)	Pb 35.0					Pb 20.8					Pb 34.1				
	Cd 35.0					Cd 20.8					Cd 34.1				
	Pb×Cd 70.0					Pb×Cd 41.6					Pb×Cd 68.2				
Toona ciliata															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	2583^{a}	2293^{b}	1982^{de}	1735^{gh}	2148^{A}	1394^{a}	1308^{b}	1213^{cde}	1154^{de}	1267^{A}	2489^{a}	2200^{b}	1968^{cd}	1908^{e}	2141^{A}
Cd_5	2192^{bc}	2083^{cd}	1981^{de}	1924^{ef}	2045^{B}	1305^{b}	1307^{b}	1242^{bcd}	1223^{bcde}	1269^{A}	2071^{c}	1849^{de}	1874^{e}	1891^{e}	1921^{B}
Cd_{15}	1893^{ef}	1791^{fg}	1632^{hi}	1608^{hij}	1731^{C}	1221^{bcde}	1264^{bc}	1204^{cde}	1162^{de}	1213^{B}	1838^{e}	1670^{f}	1685^{fg}	1555^{gh}	1687^{C}
Cd_{25}	1569^{ij}	1492^{jk}	1417^{k}	1248^{l}	1432^{D}	1205^{cde}	1167^{de}	1197^{cde}	1139^{e}	1177^{C}	1703^{f}	1586^{fg}	1430^{hi}	1358^{i}	1519^{D}
Mean	2059^{A}	1915^{B}	1753^{C}	1629^{D}		1281^{A}	1261^{A}	1214^{B}	1169^{C}		2025^{A}	1826^{B}	1739^{C}	1678^{D}	
LSD ($p\leq0.05$)	Pb 44.3					Pb 25.9					Pb 40.3				
	Cd 44.3					Cd 25.9					Cd 40.3				
	Pb×Cd 88.6					Pb×Cd 51.8					Pb×Cd 80.6				

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

In *Toona*, the significant decrease in sulphur content of all plant parts was also observed with increasing concentrations of Pb, Cd and their combinations (Table 4.25). Among Pb concentrations, the mean value for S content was maximum in control plants i.e. Pb_0 (2059 mg/kg in roots, 1281 mg/kg in stem and 2025 mg/kg in leaves) which decreased significantly with increase in Pb concentration, thus minimum S content was recorded with highest Pb concentration i.e. Pb_{300} (1629 mg/kg in roots, 1169 mg/kg in stem and 1678 mg/kg in leaves). Similarly, among Cd concentrations, maximum mean S content was observed in control (Cd_0, 2148 mg/kg in roots, 1267 mg/kg in stem and 2141 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (2045 mg/kg in roots, 1269 mg/kg in stem and 1921 mg/kg in leaves) to Cd_{25} (1432 mg/kg in roots, 1177 mg/kg in stem and 1519 mg/kg in leaves). Among combinations, maximum S content was observed in control Pb_0Cd_0 (2583 mg/kg in root, 1394 mg/kg in stem, 2489 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (1248 mg/kg in root, 1139 mg/kg in stem, 1358 mg/kg in leaves).

Thus, Pb, Cd and their combination concentrations negatively affect the S content of both *Salix* and *Toona* plant parts. For both the species, similar trend was observed that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd > Pb. Overall, among different plant parts of *Salix* and *Toona,* the higher S content was observed in roots than stem and leaves in an order as roots > leaves > stem.

g. Manganese (Mn)

The data presented in Table 4.26 reveals the negative effect of different concentrations of Pb, Cd and their combinations on manganese (Mn) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, significant decrease in Ca content in both *Salix* and *Toona* was recorded.

In *Salix,* significant decrease in manganese (Mn) content of all plant parts was observed in response to Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for Mn was maximum in control i.e. Pb_0 (350 mg/kg in roots, 109 mg/kg in stem and 266 mg/kg in leaves) which decreased significantly with increase in concentration from Pb_{100} (282 mg/kg in roots, 82.2 mg/kg in stem and 229 mg/kg in leaves) to Pb_{300} (155 mg/kg in roots, 40.4 mg/kg in stem and 98.8 mg/kg in leaves). Similar trend was observed for Cd concentrations where maximum mean Mn content was observed in control (Cd_0, 390 mg/kg in roots, 99.4 mg/kg in stem and 280 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (362 mg/kg in roots, 79.1 mg/kg in stem and 240 mg/kg in leaves) to Cd_{25} (65.5 mg/kg in roots, 45.1 mg/kg in stem and 62.4 mg/kg in leaves).

Table 4.26: Effect of heavy metals on manganese content in different plant parts of *Salix alba* and *Toona ciliata*

Manganese (mg/kg)															
Salix alba															
	Root					**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	605^{a}	478^{b}	326^{d}	149^{h}	390^{A}	174^{a}	105^{c}	69.0^{g}	49.0^{ij}	99.4^{A}	469^{a}	396^{b}	158^{f}	96.4^{g}	280^{A}
Cd_5	489^{b}	356^{c}	308^{de}	295^{e}	362^{B}	114^{b}	98.4^{d}	58.6^{h}	45.3^{jk}	79.1^{B}	306^{c}	286^{d}	205^{e}	164^{f}	240^{B}
Cd_{15}	208^{f}	205^{fg}	184^{g}	149^{h}	187^{C}	86.4^{i}	75.3^{f}	50.9^{j}	39.5^{l}	63.0^{C}	195^{e}	159^{f}	108^{g}	96.4^{g}	140^{C}
Cd_{25}	98.0^{i}	87.0^{i}	50.0^{j}	26.9^{k}	65.5^{D}	60.5^{h}	49.6^{ij}	42.3^{kl}	27.8^{m}	45.1^{D}	93.0^{g}	74.3^{h}	43.9^{i}	38.6^{i}	62.4^{D}
Mean	350^{A}	282^{B}	217^{C}	155^{D}		109^{A}	82.2^{B}	55.2^{C}	40.4^{D}		266^{A}	229^{B}	129^{C}	98.8^{D}	
LSD ($p\leq0.05$)	Pb	7.90				Pb	1.53				Pb	5.67			
	Cd	7.90				Cd	1.53				Cd	5.67			
	Pb×Cd	15.8				Pb×Cd	3.07				Pb×Cd	11.3			
Toona ciliata															
	Root					**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	629^{a}	519^{b}	393^{d}	308^{f}	462^{A}	295^{a}	193^{c}	142^{ef}	94.2^{h}	181^{A}	398^{a}	294^{b}	173^{e}	89.4^{k}	239^{A}
Cd_5	486^{c}	408^{d}	372^{e}	316^{f}	396^{B}	283^{b}	186^{c}	138^{f}	121^{g}	182^{A}	285^{c}	257^{d}	151^{g}	83.9^{k}	194^{B}
Cd_{15}	320^{f}	306^{f}	264^{g}	252^{g}	286^{C}	194^{c}	184^{c}	165^{d}	140^{f}	170^{B}	173^{e}	158^{f}	115^{i}	68.3^{m}	129^{C}
Cd_{25}	189^{h}	163^{i}	132^{j}	92.3^{k}	144^{D}	150^{e}	116^{g}	90.3^{h}	86.3^{h}	111^{C}	133^{h}	96.9^{j}	75.3^{l}	60.9^{n}	91^{D}
Mean	406^{A}	349^{B}	290^{C}	242^{D}		230^{A}	170^{B}	134^{C}	110^{D}		247^{A}	201^{B}	129^{C}	76.0^{D}	
LSD ($p\leq0.05$)	Pb	7.09				Pb	3.54				Pb	2.32			
	Cd	7.09				Cd	3.54				Cd	2.32			
	Pb×Cd	14.1				Pb×Cd	7.09				Pb×Cd	4.64			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Among combinations, maximum Mn content was observed in control Pb_0Cd_0 (605 mg/kg in root, 174 mg/kg in stem, 469 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (26.9 mg/kg in root, 27.8 mg/kg in stem, 38.6 mg/kg in leaves).

In case of *Toona*, the significant decrease in manganese content in all plant parts was observed with Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for Mn was maximum in control plants i.e. Pb_0 (406 mg/kg in roots, 230 mg/kg in stem and 247 mg/kg in leaves) which decreased significantly with increase in Pb concentration, thus minimum Mn content was recorded with highest Pb concentration i.e. Pb_{300} (242 mg/kg in roots, 110 mg/kg in stem and 76 mg/kg in leaves). Similarly, among Cd concentrations, maximum mean Mn content was observed in control (Cd_0, 462 mg/kg in roots, 181 mg/kg in stem and 239 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (396 mg/kg in roots, 182 mg/kg in stem and 194 mg/kg in leaves) to Cd_{25} (144 mg/kg in roots, 111 mg/kg in stem and 91 mg/kg in leaves). Among combinations, maximum Mn content was observed in control Pb_0Cd_0 (629 mg/kg in root, 295 mg/kg in stem, 398 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (92.3 mg/kg in root, 86.3 mg/kg in stem, 60.9 mg/kg in leaves).

Hence, the Mn content of both *Salix* and *Toona* plant parts (root, stem and leaves) was negatively affected in response to Pb, Cd and their combination concentrations. Similar trend was observed for both *Salix* and *Toona* that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd > Pb. Overall, among different plant parts, the higher Mn content was observed in roots than leaves and stem in an order as roots > leaves > stem.

h. Copper (Cu)

The data pertaining to Table 4.27 reveals the effect of different concentrations of Pb, Cd and their combinations on copper (Cu) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, significant decrease in Cu content in both *Salix* and *Toona* was recorded.

In *Salix*, significant decrease in copper content of all plant parts was observed in response to Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for Cu was maximum in control i.e. Pb_0 (61.6 mg/kg in roots, 48.5 mg/kg in stem and 44.7 mg/kg in leaves) which decreased significantly with increase in concentration from Pb_{100} (52.1 mg/kg in roots, 40.8 mg/kg in stem and 41.6 mg/kg in leaves) to Pb_{300} (33.3 mg/kg in roots, 24.9 mg/kg in stem and 22.0 mg/kg in leaves).

Table 4.27: **Effect of heavy metals on copper content in different plant parts of *Salix alba* and *Toona ciliata***

Copper (mg/kg)															
Salix alba															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	107^{a}	82.4^{b}	69.6^{d}	35.4^{gh}	73.6^{A}	83.6^{a}	64.2^{b}	52.7^{d}	38.6^{e}	59.8^{A}	74.6^{a}	68.5^{b}	49.6^{e}	33.5^{g}	56.6^{A}
Cd_5	76.3^{c}	68.3^{d}	63.9^{e}	52.5^{f}	65.3^{B}	64.3^{b}	58.4^{c}	50.6^{d}	32.4^{fg}	51.4^{B}	64.5^{c}	60.4^{d}	43.6^{f}	30.6^{gh}	49.8^{B}
Cd_{15}	38.4^{g}	35.3^{gh}	32.4^{h}	28.9^{i}	33.8^{C}	33.7^{f}	30.4^{g}	25.4^{h}	21.4^{i}	27.7^{C}	30.2^{h}	28.3^{h}	22.3^{i}	18.4^{j}	24.8^{C}
Cd_{25}	24.6^{j}	22.3^{j}	18.8^{k}	16.4^{k}	20.5^{D}	12.3^{j}	10.3^{j}	10.3^{j}	7.18^{k}	10.0^{D}	9.32^{k}	9.23k	6.42^{kl}	5.33^{l}	7.58^{D}
Mean	61.6^{A}	52.1^{B}	46.2^{C}	33.3^{D}		48.5^{A}	40.8^{B}	34.8^{C}	24.9^{D}		44.7^{A}	41.6^{B}	30.5^{C}	22.0^{D}	
LSD ($p\leq0.05$)	Pb	1.06				Pb	0.771				Pb	1.05			
	Cd	1.06				Cd	0.771				Cd	1.05			
	Pb×Cd	2.14				Pb×Cd	1.54				Pb×Cd	2.11			
Toona ciliata															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	112.5^{a}	90.9^{b}	80.8^{cd}	44.1^{gh}	82.1^{A}	94.9^{a}	72.2^{b}	60.9^{d}	46.4^{e}	68.6^{A}	110.8^{a}	70.3^{b}	55.8^{e}	42.5^{h}	69.9^{A}
Cd_5	83.6^{c}	77.6^{d}	72.8^{e}	61.6^{f}	73.9^{B}	72.6^{b}	66.7^{c}	58.3^{d}	41.2^{fg}	59.7^{B}	68.9^{b}	63.9^{c}	59.5^{d}	52.8^{f}	61.3^{B}
Cd_{15}	47.1^{g}	44.6^{gh}	41.6^{hi}	37.8^{i}	42.8^{C}	42.5^{f}	38.3^{g}	33.5^{h}	29.4^{i}	35.9^{C}	49.4^{gh}	42.9^{g}	35.8^{i}	28.0^{j}	39.0^{C}
Cd_{25}	32.5^{j}	32.2^{j}	27.3^{k}	25.4^{k}	29.4^{D}	20.5^{j}	18.2^{jk}	18.1^{jk}	15.0^{k}	18.0^{D}	22.9^{k}	19.7^{l}	15.9^{m}	13.9^{m}	18.1^{D}
Mean	68.9^{A}	61.4^{B}	55.6^{C}	42.2^{D}		57.6^{A}	48.9^{B}	42.7^{C}	33.0^{D}		63.0^{A}	49.2^{B}	41.8^{C}	34.3^{D}	
LSD ($p\leq0.05$)	Pb	1.48				Pb	1.13				Pb	0.828			
	Cd	1.48				Cd	1.13				Cd	0.828			
	Pb×Cd	2.97				Pb×Cd	2.27				Pb×Cd	1.658			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Similar trend was observed for Cd concentrations where maximum mean Cu content was observed in control (Cd_0, 73.6 mg/kg in roots, 59.8 mg/kg in stem and 56.6 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (65.3 mg/kg in roots, 51.4 mg/kg in stem and 49.8 mg/kg in leaves) to Cd_{25} (20.5 mg/kg in roots, 10.0 mg/kg in stem and 7.58 mg/kg in leaves). Among combinations, maximum Cu content was observed in control Pb_0Cd_0 (107 mg/kg in root, 8.36 mg/kg in stem, 74.6 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (16.4 mg/kg in root, 7.18 mg/kg in stem, 5.33 mg/kg in leaves).

In case of *Toona*, the significant decrease in copper content of all plant parts was observed with Pb, Cd and their combination concentrations (Table 4.27). Among Pb concentrations, the mean value for Cu was maximum in control plants i.e. Pb_0 (68.9 mg/kg in roots, 57.0 mg/kg in stem and 63.0 mg/kg in leaves) which decreased significantly with increase in Pb concentration, thus minimum Cu content was recorded with highest Pb concentration i.e. Pb_{300} (42.2 mg/kg in roots, 33.3 mg/kg in stem and 34.3 mg/kg in leaves). Similarly, among Cd concentrations, maximum mean Cu content was observed in control (Cd_0, 82.1 mg/kg in roots, 68.6 mg/kg in stem, 69.9 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (73.9 mg/kg in roots, 59.7 mg/kg in stem and 61.3 mg/kg in leaves) to Cd_{25} (29.4 mg/kg in roots, 18 mg/kg in stem and 18.1mg/kg in leaves). Among combinations, maximum Cu content was observed in control Pb_0Cd_0 (112.5 mg/kg in root, 94.9 mg/kg in stem, 110.8 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (25.4 mg/kg in root, 15.0 mg/kg in stem, 13.9 mg/kg in leaves).

Hence, the Cu content of both *Salix* and *Toona* plant parts (root, stem and leaves) was negatively affected in response to Pb, Cd and their combination concentrations. However, similar trend was observed for both *Salix* and *Toona* that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd > Pb. Overall, among different plant parts, the higher Cu content was observed in roots than leaves and stem in an order as roots > leaves > stem.

i. **Iron (Fe)**

The data presented in Table 4.28 shows the effect of different concentrations of Pb, Cd and their combinations on iron (Fe) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, significant decrease in Fe content in both *Salix* and *Toona* was recorded.

Table 4.28: Effect of heavy metals on iron content in different plant parts of *Salix alba* and *Toona ciliata*

Iron (mg/kg)															
Salix alba															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	548^{a}	496^{b}	379^{d}	119^{h}	386^{A}	371^{a}	203^{d}	$192d^{e}$	142^{f}	227^{A}	249^{a}	178^{c}	83.6^{fg}	68.9^{j}	145^{A}
Cd_5	398^{c}	348^{e}	295^{f}	246^{g}	322^{B}	296^{b}	215^{c}	198^{d}	184^{e}	224^{B}	193^{b}	94.2^{e}	78.3^{fghi}	75.9^{ghij}	110^{B}
Cd_{15}	108^{hi}	98.5^{i}	92.3^{i}	72.3^{j}	92.9^{C}	112^{g}	98.3^{h}	94.2^{h}	88.3^{hi}	98.3^{C}	146^{d}	84.3^{f}	80.3^{fgh}	74.6^{hij}	96.0^{C}
Cd_{25}	64.3^{jk}	52.3^{k}	51.6^{k}	48.7^{k}	54.2^{D}	98.4^{h}	82.9^{ij}	74.6^{j}	62.4^{k}	79.6^{D}	72.4^{hij}	70.3^{ij}	56.3^{k}	45.6^{l}	61.0^{D}
Mean	280^{A}	249^{B}	205^{C}	122^{D}		220^{A}	150^{B}	140^{C}	119^{D}		165^{A}	107^{B}	75.1^{C}	66.2^{D}	
LSD ($p\leq0.05$)	Pb	6.16				Pb	3.66				Pb	2.56			
	Cd	6.16				Cd	3.66				Cd	2.56			
	Pb×Cd	12.32				Pb×Cd	7.32				Pb×Cd	5.11			
Toona ciliata															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	381^{a}	304^{b}	184^{e}	96.5^{h}	242^{A}	285^{a}	218^{b}	185^{e}	129^{gh}	204^{A}	194^{a}	169^{b}	129^{d}	94.2^{g}	147^{A}
Cd_5	282^{c}	218^{d}	172^{e}	89.4^{hi}	191^{B}	225^{b}	209^{c}	195^{d}	166^{f}	198^{B}	152^{c}	125^{d}	113^{e}	84.3^{h}	119^{B}
Cd_{15}	149^{f}	120^{g}	94.3^{hi}	80.5^{ij}	111^{C}	157^{f}	137^{g}	124^{h}	97.5^{i}	129^{C}	129^{d}	105^{f}	95.4^{g}	79.5^{hi}	102^{C}
Cd_{25}	84.3^{hi}	70.4^{jk}	68.5^{jk}	62.9^{k}	71.5^{D}	96.5^{i}	80.5^{j}	79.9^{j}	74.3^{j}	82.8^{D}	93.5^{g}	82.4^{hi}	75.3^{ij}	69.4^{j}	80.1^{D}
Mean	224^{A}	178^{B}	129^{C}	82.3^{D}		191^{A}	161^{B}	146^{C}	117^{D}		142^{A}	120^{B}	103^{C}	81.8^{D}	
LSD ($p\leq0.05$)	Pb	4.56				Pb	3.21				Pb	2.36			
	Cd	4.56				Cd	3.21				Cd	2.36			
	Pb×Cd	9.12				Pb×Cd	6.42				Pb×Cd	4.73			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

In *Salix*, significant decrease in iron content of all plant parts was observed in response to Pb, Cd and their combination concentrations (Table 4.28). Among Pb concentrations, the mean value for Fe was maximum in control i.e. Pb_0 (280 mg/kg in roots, 220 mg/kg in stem and 165 mg/kg in leaves) which decreased significantly with increase in Pb concentration, thus minimum Fe content was recorded with highest Pb concentration i.e. Pb_{300} (122 mg/kg in roots, 119 mg/kg in stem and 66 mg/kg in leaves). Similar trend was observed for Cd concentrations where maximum mean Fe content was in control (Cd_0, 386 mg/kg in roots, 227 mg/kg in stem and 145 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (322 mg/kg in roots, 224 mg/kg in stem and 110 mg/kg in leaves) to Cd_{25} (54.2 mg/kg in roots, 79.6 mg/kg in stem and 61 mg/kg in leaves). Among combinations, maximum Fe content was observed in control Pb_0Cd_0 (548 mg/kg root, 371 mg/kg in stem, 249 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (48.7 mg/kg in root, 62.4 mg/kg in stem, 45.6 mg/kg in leaves).

In case of *Toona*, the significant decrease in iron content of all plant parts was observed because of Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for Fe content was maximum in control plants i.e. Pb_0 (224.4 mg/kg in roots, 190.9 mg/kg in stem and 142.4 mg/kg in leaves) which decreased significantly with increase in Pb concentration, thus minimum Fe content was recorded with highest Pb concentration i.e. Pb_{300} (82.3 mg/kg in roots, 116.8 mg/kg in stem and 81.8 mg/kg in leaves). Similarly, among Cd concentrations, maximum mean Fe content was observed in control (Cd_0, 242 mg/kg in roots, 204 mg/kg in stem and 147 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (191 mg/kg in roots, 198 mg/kg in stem and 119 mg/kg in leaves) to Cd_{25} (71.5 mg/kg in roots, 82.8 mg/kg in stem and 80.1 mg/kg in leaves. Among combinations, maximum Fe content was observed in control Pb_0Cd_0 (381 mg/kg in root, 285 mg/kg in stem, 194 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (62.9 mg/kg in root, 74.3 mg/kg in stem, 69.4 mg/kg in leaves).

Thus, Pb, Cd and their combination concentrations negatively affect the Fe content of both *Salix* and *Toona* plant parts. For both the species, similar trend was observed that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd > Pb. Overall, among different plant parts, the higher Fe content was observed in roots than stem and leaves in an order as leaves > stem ≥ roots.

j. Zinc (Zn)

The data presented in Table 4.29 reveals the negative effect of different concentrations of Pb, Cd and their combinations on zinc (Zn) content of *Salix* and *Toona* plants parts (root stem and leaves). With increasing concentrations of Pb and Cd, significant decrease in Zn content of both *Salix* and *Toona* was recorded.

In *Salix*, significant decrease in zinc (Zn) content of all plant parts was observed in response to Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for Zn was maximum in control i.e. Pb_0 (36.5 mg/kg in roots, 27.2 mg/kg in stem and 26.5 mg/kg in leaves) which decreased significantly with increase in concentration from Pb_{100} (31 mg/kg in roots, 24.5 mg/kg in stem and 23.3 mg/kg in leaves) to Pb_{300} (19.3 mg/kg in roots, 14.7 mg/kg in stem and 13.7 mg/kg in leaves). Similar trend was observed for Cd concentrations where maximum mean Zn content was observed in control (Cd_0, 46.6 in roots, 30.3 mg/kg in stem and 34.6 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (35.7 mg/kg in roots, 29.4 mg/kg in stem and 24.3 mg/kg in leaves) to Cd_{25} (10.6 mg/kg in roots, 10.1 mg/kg in stem and 8.4 mg/kg in leaves). Among combinations, maximum Zn content was observed in control Pb_0Cd_0 (66.4 mg/kg in root, 42.4 mg/kg in stem, 45.9 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (4.89 mg/kg in root, 7.17 mg/kg in stem, 6.15 mg/kg in leaves).

In case of *Toona*, the significant decrease in zinc among all plant parts was observed with Pb, Cd and their combination concentrations. Among Pb concentrations, the mean value for Zn content was maximum in control plants i.e. Pb_0 (52.3 mg/kg in roots, 65.9 mg/kg in stem and 38.4 mg/kg in leaves) which decreased significantly with increase in Pb concentration, thus minimum zinc was recorded with highest Pb concentration i.e. Pb_{300} (29.8 mg/kg in roots, 38.2 mg/kg in stem and 25.7 mg/kg in leaves). Similarly, among Cd concentrations, maximum mean Zn content was observed in control (Cd_0, 61.4 mg/kg in roots, 70.5 mg/kg in stem and 46.3 mg/kg in leaves) which decreased significantly with increase in concentration from Cd_5 (51.9 mg/kg in roots, 62.4 mg/kg in stem and 36.2 mg/kg in leaves) to Cd_{25} (19.6 mg/kg in roots, 29.5 mg/kg in stem and 20.4 mg/kg in leaves). Among combinations, maximum Zn content was observed in control Pb_0Cd_0 (83.3 mg/kg in root, 90.5 mg/kg in stem, 57.1 mg/kg in leaves) which also decreased significantly with increase in concentration and recorded to be minimum with highest combination i.e. $Pb_{300}Cd_{25}$ (18.5 mg/kg in root, 23.6 mg/kg in stem, 18.1 mg/kg in leaves).

Table 4.29: Effect of heavy metals on zinc content in different plant parts of *Salix alba* and *Toona ciliata*

Zinc (mg/kg)															
Salix alba															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	66.4^{a}	52.4^{b}	39.4^{d}	28.3^{f}	46.6^{A}	42.4^{a}	39.8^{b}	20.5^{g}	18.3^{h}	30.3^{A}	45.9^{a}	39.4^{b}	32.9^{c}	20.3^{f}	34.6^{A}
Cd_5	42.8^{c}	38.9^{d}	32.4^{e}	28.5^{f}	35.7^{B}	35.4^{c}	30.9^{d}	28.3^{e}	22.8^{f}	29.4^{B}	31.4^{d}	28.9^{e}	20.8^{f}	15.9^{h}	24.3^{B}
Cd_{15}	22.5^{g}	20.4^{h}	18.3^{i}	15.4^{j}	19.2^{C}	18.4^{h}	15.9^{i}	15.2^{i}	10.5^{jk}	15.0^{C}	18.3^{g}	15.2^{h}	12.4^{i}	12.3^{i}	14.6^{C}
Cd_{25}	14.3^{j}	12.4^{k}	10.7^{l}	4.89^{m}	10.6^{D}	12.4^{j}	11.5^{j}	9.40^{k}	7.17^{l}	10.1^{D}	10.4^{j}	9.88^{j}	7.32^{k}	6.15^{l}	8.4^{D}
Mean	36.5^{A}	31.0^{B}	25.2^{C}	19.3^{D}		27.2^{A}	24.5^{B}	18.4^{C}	14.7^{D}		26.5^{A}	23.3^{B}	18.4^{C}	13.7^{D}	
LSD ($p\leq0.05$)	Pb	0.561				Pb	0.646				Pb	0.302			
	Cd	0.561				Cd	0.646				Cd	0.302			
	Pb×Cd	1.021				Pb×Cd	1.292				Pb×Cd	0.604			
Toona ciliata															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	83.3^{a}	72.9^{b}	50.7^{e}	38.5^{f}	61.4^{A}	90.5^{a}	79.5^{b}	63.4^{e}	48.4^{g}	70.5^{A}	57.1^{a}	51.1^{b}	44.3^{c}	32.5^{ef}	46.3^{A}
Cd_5	65.0^{c}	60.4^{d}	48.7^{e}	33.5^{g}	51.9^{B}	74.7^{c}	69.4^{d}	57.3^{f}	48.3^{g}	62.4^{B}	42.9^{c}	40.8^{d}	32.7^{e}	28.2^{g}	36.2^{B}
Cd_{15}	39.5^{f}	35.4^{g}	30.6^{h}	29.0^{h}	33.6^{C}	58.9^{f}	50.4^{g}	37.5^{h}	32.4^{i}	44.8^{C}	30.9^{f}	27.8^{g}	24.2^{h}	23.9^{hi}	26.7^{C}
Cd_{25}	21.5^{i}	19.4^{ij}	19.0^{j}	18.5^{j}	19.6^{D}	39.5^{h}	29.5^{i}	25.4^{j}	23.6^{j}	29.5^{D}	22.5^{i}	22.1^{i}	19.2^{j}	18.1^{j}	20.4^{D}
Mean	52.3^{A}	47.0^{B}	37.2^{C}	29.8^{D}		65.9^{A}	57.2^{B}	45.9^{C}	38.2^{D}		38.4^{A}	35.5^{B}	30.1^{C}	25.7^{D}	
LSD ($p\leq0.05$)	Pb	0.766				Pb	1.23				Pb	0.701			
	Cd	0.766				Cd	1.23				Cd	0.701			
	Pb×Cd	1.53				Pb×Cd	2.46				Pb×Cd	1.40			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Hence, the Zn content of both *Salix* and *Toona* plant parts (root, stem and leaves) was negatively affected in response to Pb, Cd and their combination concentrations. However, similar trend was observed for both *Salix* and *Toona* that the effect of combination concentrations were more pronounced than individual Pb and Cd concentrations and follows an order Pb+Cd > Cd > Pb. Overall, among different plant parts, the higher Zn content was observed in stem than roots and leaves in an order as leaves > roots > stem.

Heavy metal contaminated soil affects the ability of plants to absorb water and nutrients which further leads to mineral and nutrient deficiencies in plants (Ali *et al* 2015). Heavy metal and nutrient interrelationship is a complex phenomenon, which depends on the type of soil and varies among species to species (Hossain *et al* 2012). In the present study, the decrease in macro- (N, P, K, Ca, Mg, S) and micro- (Mn, Cu, Fe and Zn) nutrients of both *Salix* and *Toona* are observed in response to different Pb and Cd concentrations.

These results are in accordance with previous research studies reported by several researchers such as Ahmad *et al* (2011) reported that Pb concentration (0.01-1.0mg/ L) significantly reduces K and Cu concentration in root, shoot and leaves of maize genotypes. Li *et al* (2016) reported that heavy metal interacts with other nutrients such as Zn, Fe, Cu, Ca, N, P and K, and malfunctioned the absorption of nutrients by roots either through immobilization or decreased uptake which results in nutrient deficiency in plants. Gupta *et al* (2013) reported that cadmium contamination induce inhibitory affect on Mn accumulation and total amount of mineralized carbon status of plants and soil. Similarly, it has been demonstrated that Cu, Zn, Ni and Cd compete for same membrane carriers for translocation in plants. Lamhamdi *et al* (2013) reported that Pb stress reduced that uptake of micronutrients such as Na, K, Ca, P, Mg, Fe, Cu and Zn in *Triticum aestivum* and *Spinacia oleracea*. Wang *et al* (2011) found that Pb contamination leads to decreased concentration of P, K and Mn in *Vallisneria natans* and suggests antagonistic relationship among them. Liu *et al* (2013) found that Cd stress negatively correlates with Mn^{2+} ions and further Mn deficiency leads to chlorosis and necrotic lesions in leaves of *Oryza sativa.* Yoshihara *et al* (2006) reported that Cd stress induce Fe deficiency along with reduced uptake of K and Ca ions in *Nicotiana tabacum*.

In present study, the decrease in nutrient content of plants (*Salix* and *Toona*) in response to Pb and Cd concentrations might be due to abnormal root growth such as reduced root number, root length and root biomass (Fig. 4.6 and Fig. 4.7). Roots and root hairs are primary organs that directly come in contact with soil heavy metals, which get injured due to high concentration of heavy metal that may also results to abnormalities in optimum water and nutrient uptake and ultimately cause nutrient deficiency and water deficit (Singh *et al* 2016). Thus, all these abnormalities further responsible for reduced plant growth and limited production.

4.2.4 Fourier transformed infrared spectroscopy (FTIR) analysis

Heavy metal elements interact and bind with the functional groups of biomolecules present inside the plant tissues i.e. polysaccharides, proteins and nucleic acids which can be determined under infrared spectroscopy (Usman *et al* 2019). The region of functional group alterations in response to heavy metal was determined using control spectra and peaks which are allocated using previous reports (Largo-Gosens *et al* 2014, Pandey *et al* 2014, Sivakumar 2016, Peng *et al* 2020).

The morpho-physiological parameters and metal accumulation patterns of both *Salix* and *Toona* reveals the adverse effect with highest concentration of heavy metals such as Pb and Cd combination i.e. $Pb_{300}Cd_{25}$, with respect to control. Hence, this concentration ($Pb_{300}Cd_{25}$) was selected for FTIR analysis to study the functional group alterations along with modifications in their associated chemical compounds as compared to control (Pb_0Cd_0). These samples were analyzed at ATR (Attenuated Total Reflection) mode by using Raman spectrophotometer at electron microscopy and nanoscience (EMN) laboratory, Department of soil science, Punjab Agricultural University, Ludhiana; data recorded within 400-4000 Cm^{-1} range.

a. FTIR analysis of *Salix alba*

All the spectra (Fig. 4.12) depicted six major bands corresponding to heavy metal interactions in contaminated soils as well different parts of *Salix*, which were categorized in four major groups i.e. lipid region (3000-2000 cm^{-1}), proteins (1800-1500 cm^{-1}), carbohydrate (1500-1200 cm^{-1}) and cell wall components (1000-600 cm^{-1}). Selected peaks having variable wave numbers in their respective regions for control and heavy metal treated soils and plant parts are represented in Table 4.30. The transmittance spectra values of treated samples were observed low in comparison to control spectra, which might be due to changes in the vibrational frequency of several functional groups owing to their interaction with Pb and Cd in respective plant tissues.

The first marked variable transmittance peak of soil spectra observed at 3473.5 cm^{-1} for control and 3473.3 cm^{-1} for treated soils corresponds to the presence of functional groups of alcohol and phenols, which predict the change due to the vibrations of hydroxyl groups (Fig.4.12 a). Similar type of band shift with different peak values was observed in roots (3605.4 cm^{-1}, 3594.4 cm^{-1}), stems (3627.3 cm^{-1}, 3607.3 cm^{-1}) and leaves (3620.3 cm^{-1}, 3567.5 cm^{-1}) that represents the change in functional groups of alcohol and phenol in cellulose structure owing to their interaction with Pb and Cd ions.

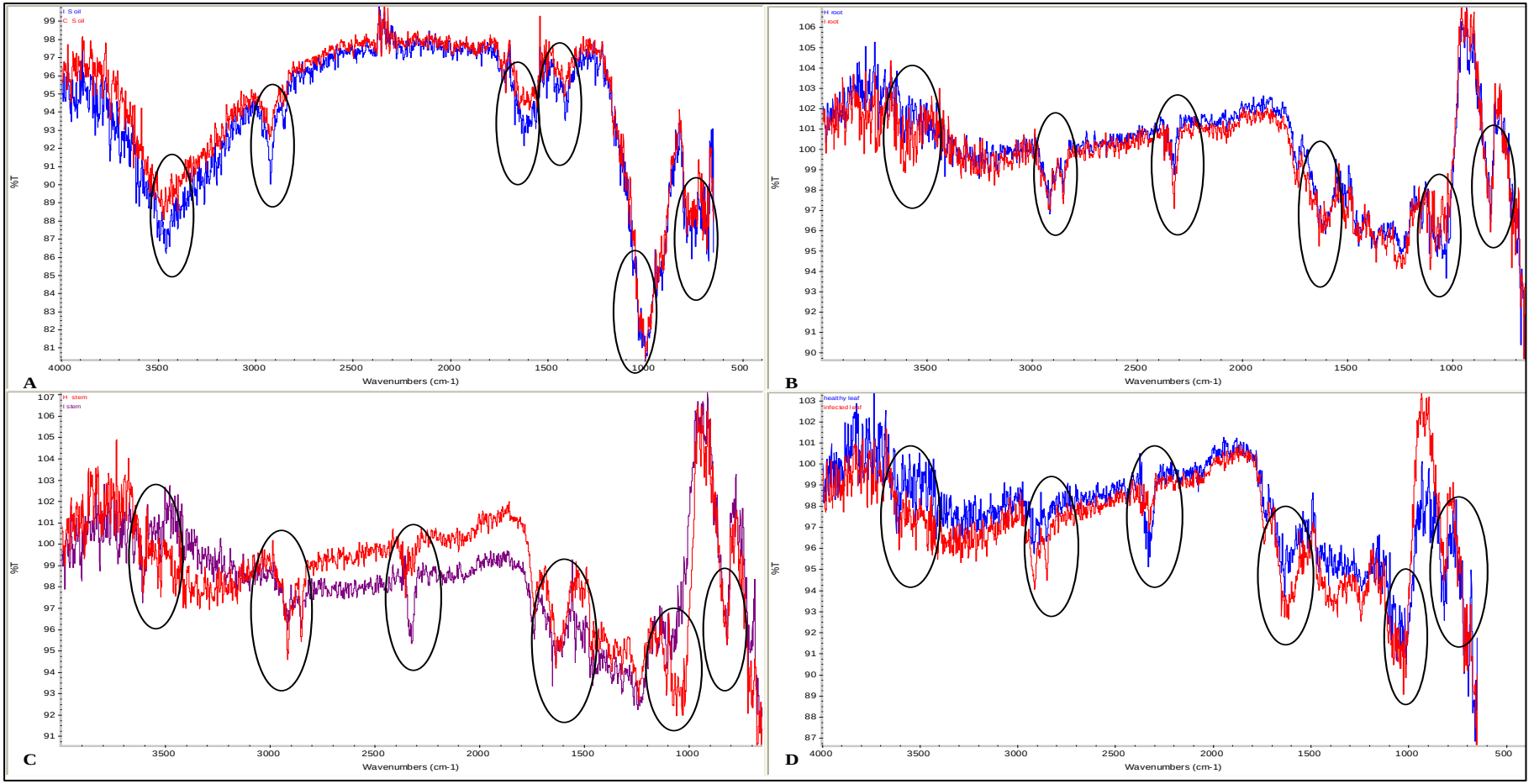


Fig. 4.12: Fourier transformed infra-red (FTIR) spectra for soil and plant parts of *Salix alba*

A. Soil control (Pb_0Cd_0, red line) vs treated ($Pb_{300}Cd_{25}$, blue line)
B. Roots control (Pb_0Cd_0, blue line) vs treated ($Pb_{300}Cd_{25}$, red line)
C. Stems control (Pb_0Cd_0, red line) vs treated ($Pb_{300}Cd_{25}$, blue line)
D. Leaves control (Pb_0Cd_0, blue line) vs treated ($Pb_{300}Cd_{25}$, red line).

Table 4.30: Mean peak values of selected FTIR spectral regions for control (Pb_0Cd_0) and heavy metal treated ($Pb_{300}Cd_{25}$) soil and plant parts of *Salix alba*

Sr No	FTIR Soil					FTIR Root				
	Pb_0Cd_0 Wavenumber (cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber (cm^{-1})	Chemical assignment	Functional group	Compound	Pb_0Cd_0 Wavenumber (cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber (cm^{-1})	Chemical assignment	Functional group	Compound
1	3473.5	3473.3	O-H	Alcohols Phenols	Phenolic compounds, Cellulose	3605.4	3594.4	O-H	Alcohols Phenols	Phenolic compounds, Cellulose
2	2924.9	2857.2	S, C-H, O-H	Alkanes Carboxylic acid	Lipid region,hydrocarbon	2917.3	2895.5	S, C-H, O-H	Alkanes Carboxylic acid	Glycerolipid, wax, hydrocarbon
3	1645.7	1622.7	C=C, C-O, N-H, C=O	Alkenes, Aromatics Amines, Benzene	Amides of protein, Proline	2348.3	2325.7	C=O, P-H	Alkenes, Phosphine	Carboxy-amino compounds, phosphates
4	1418.7	1412.2	C-H, C-C, C-O, N=O	Alkanes, Aromatics	Polysaccharides	1604.7	1635.8	C=C, C-C, N-H, C=O	Alkenes, Benzene, Aromatics, Amides	Amides of protein
5	997.4	997.3	C-H, C-O-P, S=O, =C-H	Alcohols, Aliphatic amines, Alkenes	Phosphates, Sulphoxides	1296.1	1266.7	C-O/C-C	Methoxy, Esters, Amines, Carbonyl, Carboxylic acid	Lignin, ligno-cellulose
6	754.6	707.1	C-O-P, =C-H, C-N	Alkynes, Benzene, Amines	Polysaccharides, Xyloglucans	833.1	819.6	N-H	Alkyl halides, Benzene, Amines	Chlorides

Sr No	FTIR Stem					FTIR Leaf				
	Pb_0Cd_0 Wavenumber (cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber (cm^{-1})	Chemical assignment	Functional group	Compound	Pb_0Cd_0 Wavenumber (cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber (cm^{-1})	Chemical assignment	Functional group	Compound
1	3627.3	3607.3	O-H	Phenols, Alcohols	Phenolic compounds, Cellulose	3620.3 3606.6 3330.0	3567.5 3576.9 3362.3	O-H	Phenols	Phenolic compounds, Cellulose
2	2931.9	2324.9	S, C-H, O-H	Alkenes Carboxylic acid	Glycerolipid, wax, hydrocarbon	2916.7 2890.6 2652.9	2915.3 2888.7	S, C-H, O-H	Alkanes Carboxylic acid	Chlorophyll, Glycerolipid, wax, hydrocarbon
3	2354.8	2324.9	O-H, N-H	Alkenes, Amines	Carboxy-amino compounds	2345.0 2333.6	2330.6	O-H, N-H	Alkenes	Carboxy-amino compounds
4	1734.2	1652.9	C = C, C-C, C=O, N-H	Alkenes, Amines, Saturated aldehydes	Amides of protein	1652.5 1656.7	1623.9 1616.0	C=C, C-C, N-H	Alkenes, Aromatics, Amines	Amides of proteins
5	1239.8	1117.5	C-O/C-C, C-N	Methoxy, carbonyl, carboxylic acid	Lignin, ligno-cellulose	1033.7 1027.9	1014.0 1034.1	C-O	Esters, ethers, alcohols, carboxylic acids	Carbohydrate
6	813.9	806.5	=C-H, C-N	Alkenes, Benzene, Amine	Cellulose, Cell wall components	875.4 925.6	818.8 807.0	=C-H, C-N, C-C	Alkenes, Benzene	Carotenoids Cellulose, Chlorides

The significant difference between the second transmittance peaks of control (Pb_0Cd_0) and treated ($Pb_{300}Cd_{25}$) samples of soils (2924.9 cm^{-1}, 2857.2 cm^{-1}), root (2917.3 cm^{-1}, 2895.5 cm^{-1}), stem (2931.9 cm^{-1}, 2324.9 cm^{-1}) and leaves (2890.6 cm^{-1}, 2888.7 cm^{-1}) denotes modifications in lipid regions containing glycerolipids, waxes and hydrocarbons with alteration in vibration frequency by strong narrow stretching of C-H and O-H bonds having carboxylic acids and alkanes as functional groups. The third band of plants root (2348.3 cm^{-1}, 2325.7 cm^{-1}), stem (2354.8 cm^{-1}, 2324.9 cm^{-1}) and leaves (2345.0 cm^{-1}, 2330.6 cm^{-1}) spectra represents the carboxy-amino compounds and other compounds having alkene groups that showed strong sharp stretching of C=O. The third peak of soil samples (1645.7 cm^{-1}, 1622.7 cm^{-1}) and fourth peaks of root (1640.7 cm^{-1}, 1635.8 cm^{-1}), stem (1734.2 cm^{-1}, 1652.9 cm^{-1}) and leaves (1652.5 cm^{-1}, 1623.9 cm^{-1}) showed the presence of protein amides in control and treated samples having strong broad stretching of C=O, N-H, C-H and C=C chemical bonds due to change in their vibrational frequency.

The fifth peak of plants root (1296.1 cm^{-1}, 1244.9 cm^{-1}) and stem (1239.8 cm^{-1}, 1117.5 cm^{-1}) showed common region of spectra representing narrow bend and variable stretching of C-O/C-C in lignin compounds, meanwhile leave sample showed sharp bend back in C-O, C-N corresponds to esters, ethers, alcohol and carboxylic acids containing carbohydrates. The sixth sharp peak of soil (754.6 cm^{-1}, 707.1 cm^{-1}) root (833.1 cm^{-1}, 819.6 cm^{-1}), stem (813.9 cm^{-1}, 806.5 cm^{-1}) and leaves (875.4 cm^{-1}, 818.8 cm^{-1}) denotes variable and sharp stretching of =C-H resulting in modifications in wave number for aromatic compounds, phosphate, primary and secondary amines and alkenes. The bend back peak of leaves sample in the respective region represents change in structure of carotenoids in plant

b. FTIR analysis of *Toona ciliata*

The FTIR spectra of *Toona* (Fig.4.13) exhibits eight major bands indicating the alterations induced due to heavy metal interactions in *Toona* and treated soils. The transmittance spectra's of heavy metal treated plant parts were observed to be low in comparison to control spectra values, whereas the transmittance spectra for soil indicate fluctuations among control and heavy metal treated soils. The major peaks having variable wave number values in their respective regions for control (Pb_0Cd_0) and heavy metal treated ($Pb_{300}Cd_{25}$) soils and plant parts are represented in Table 4.31.

The first transmittance peak of all plant parts and soil spectra were observed in the range of 3784 cm^{-1} to 3789 cm^{-1} indicated the non-bonded region of metabolites. The second peak for control (3373 cm^{-1}) and heavy metal treated soils (3371.9 cm^{-1}) corresponds to the presence of functional groups of alcohol and phenols, which predict the modifications due to the vibrations of hydroxyl groups (Fig.4.13 A). Similar type of band shift with different peak values was observed in roots (3314.4 cm^{-1}, 3324.3 cm^{-1}), stems (3333.2 cm^{-1}, 3313.4 cm^{-1}) and leaves (3296.1 cm^{-1}, 3296.3 cm^{-1}) that represents the change in functional groups of alcohol and phenols due to their interaction with Pb and Cd ions.

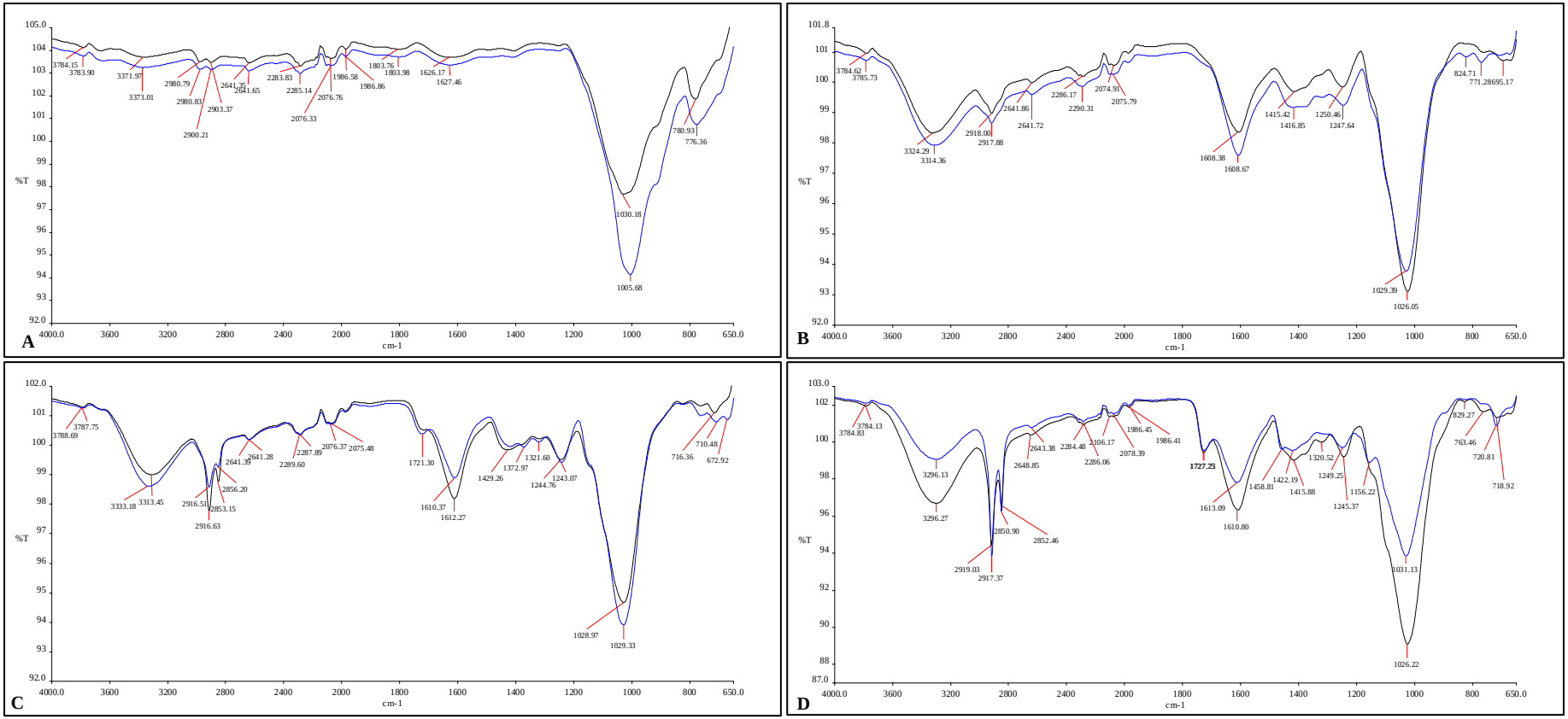


Fig. 4.13: Fourier transformed infra-red (FTIR) spectra for soil and plant parts of *Toona ciliata*

A. Soil control (Pb_0Cd_0, blue line) vs treated ($Pb_{300}Cd_{25}$, black line)
B. Roots control (Pb_0Cd_0, blue line) vs treated ($Pb_{300}Cd_{25}$, black line)
C. Stems control (Pb_0Cd_0, blue line) vs treated ($Pb_{300}Cd_{25}$, black line)
D. Leaves control (Pb_0Cd_0, blue line) vs treated ($Pb_{300}Cd_{25}$, black line

Table 4.31: Mean peak values of selected FTIR spectral regions for control (Pb_0Cd_0) and heavy metal treated ($Pb_{300}Cd_{25}$) soil and plant parts of *Toona ciliata*

Sr No.	FTIR Soil					FTIR Root				
	Pb_0Cd_0 Wavenumber (cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber (cm^{-1})	Functional group	Chemical assignment	Compound	Pb_0Cd_0 Wavenumber (cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber (cm^{-1})	Functional group	Chemical assignment	Compound
1	3783.9	3784.2	Non-bonded	-	-	3785.7	3784.6	Non-bonded	-	-
2	3373.0	3371.9	O-H	Alcohols Phenols	Phenolic compounds, Cellulose	3314.4	3324.3	O-H	Alcohols Phenols	Phenolic compounds, Cellulose
3	2980.8 2900.2	2980.8 2903.4	S, C-H, O-H	Alkanes Carboxylic acid	Lipids	2917.8 2641.7	2918.0 2641.8	S, C-H, O-H	Alkanes Carboxylic acid	Glycerolipid, wax, hydrocarbon
4	2285.1 2076.3	2283.8 2076.8	C≡C,C=N	Alkynes	Lipids, Nitriles	2290.3 2075.8	2286.2 2074.9	C=O, P-H	Alkenes, Phosphine	Carboxy-amino compounds, phosphates
5	1803.9	1803.8	C=O	Esters Carboxylic acids	Polysaccharides Transition metal complexes	1608.7	1608.4	C=C, C-C, N-H, C=O	Alkenes, Benzene, Aromatics, Amides	Amides of protein
6	1627.5	1626.2	C=C, C-O, N-H, C=O	Alkenes, Aromatics, Amines, Benzene	Amides of protein, Proline	1416.8 1247.6	1415.4 1250.5	C-O/C-C	Methoxy, Amines, Carbonyl, Carboxylic acid	Lignin, ligno-cellulose
7	1005.1	1030.2	C-O-P, S=O	Alcohols, Aliphatic amines, Alkenes	Phosphates, Sulphoxides	1026.1	1029.4	C-O	Carbonyl	Carbohydrates
8	776.4	780.9	C-O-P, =C-H, C-N	Alkynes, Benzene, Amines	Polysaccharides, Xyloglucans	824.7 771.3	695.2	N-H	Alkyl halides, Benzene, Amines	Cell wall components

Sr No.	FTIR Stem					FTIR Leaf				
	Pb_0Cd_0 Wavenumber (cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber (cm^{-1})	Functional group	Chemical assignment	Compound	Pb_0Cd_0 Wavenumber (cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber (cm^{-1})	Functional group	Chemical assignment	Compound
1	3788.7	3787.7	Non bonded	-	-	3784.8	3784.1	Non bonded	-	-
2	3333.2	3313.4	O-H	Phenols, Alcohols	Phenolic compounds, Cellulose	3296.1	3296.3	O-H	Phenols	Phenolic compounds, Cellulose
3	2916.5 2856.2	2916.6 2853.1	S, C-H, O-H	Alkenes Carboxylic acid	Glycerolipid, wax, hydrocarbon	2917.4 2850.9	2919.0 2852.5	S, C-H, O-H	Alkanes Carboxylic acid	Chlorophyll, Glycerolipid, wax, hydrocarbon
4	2287.9 2075.5	2289.6 2076.4	O-H, N-H	Alkenes, Amines	Carboxy-amino compounds	2643.4 2286.1 2078.4	2648.8 2284.5 2106.2	O-H, N-H	Alkenes	Carboxy-amino compounds
5	1610.4	1612.3	C = C, C-C, C=O, N-H	Alkenes, Amines, Aldehydes	Amides of protein	1613.1	1610.8	C=C, C-C, N-H	Alkenes, Aromatics, Amines	Amides of proteins
6	1372.9 1243.1	1429.3 1244.8	C-O/C-C, C-N	Methoxy, carbonyl, carboxylic acid	Lignin, ligno-cellulose	1249.2	1245.4	C-O/C=O, C-C	Esters, ethers, carboxylic acids	Lignin
7	1029.3	1028.9	C-O	Carbonyl	Carbohydrates	1031.1	1026.2	C-O	Carbonyl	Carbohydrates
8	710.5 672.9	716.4	=C-H	Carboxylic acids	Polysaccharides	720.8	718.9	=C-H	Carboxylic acids	Polysaccharides

The third transmittance peaks of control (Pb_0Cd_0) and treated ($Pb_{300}Cd_{25}$) samples of soils (2900 cm^{-1} to 2980.8 cm^{-1}), root (2641.7 cm^{-1} to 2918 cm^{-1}), stem (2853.1cm^{-1} to 2916.6 cm^{-1}) and leaves (2850.9 cm^{-1} to 2919 cm^{-1}) denotes vibrational alterations due to sharp narrow stretching of C-H and O-H bonds having carboxylic acids and alkanes functional groups in lipid regions containing glycerolipids, waxes and hydrocarbons. The fourth stretch band of control (2285.1 cm^{-1}, 2076.3 cm^{-1}) and heavy metal treated soil (2283.8 cm^{-1}, 2076.8 cm^{-1}) corresponds to lipids and nitrile compounds with vibrational fluctuations in C≡C and C=N functional groups. Meanwhile the fourth band of control and heavy metal treated plant parts i.e. root (2290.3 cm^{-1}, 2286.2cm^{-1}), stem (2287.8, 2289.6cm^{-1}) and leaves (2643.4 cm^{-1}, 2648.85 cm^{-1}) represents the carboxy-amino compounds or other compounds having alkene, phosphine and amine groups.

The fifith peak of control (1803.9 cm^{-1}) and heavy metal treated (1803.8 cm^{-1}) soils indicate the fluctuation in C=O functional groups of esters and carboxylic acids. Further, the sixth band shift of soil and fifth bands of transmittance peak showed vibrational frequency in range of 1608 cm^{-1} to 1627 cm^{-1} which corresponds to protein region containing substituents of proline and amides of proteins. The sixth peak of plant samples (1250.4 cm^{-1} to1416.8 cm^{-1}) showed broad curved shift of C-O/C-C and C-N functional groups corresponds to lignin and lingo-cellulose compounds.

The seventh sharp transmittance peak of control (1005.1 cm^{-1}) and heavy metal treated (1030.2 cm^{-1}) soils represents the vibrational alterations in C-O-P and S=O functional groups of phosphates and sulphoxides. Meanwhile, the seventh peak of root (1026.0 cm^{-1}, 1029.4 cm^{-1}) and stem (1029.3 cm^{-1}, 1028.9 cm^{-1}) and leaves (1031.1 cm^{-1}, 1026.2 cm^{-1}) showed common region of spectra representing sharp bend of carbonyl (C-O) compounds of carbohydrate groups.

The eighth bend peaks of control (776.4 cm^{-1}) and heavy metal treated soil (780.9 cm^{-1}) indicates the modification in C-O-P, =C-H and C-N functional groups which corresponds to polysaccharides and xyloglucans compounds. In case of plant samples, control and heavy metal treated roots (824.7 cm^{-1}, 695.2 cm^{-1} respectively) showed minor band shifts corresponds to the region of cell wall components, whereas in stem (710.5 cm^{-1}, 716.4 cm^{-1}) and leaves (720.8 cm^{-1}, 718.9 cm^{-1}) denotes random variable stretching of =C-H resulting in modifications in wave number of carboxylic groups of polysaccharides.

Thus, the FTIR results of *Salix* and *Toona* conferred that Pb and Cd metal interaction within plant tissues was mostly confined to carboxyl and amino functional groups. The interactions of functional groups in plants with metal cation exchange led to modifications in cellulose, lipids, carbohydrates and protein structural moieties resulting in disruption of plant growth and development patterns.

Further, they confirmed the binding relationship of amide, hydroxyl, phosphate and

carboxyl groups with Pb and Cd ions in both *Salix* and *Toona*. The availability of binding sites determines the affinity of different plant tissues for specific metal ions. The ion exchange via carboxyl groups in the plant tissues is considered as a primary mechanism for phytoremediation (Usman *et al* 2019). The transmittance value of metal treated soil and plants were recorded to be higher as compared to control, reflecting the strong binding of heavy metal ions with their respective functional groups. The strong functional group binding with the corresponding metal ion is desired in order to maintain the availability of heavy metals in soil (rhizosphere) and their accumulation in plants in order to prevent the heavy metal leaching and releasing back to the contaminated media (Sangeetha *et al* 2019).

Peng *et al* (2020) noticed similar trend of FTIR spectra for chlorophyll and carotenoid stretching in contaminated samples which were correlated with leaf chlorosis due to heavy metal toxicity. Findings of D'Souza *et al* (2008) reported that the heavy metal ions interaction with the hydroxyl ions band shift in the range of 3627.3 to 32456.5cm^{-1} corresponds to metal and oxygen binding. Further, metal ion accumulation in plant biomass affects the general ligno-cellulosic content in tissues (Al-Ghouti *et al* 2010) and these findings are in conformity with present results. The strong metal band shift in response to accumulation of heavy metals in plant depicts the behavior of chemical constituents and their reactions with heavy metal ions. Similarly, Usman *et al* (2019) reported that metal accumulation in plant tissues cause in alteration in plant cell wall components which are responsible for metal ion exchange, interaction and binding, also limits the metal translocation to other tissues in *Tetraena qataranse*.

Experiment No.3

4.3.1To investigate the effect of heavy metals on physico-chemical properties of soil

In the present study, the effect of heavy metals on physico-chemical properties such as pH, electrical conductivity (EC), organic carbon (OC) and cation exchange capacity (CEC) are estimated from heavy metal equilibrated soil (before planting) and after uprooting of *Salix alba* and *Toona ciliata*.

a. pH

pH is one of the most important soil property that affects the nutrient availability, microbial diversity and also impacts the plant response (Król *et al* 2020). The data presented in Table 4.32 shows the variation in soil pH in response to different concentrations of Pb, Cd and their combinations. Before planation of *Salix* and *Toona*, the pH value increased significantly with increase in Pb and Cd concentrations in soil. Thus, minimum pH value was observed in control (Pb_0Cd_0, pH 7.65) which increased significantly and recorded to be maximum with highest concentration i.e. $Pb_{300}Cd_{25}$ (pH 8.36).

Table 4.32: **Effect of heavy metals on soil pH and electrical conductivity before planting and after uprooting of *Salix alba* and *Toona ciliata***

pH															
Before planting						**After uprooting of *Salix alba***					**After uprooting of *Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	7.65^{f}	7.76^{def}	7.81^{cdef}	8.06^{abcdef}	7.82^{C}	8.03^{a}	8.24^{a}	8.29^{a}	8.32^{a}	8.22^{A}	8.10^{a}	8.31^{a}	8.36^{a}	8.39^{a}	8.29
Cd_5	8.15^{abcd}	7.70^{ef}	7.79^{cdef}	7.85^{cdef}	7.87^{B}	8.22^{a}	8.25^{a}	8.34^{a}	8.37^{a}	8.30^{A}	8.29^{a}	8.32^{a}	8.41^{a}	8.44^{a}	8.37
Cd_{15}	8.12^{abcd}	7.81^{cdef}	7.87^{bcdef}	8.12^{abcd}	7.98^{B}	8.45^{a}	8.38^{a}	8.42^{a}	8.45^{a}	8.43^{A}	8.52^{a}	8.45^{a}	8.49^{a}	8.52^{a}	8.50
Cd_{25}	8.28^{a}	8.18^{abc}	8.26^{ab}	8.36^{a}	8.27^{A}	8.45^{a}	8.38^{a}	8.47^{a}	8.47^{a}	8.44^{A}	8.52^{a}	8.45^{a}	8.56^{a}	8.60^{a}	8.53
Mean	8.05^{AB}	7.86^{C}	7.93^{BC}	8.10^{A}		8.29^{A}	8.31^{A}	8.38^{A}	8.40^{A}		8.36	8.38	8.46	8.49	
LSD ($p\leq0.05$)	Pb	0.117				Pb	NS				Pb	0.151			
	Cd	0.117				Cd	NS				Cd	NS			
	Pb×Cd	0.237				Pb×Cd	NS				Pb×Cd	NS			
Electrical conductivity (dS/m)															
Before planting						**After uprooting of *Salix alba***					**After uprooting of *Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.483^{e}	0.483^{e}	0.517^{de}	0.621^{abc}	0.526^{D}	0.658^{e}	0.724^{d}	0.775^{bc}	0.813^{ab}	0.743^{C}	0.694^{c}	0.760^{b}	0.811^{a}	0.849^{a}	0.779^{C}
Cd_5	0.529^{d}	0.547^{d}	0.553^{d}	0.551^{d}	0.545^{C}	0.731^{cd}	0.731^{cd}	0.775^{bc}	0.815^{ab}	0.763^{B}	0.767^{b}	0.770^{a}	0.811^{ab}	0.851^{a}	0.800^{B}
Cd_{15}	0.588^{c}	0.588^{c}	0.595^{bc}	0.597^{bc}	0.592^{B}	0.816^{ab}	0.816^{ab}	0.821^{ab}	0.828^{a}	0.820^{A}	0.852^{a}	0.858^{a}	0.857^{a}	0.864^{a}	0.858^{A}
Cd_{25}	0.628^{ab}	0.647^{a}	0.652^{a}	0.654^{a}	0.645^{A}	0.824^{a}	0.829^{a}	0.832^{a}	0.832^{a}	0.829^{A}	0.860^{a}	0.856^{a}	0.846a	0.865^{a}	0.857^{A}
Mean	0.557^{C}	0.566^{BC}	0.579^{B}	0.606^{A}		0.757^{D}	0.775^{C}	0.801^{B}	0.822^{A}		0.793^{D}	0.811^{C}	0.831^{B}	0.857^{A}	
LSD ($p\leq0.05$)	Pb	0.011				Pb	0.014				Pb	0.016			
	Cd	0.011				Cd	0.014				Cd	0.016			
	Pb×Cd	0.022				Pb×Cd	0.028				Pb×Cd	0.032			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

However, after uprooting of *Salix* and *Toona,* the soil pH significantly decreased as compared to equilibrated soils. Soil pH ranged from 8.03-8.47 for Salix that were non-significant among all Pb, Cd and their combination concentrations. Similarly, in case of *Toona* the soil pH values ranged non-significantly from 8.10-8.60. Significant increase in soil pH with increasing Pb and Cd concentration in heavy metal equilibrated soils (before planting), might be due to lead nitrate and cadmium nitrate salt used for heavy metal treatments. These findings are in agreement with Wang *et al* (2006), who reported that Cd and Zn contamination results to increase in soil pH that decrease the nutrient availability to roots of *Thlaspi caerulescens*. Lenart and Wolny-Koladka (2013) reported that soil pH turns slightly alkaline (>pH) due to the hindered microbial abundance under heavy metal stress, these results are also in line with present study. However, after uprooting of *Salix* and *Toona,* pH values showed non-significant difference among Pb, Cd and their combination concentrations.

b. Electrical conductivity (EC)

The data presented in Table 4.32 reveals the significant increase in soil electrical conductivity (EC) in response to different concentrations of Pb, Cd and their combinations. Before planation of *Salix* and *Toona*, the EC value was minimum in control soils (Pb_0Cd_0, 0.483 dS/m) which increased significantly with increase in Pb and Cd concentrations, thus maximum EC values were recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (0.654 dS/m). Similar trend was recorded in soils after uprooting of *Salix* and *Toona,* where EC values increased significantly with increase in Pb and Cd concentrations. Overall, the EC range was recorded to be less in heavy metal equilibrated soil (0.483-0.654) than the soils after uprooting of *Salix* (0.658-0.832 dS/m) and *Toona* (0.694-0.823 dS/m).

Soil pH and EC are the important factors which affects the heavy metal bioavailability to plants (Król *et al* 2020). Under heavy metal stress, the increased soil EC might be due to the $Pb(NO_3)_2$ and $Cd(NO_3)_2$ salts used for spiking, hence the soil conductivity increased as the cationic metal of rhizosphere increased in the unsaturated medium. Nouri *et al* (2009) reported the positive correlation between EC values and Zn accumulation in *Cousinia* sp. and *C. congestum* and these results are in accordance with present study.

c. Soil organic carbon (SOC)

The significant increase in soil organic carbon (SOC) observed with different Pb and Cd concentrations (Table 4.33). Before planation of *Salix* and *Toona*, the minimum SOC value was observed in control (Pb_0Cd_0, 0.421 %) which increased significantly with increase in Pb and Cd concentrations, thus maximum SOC recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (0.731 %). Similarly, after uprooting of *Salix* and *Toona* SOC increased significantly with increase in Pb and Cd concentrations. Overall, the SOC range was recorded to be lower in heavy metal equilibrated soil (0.421-0.731%) than the soils after uprooting of *Salix* (0.524-0.752 %) and *Toona* (0.536-0.751%).

Table 4.33: **Effect of heavy metals on soil organic carbon and cation exchange capacity before planting and after uprooting of *Salix alba* and *Toona ciliata***

Soil organic carbon (%)															
Before planting						**After uprooting of *Salix alba***					**After uprooting of *Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.421^{f}	0.468^{e}	0.670^{b}	0.702^{ab}	0.565^{D}	0.524^{e}	0.534^{e}	0.671^{c}	0.689^{bc}	0.605^{D}	0.536^{d}	0.536^{d}	0.682^{bc}	0.702^{abc}	0.614^{C}
Cd_5	0.451^{ef}	0.516^{d}	0.670^{b}	0.702^{ab}	0.585^{C}	0.534^{e}	0.582^{d}	0.681^{bc}	0.723^{ab}	0.630^{B}	0.568^{d}	0.568^{d}	0.668^{c}	0.706^{abc}	0.628^{C}
Cd_{15}	0.582^{c}	0.621^{c}	0.706^{ab}	0.706^{ab}	0.654^{B}	0.682^{bc}	0.692^{bc}	0.715^{abc}	0.715^{abc}	0.701^{C}	0.712^{abc}	0.719^{ab}	0.721^{ab}	0.721^{ab}	0.718^{B}
Cd_{25}	0.718^{ab}	0.718^{ab}	0.731^{a}	0.731^{a}	0.725^{A}	0.748^{a}	0.748^{a}	0.748^{a}	0.752^{a}	0.749^{A}	0.732^{ab}	0.732^{ab}	0.751^{a}	0.751^{a}	0.742^{A}
Mean	0.543^{C}	0.581^{B}	0.694^{A}	0.710^{A}		0.622^{D}	0.639^{C}	0.704^{B}	0.720^{A}		0.637^{B}	0.639^{B}	0.706^{A}	0.720^{A}	
LSD ($p\leq0.05$)	Pb	0.0143				Pb	0.0136				Pb	0.0151			
	Cd	0.0143				Cd	0.0136				Cd	0.0151			
	Pb×Cd	0.0286				Pb×Cd	0.0272				Pb×Cd	0.0301			
Cation exchange capacity (cmol(+)/kg)															
Before planting						**After uprooting of *Salix alba***					**After uprooting of *Toona ciliata***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	5.69^{i}	6.55^{h}	7.94^{fg}	9.31^{ab}	7.37^{D}	6.13^{h}	6.34^{g}	6.69^{ef}	7.03^{cd}	6.55^{D}	6.08^{i}	6.29^{h}	6.64^{fg}	6.98^{cd}	6.50^{D}
Cd_5	7.46^{g}	7.69^{fg}	8.17^{ef}	9.31^{ab}	8.16^{C}	6.57^{f}	6.63^{f}	6.74^{ef}	7.03^{cd}	6.74^{C}	6.53^{g}	6.58^{g}	6.70^{fg}	6.98^{cd}	6.71^{C}
Cd_{15}	7.93^{fg}	8.57^{de}	8.71^{cd}	9.17^{bc}	8.59^{B}	6.68^{ef}	6.85^{de}	7.13^{bc}	7.24^{ab}	6.97^{B}	6.64^{fg}	6.80^{ef}	7.08^{bc}	7.20^{ab}	6.93^{B}
Cd_{25}	7.97^{fg}	9.13^{bc}	9.13^{bc}	9.79^{a}	9.01^{A}	6.95^{d}	7.23^{ab}	7.27^{ab}	7.40^{a}	7.21^{A}	6.90^{de}	7.19^{ab}	7.19^{ab}	7.36^{a}	7.16^{A}
Mean	7.26^{D}	7.98^{C}	8.49^{B}	9.39^{A}		6.58^{D}	6.76^{C}	6.96^{B}	7.17^{A}		6.54^{D}	6.72^{C}	6.91^{B}	7.13^{A}	
LSD ($p\leq0.05$)	Pb	0.168				Pb	0.058				Pb	0.057			
	Cd	0.168				Cd	0.058				Cd	0.057			
	Pb×Cd	0.336				Pb×Cd	0.116				Pb×Cd	0.115			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Heavy metal contamination reduces the soil carbon utilization speed due to inhibition of microbial population which further results accumulation of soil organic carbon and increased cation exchange capacity of soil (Hou *et al* 2019). These findings are in line with present results that Pb and Cd concentrations leads accumulation of soil organic carbon in heavy metal equilibrated soil as well as after uprooting of *Salix* and *Toona*.

d. Cation exchange capacity (CEC)

The data presented in Table 4.33 reveals the significant increase in cation exchange capacity (CEC) in response to different concentrations of Pb, Cd and their combinations. Before planation of *Salix* and *Toona*, the CEC value was minimum in control (Pb_0Cd_0, 5.69 cmol(+)/kg) which increased significantly with increase in Pb and Cd concentrations, thus maximum CEC values were recorded with highest concentration i.e. $Pb_{300}Cd_{25}$ (9.79 cmol(+)/kg soil. After uprooting of *Salix* and *Toona*, CEC increased significantly with increase in Pb and Cd concentrations. Overall, the CEC range in heavy metal equilibrated soil was in range of 5.69 - 9.79 cmol (+)/kg, meanwhile the CEC range in soils after uprooting of *Salix* was 6.13-7.40 cmol(+)/kg) and in *Toona* was 6.08- 7.36 cmol(+)/kg soil). Thus, CEC range (5.69-9.79 cmol(+)/kg) was almost similar in heavy metal equilibrated soil (before planting), after uprooting of *Salix* and *Toona*.

Cation exchange capacity (CEC) is considered as a dominant factor to study heavy metal retention in soil (Lorenz *et al* 2006). Malandrino *et al* (2006) suggested the ability of soils to adsorb heavy metals can be linked with their CEC, i.e. greater CEC of soil indicates the more exchange sites on soil minerals accessible for metal retention. The increased soil CEC can also be correlated with organic carbon accumulation and increased electrical conductivity. In soils, competing ions also have a significant impact on ion sorption, as some of the metal cations (Cu, Zn, Cd and Pb) compete with more common soil cations such as Ca^{2+} and Mg^{2+} for specific as well as nonspecific exchangeable sites (Chen 2012).

4.3.2 Soil nutrient analysis

The effect of heavy metals on soil total and available macro (Nitrogen, phosphorous, potassium, calcium, magnesium, sulfur) and micro nutrients (Manganese, copper, iron and zinc) before (heavy metal equilibrated soil) and after uprooting of *Salix* and *Toona* were investigated and discussed below:

a. Nitrogen (N)

Data presented in Table 4.34 shows the effect of different concentrations of Pb and Cd on total and available nitrogen (N) of heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona*.

Table 4.34: **Effect of heavy metals on total and available nitrogen in soil before planting and after uprooting of *Salix alba* and *Toona ciliata***

Total nitrogen (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	320^{a}	310^{a}	279^{bc}	209^{fg}	280^{A}	309^{a}	302^{ab}	296^{abc}	215^{f}	281^{A}	314^{a}	306^{a}	288^{b}	212^{e}	280^{A}
Cd_5	288^{b}	285^{b}	269^{c}	203^{fg}	261^{B}	295^{abc}	287^{bcd}	283^{cde}	209^{f}	269^{B}	291^{b}	286^{b}	276^{b}	206^{ef}	265^{B}
Cd_{15}	278^{bc}	249^{d}	225^{e}	215^{ef}	242^{C}	285^{bcde}	272^{de}	268^{e}	209^{f}	259^{C}	282^{b}	261^{c}	247^{d}	212^{e}	250^{C}
Cd_{25}	202^{fg}	204^{fg}	196^{gh}	186^{h}	197^{D}	209^{f}	209^{f}	205^{f}	205^{f}	207^{D}	205^{ef}	206^{ef}	201^{ef}	196^{f}	202^{D}
Mean	272^{A}	262^{B}	242^{C}	203^{D}	**245**	274^{A}	268^{B}	263^{B}	210^{C}	**253**	273^{A}	265^{B}	253^{C}	207^{D}	**250**
LSD ($p \leq 0.05$)	Pb	4.89				Pb	5.53				Pb	4.76			
	Cd	4.89				Cd	5.53				Cd	4.76			
	Pb×Cd	9.79				Pb×Cd	11.06				Pb×Cd	9.52			
Available nitrogen (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	183^{a}	162^{b}	112^{e}	92.0^{g}	137^{A}	180^{a}	172^{ab}	144^{d}	109^{f}	151^{A}	182^{a}	167^{b}	128^{de}	101^{g}	144^{A}
Cd_5	157^{b}	142^{c}	102^{f}	92.1^{g}	123^{B}	169^{b}	155^{c}	139^{de}	111^{f}	143^{B}	163^{b}	148^{c}	121^{ef}	101^{g}	133^{B}
Cd_{15}	128^{d}	107^{ef}	92.3^{g}	81.2^{h}	102^{C}	135^{de}	130^{e}	102^{fg}	98.5^{g}	117^{C}	131^{d}	119^{f}	97.2^{gh}	89.8^{hi}	109^{C}
Cd_{25}	86.0^{gh}	70.0^{i}	63.3^{j}	52.7^{k}	68.0^{D}	99.5^{g}	96.6^{g}	96.6^{g}	93.7^{g}	96.6^{D}	92.8^{gh}	83.3^{ij}	80.0^{jk}	73.2^{k}	82.3^{D}
Mean	139^{A}	120^{B}	92.3^{C}	79.4^{D}	**108**	146^{A}	139^{B}	120^{C}	103^{D}	**127**	142^{A}	129^{B}	106^{C}	91.2^{D}	**117**
LSD ($p \leq 0.05$)	Pb	2.15				Pb	3. 02				Pb	2.88			
	Cd	2.15				Cd	3.02				Cd	2.88			
	Pb×Cd	4.30				Pb×Cd	6.05				Pb×Cd	5.76			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

Before planting, heavy metal equilibrated soil showed significant decline in both total and available nitrogen content compared to control. Under Pb concentrations, the total and available N was maximum in control i.e. Pb_0 (272 mg/kg and 139 mg/kg respectively) that decreased significantly with increase in Pb concentration, while minimum N (both total and available) was recorded under highest Pb concentration i.e. Pb_{300} (203 mg/kg and 79.4 mg/kg). Similarly, with Cd application both total and available N content was highest in control (280mg/kg and 137mg/kg respectively) that decreased significantly with increase in concentration from Cd_5 (261mg/kg and 123mg/kg respectively) to Cd_{25} (197mg/kg and 68mg/kg respectively).

Similar trend was observed after uprooting of *Salix* and *Toona* that significant decrease in total and available N of soil was recorded in response to Pb and Cd concentrations. On an average, total N of heavy metal equilibrated soil remains unaffected after uprooting of *Salix* and *Toona*. Meanwhile, available nitrogen was found to increase after uprooting of *Salix* and *Toona* by 8 % and 14.9% respectively with respect to heavy metal equilibrated soils.

b. Phosphorous (P)

The significant effect of different concentrations of Pb, Cd and their combinations on total and available phosphorous (P) of heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona* is depicted in Table 4.35. The significant decrease in total and phosphorous content was observed with increasing Pb and Cd concentrations.

The heavy metal equilibrated soil (before planting) exhibited significant decline in both total and available P in comparison to control. Under Pb concentrations, the total and available P was maximum in control i.e. Pb_0 (529 mg/kg and 44.8 mg/kg respectively) that decreased significantly with increase in concentration from Pb_{100} (493 mg/kg and 41.6 mg/kg respectively) to Pb_{300} (426 mg/kg and 33.1 mg/kg). Similarly, with Cd concentrations both total and available P content was maximum in control (518 mg/kg and 44.5 mg/kg respectively) which decreased significantly with increase in Cd concentration, thus recorded to be minimum with highest concentration i.e. Cd_{25} (395 mg/kg and 29.8 mg/kg respectively).

Similarly, after the uprooting of *Salix* and *Toona*, significant decrease in total and available P of soil was recorded in response to Pb and Cd concentrations. However, comparing the soil available phosphorous in before planting and after uprooting, a significant increase was found. On an average, available P in heavy metal equilibrated soil showed 15 % and 8.15 % increase after uprooting of *Salix* and *Toona* respectively. This may be attributed to comparatively more addition of organic matter and root exudates after plantation.

Table 4.35: Effect of heavy metals on total and available phosphorous in soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total phosphorous (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	597^{a}	543^{b}	485^{c}	448^{de}	518^{A}	602^{a}	564^{b}	508^{c}	471^{de}	536^{A}	599^{a}	553^{b}	496^{c}	459^{de}	527^{A}
Cd_5	578^{a}	512^{bc}	454^{d}	436^{de}	495^{B}	601^{a}	535^{bc}	477^{d}	459^{de}	518^{B}	589^{a}	523^{bc}	465^{d}	447^{de}	506^{B}
Cd_{15}	525^{b}	518^{b}	447^{de}	438^{de}	482^{C}	548^{b}	541^{b}	470^{de}	461^{de}	505^{C}	536^{b}	529^{b}	458^{de}	449^{de}	493^{C}
Cd_{25}	417^{ef}	398^{fg}	383^{g}	383^{g}	395^{D}	440^{ef}	421^{f}	421^{f}	417^{f}	424^{D}	428^{ef}	409^{f}	402^{f}	400^{f}	410^{D}
Mean	529^{A}	493^{B}	442^{C}	426^{D}	**473**	547^{A}	515^{B}	469^{C}	452^{D}	**496**	538^{A}	504^{B}	455^{C}	439^{D}	**484**
LSD ($p \leq 0.05$)	Pb	9.84				Pb	10.1				Pb	9.90			
	Cd	9.84				Cd	10.1				Cd	9.90			
	Pb×Cd	19.7				Pb×Cd	20.1				Pb×Cd	19.8			
Available phosphorous (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	50.9^{a}	46.3^{c}	42.5^{ef}	38.2^{g}	44.5^{A}	55.3^{a}	53.7^{ab}	49.9^{cd}	45.6^{ef}	51.1^{A}	53.1^{a}	50.0^{bc}	46.2^{de}	41.9^{fg}	47.8^{A}
Cd_5	48.3^{b}	45.8^{cd}	40.6^{f}	36.9^{g}	42.9^{B}	54.4^{a}	53.2^{ab}	48.0^{de}	44.3^{fg}	50.0^{B}	51.3^{ab}	49.5^{bc}	44.3^{ef}	40.6^{gh}	46.4^{B}
Cd_{15}	45.3^{cd}	43.8^{de}	40.7^{f}	31.6^{i}	40.4^{C}	52.7^{ab}	51.2^{bc}	48.1^{de}	39.0^{h}	47.8^{C}	49.0^{bcd}	47.5^{cd}	44.4^{ef}	35.3^{ef}	44.1^{C}
Cd_{25}	34.5^{h}	30.3^{ij}	28.6^{j}	25.8^{k}	29.8^{D}	41.9^{g}	37.7^{hi}	36.0^{i}	33.2^{j}	37.2^{D}	38.2^{h}	34.0^{i}	32.3^{i}	29.5^{j}	33.5^{D}
Mean	44.8^{A}	41.6^{B}	38.1^{C}	33.1^{D}	**39.4**	51.1^{A}	49.0^{B}	45.5^{C}	40.5^{D}	**46.5**	47.9^{A}	45.3^{B}	41.8^{C}	36.8^{D}	**42.9**
LSD ($p \leq 0.05$)	Pb	0.693				Pb	0.859				Pb	0.891			
	Cd	0.693				Cd	0.859				Cd	0.891			
	Pb×Cd	1.38				Pb×Cd	1.72				Pb×Cd	1.78			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

c. Potassium (K)

The data pertains to Table 4.36 depicts the effect of different concentrations of Pb and Cd on total and available potassium (K) of heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona*. The significant decrease in total and available K was observed with increasing Pb and Cd concentrations in soils.

Before planting, heavy metal equilibrated soil showed significant decline in both total and available potassium as compared to control. Under Pb concentrations, the total and available K was maximum in control i.e. Pb_0 (1057 mg/kg and 112 mg/kg respectively) that decreased significantly with increase in Pb concentration, while minimum K (both total and available) was recorded under highest Pb concentration i.e. Pb_{300} (774 mg/kg and 76.7 mg/kg). Similarly, with Cd application both total and available K content was maximum in control (1015 mg/kg and 107mg/kg respectively) and minimum in Cd_{25} treated soil (747.5 mg/kg and 78.7 mg/kg respectively).

Similar trend was observed after uprooting of *Salix* and *Toona* that significant decrease in total and available K was recorded in response to Pb and Cd concentrations. On an average, total K of heavy metal equilibrated soil showed 11 % and 5.85 % increase after uprooting of *Salix* and *Toona* respectively, similarly available K was also recorded to be increased after uprooting of *Salix* and *Toona* by 14.7 % and 9 % respectively with respect to heavy metal equilibrated soils.

d. Calcium (Ca)

Data presented in Table 4.37 reveals the negative effect of different concentrations of Pb, Cd and their combinations on total and available calcium (Ca) in heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona*.

The significant decline in both total and available Ca content was observed in heavy metal equilibrated soil (before planting) compared to control. Among Pb concentrations, the total and available Ca was maximum in control i.e. Pb_0 (4028 mg/kg and 1161 mg/kg respectively) that decreased significantly with increase in concentration from Pb_{100} (3837 mg/kg and 951 mg/kg respectively) to Pb_{300} (3122 mg/kg and 806 mg/kg). Similarly, with Cd concentrtions both total and available Ca content was maximum in control (4158 mg/kg and 1141 mg/kg respectively) which decreased significantly with increase in Cd concentration, thus recorded to be minimum with highest concentration i.e. Cd_{25} (3150 mg/kg and 821 mg/kg respectively).

Similarly, after the uprooting of *Salix* and *Toona* significant decrease in total and available Ca was recorded in response to Pb and Cd concentrations. On an average, the total Ca in heavy metal equilibrated soil remains unaffected after uprooting of *Salix* and *Toona*, while available Ca in heavy metal equilibrated soil showed 2.5 to 5% increase after uprooting of both *Salix* and *Toona*.

Table 4.36 Effect of heavy metals on total and available potassium in soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total potassium (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	1378^{a}	1046^{c}	856^{e}	780^{gh}	1015^{A}	1401^{a}	1161^{c}	971^{e}	895^{gh}	1107^{A}	1390^{a}	1104^{c}	914^{e}	838^{fg}	1061^{A}
Cd_5	1125^{b}	975^{d}	848^{e}	780^{gh}	932^{B}	1240^{b}	1090^{d}	963^{ef}	895^{gh}	1047^{B}	1183^{b}	1033^{d}	906^{e}	838^{fg}	990^{B}
Cd_{15}	973^{d}	825^{ef}	794^{fg}	793^{fg}	846.2^{C}	1088^{d}	940^{efg}	909^{fgh}	908^{fgh}	961.25^{C}	1031	883^{ef}	852^{efg}	851^{efg}	904^{C}
Cd_{25}	752^{gh}	752^{gh}	743^{h}	743^{h}	747.5^{D}	867^{h}	860^{h}	860^{h}	858^{h}	861.25^{D}	810^{g}	806^{g}	802^{g}	801^{g}	804^{D}
Mean	1057^{A}	900^{B}	810^{C}	774^{D}	**885**	1149^{A}	1013^{B}	926^{C}	889^{D}	**994**	1103^{A}	956^{B}	868^{C}	832^{D}	**940**
LSD ($p \leq 0.05$)	Pb	13.4				Pb	19.1				Pb	20.0			
	Cd	13.4				Cd	19.1				Cd	20.0			
	Pb×Cd	26.8				Pb×Cd	38.2				Pb×Cd	40.0			
Available potassium (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Cd_0	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_5	148^{a}	110^{c}	89.5^{de}	82.7^{fg}	107^{A}	151^{a}	122^{c}	102^{ef}	101^{ef}	119^{A}	149^{a}	116^{bc}	96.0^{bcde}	91.8^{cde}	113^{A}
Cd_{15}	121.5^{b}	105.7^{c}	85.6^{efg}	80.9^{fg}	98.4^{B}	140^{b}	124^{c}	104^{def}	99.1^{f}	116^{A}	131^{ab}	115^{bc}	94.7^{bcde}	90.0^{cde}	108^{A}
Cd_{25}	93.5^{d}	90.4^{de}	82.5^{fg}	80.9^{fg}	86.8^{C}	112^{d}	109^{de}	101^{ef}	99.1^{f}	105^{B}	103^{bcd}	99.5^{bcd}	91.6^{cde}	90.0^{cde}	96.1^{B}
Mean	86.4^{ef}	86.4^{ef}	79.4^{g}	62.5^{h}	78.7^{D}	105^{def}	105^{def}	97.6^{f}	80.7^{g}	96.8^{C}	95.5^{bcde}	95.5^{bcde}	88.5^{cde}	71.6^{de}	88.3^{B}
LSD ($p \leq 0.05$)	112^{A}	98.2^{B}	84.3^{C}	76.7^{D}	**92.9**	127^{A}	115^{B}	101^{C}	94.9^{D}	**109**	120^{A}	107^{B}	92.7^{C}	85.8^{D}	**101**
	Pb	2.03				Pb	2.59				Pb	2.17			
	Cd	2.03				Cd	2.59				Cd	2.17			
	Pb×Cd	4.06				Pb×Cd	5.18				Pb×Cd	4.35			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

Table 4.37 Effect of heavy metals on total and available calcium in soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total calcium (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	5001^{a}	4385^{b}	3852^{cd}	3395^{fg}	4158^{A}	4962^{a}	4458^{b}	3925^{cd}	3468^{fg}	4203^{A}	4982^{a}	4422^{b}	3889^{cd}	3432^{fg}	4181^{A}
Cd_5	4085^{c}	3964^{cd}	3784^{d}	3081^{hi}	3729^{B}	4158^{c}	4037^{cd}	3857^{d}	3154^{hi}	3802^{B}	4122^{c}	4001^{cd}	3821^{de}	3118^{hi}	3765^{B}
Cd_{15}	3752^{de}	3745^{de}	3532^{ef}	3025^{hi}	3514^{C}	3825^{de}	3818^{de}	3605^{ef}	3098^{hi}	3587^{C}	3789^{de}	3782^{de}	3569^{ef}	3062^{hi}	3550^{C}
Cd_{25}	3275^{gh}	3254^{gh}	3085^{hi}	2986^{i}	3150^{D}	3348^{gh}	3327^{gh}	3158^{hi}	3059^{i}	3223^{D}	3312^{fgh}	3291^{ghi}	3122^{hi}	3023^{i}	3187^{D}
Mean	4028^{A}	3837^{B}	3563^{C}	3122^{D}	**3638**	4073^{A}	3910^{B}	3636^{C}	3195^{D}	**3704**	4051^{A}	3874^{B}	3600^{C}	3158^{D}	**3671**
LSD ($p \leq 0.05$)	Pb	78.6				Pb	77.4				Pb	84.9			
	Cd	78.6				Cd	77.4				Cd	84.9			
	Pb×Cd	157.2				Pb×Cd	154.8				Pb×Cd	169.8			
Available calcium (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	1710^{a}	1059^{c}	969^{de}	825^{hi}	1141^{A}	1731^{a}	1106^{c}	1014^{de}	873^{ghi}	1181^{A}	1721^{a}	1083^{c}	992^{de}	849^{ghi}	1161^{A}
Cd_5	1135^{b}	1005^{d}	925^{ef}	810^{i}	969^{B}	1183^{b}	1053^{cd}	973^{ef}	858^{hi}	1017^{B}	1159^{b}	1029^{cd}	949^{ef}	834^{hi}	993^{B}
Cd_{15}	927^{ef}	914^{fg}	895^{fg}	806^{i}	886^{C}	975^{ef}	962^{f}	943^{efg}	854^{hi}	934^{C}	951^{ef}	938^{ef}	919^{efg}	830^{hi}	910^{C}
Cd_{25}	873^{gh}	825^{hi}	803^{i}	782^{i}	821^{D}	921^{fgh}	873^{ghi}	851^{hi}	830^{i}	869^{D}	897^{fgh}	849^{ghi}	827^{hi}	806^{i}	845^{D}
Mean	1161^{A}	951^{B}	898^{C}	806^{D}	**954**	1203^{A}	999^{B}	945^{C}	854^{D}	**1000**	1182^{A}	975^{B}	922^{C}	830^{D}	**977**
LSD ($p \leq 0.05$)	Pb	16.4				Pb	23.2				Pb	24.4			
	Cd	16.4				Cd	23.2				Cd	24.4			
	Pb×Cd	32.8				Pb×Cd	46.4				Pb×Cd	48.8			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

e. Magnesium (Mg)

The data pertains to Table 4.38 shows the significant effect of Pb, Cd and their combinations on both total and available magnesium (Mg) content of heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona*. Before planting, heavy metal equilibrated soil showed significant decline in both total and available Mg as compared to control. Under Pb concentrations, the total and available Mg content was maximum in control i.e. Pb_0 (2033 mg/kg and 173 mg/kg respectively) that decreased significantly with increase in Pb concentration, while minimum Mg (both total and available) was recorded under highest Pb concentration i.e. Pb_{300} (1885 mg/kg and 93.1 mg/kg). Similarly, with Cd application both total and available Mg content was maximum in control (2030 mg/kg and 169 mg/kg respectively) at par with Cd_5 (1980mg/kg and 151 mg/kg respectively) and minimum in Cd_{25} treated soil (1862 mg/kg and 89.3 mg/kg respectively).

Similar trend was observed after uprooting of *Salix* and *Toona* that significant decrease in total and available Mg content in response to Pb and Cd concentrations. Overall, total and available Mg content of heavy metal equilibrated soil remains unaffected after uprooting of *Salix* and *Toona*.

f. Sulphur (S)

Data presented in Table 4.39 shows the effect of different concentrations of Pb and Cd on total and available nitrogen (S) of heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona*. With increasing concentrations of Pb and Cd, significant decrease in total and available S content was recorded in soils.

Before planting, heavy metal equilibrated soil showed significant decline in both total and available sulphur content compared to control. Under Pb concentrations, the total and available S was maximum in control i.e. Pb_0 (433 mg/kg and 17.6 mg/kg respectively) that decreased significantly with increase in Pb concentration, while minimum S (both total and available) was recorded under highest Pb concentration i.e. Pb_{300} (386 mg/kg and 9 mg/kg) which is statistically equivalent with Pb_{200} (395 mg/kg and 10.7 mg/kg). Similarly, with Cd application both total and available S content was highest in control (424 mg/kg and 16.9 mg/kg respectively) that decreased significantly with increase in concentration, thus maximum with highest Cd_{25} cpncentration (385 mg/kg and 9.2 mg/kg respectively).

Similar trend was observed after uprooting of *Salix* and *Toona* that significant decrease in total and available S in soil was recorded in response to Pb and Cd concentrations. On an average, total S of heavy metal equilibrated soil remains unaffected after uprooting of *Salix* and *Toona*. Meanwhile, available S content was found to increase after uprooting of *Salix* and *Toona* by 4% -7 % with respect to heavy metal equilibrated soils.

Table 4.38 Effect of heavy metals on total and available magnesium in soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total magnesium (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	2199^{a}	2058^{bc}	1939^{cde}	1922^{cde}	2030^{A}	2212^{a}	2054^{bc}	1933^{cde}	1914^{cde}	2028^{A}	2206^{a}	2056^{bc}	1936^{cde}	1918^{cde}	2028^{A}
Cd_5	2118^{ab}	2005^{bcd}	1904^{de}	1893^{de}	1980^{A}	2115^{ab}	1997^{bcd}	1897^{de}	1891^{de}	1975^{B}	2117^{ab}	2001^{bcd}	1901^{de}	1892^{de}	1978^{A}
Cd_{15}	1929^{cde}	1912^{de}	1898^{de}	1886^{de}	1906^{B}	1925^{cde}	1903^{de}	1895^{de}	1884^{de}	1901^{C}	1927^{cde}	1908^{de}	1897^{de}	1885^{de}	1904^{B}
Cd_{25}	1885^{de}	1882^{de}	1843^{e}	1837^{e}	1862^{B}	1880^{de}	1876^{de}	1836^{e}	1828^{e}	1855^{C}	1883^{de}	1879^{de}	1840^{e}	1833^{e}	1856^{B}
Mean	2033^{A}	1964^{B}	1896^{C}	1885^{D}	**1944**	2041^{A}	1958^{AB}	1890^{C}	1879^{C}	**1940**	2038^{A}	1961^{B}	1893^{C}	1882^{C}	**1946**
LSD ($p\leq0.05$)	Pb	43.8				Pb	44.4				Pb	44.2			
	Cd	43.8				Cd	44.4				Cd	44.2			
	Pb×Cd	87.7				Pb×Cd	88.7				Pb×Cd	88.3			
Available magnesium (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	248^{a}	196^{b}	135^{e}	98.5^{f}	169^{A}	239^{a}	183^{c}	157^{e}	106^{h}	171^{A}	244^{a}	190^{b}	146^{e}	102^{h}	168^{A}
Cd_5	190^{bc}	185^{c}	133^{e}	95.4^{f}	151^{B}	195^{b}	191^{b}	141^{f}	104^{h}	158^{B}	193^{b}	188^{b}	137^{f}	99.9^{h}	154^{B}
Cd_{15}	165^{d}	159^{d}	128^{e}	90.2^{f}	136^{C}	171^{d}	165^{d}	131^{g}	95.2^{i}	141^{C}	168^{c}	162^{d}	130^{g}	92.7^{i}	138^{C}
Cd_{25}	90.5^{f}	89.2^{f}	89.2^{f}	88.1^{f}	89.3^{D}	94.5^{i}	92.2^{i}	92.2^{i}	88.1^{i}	91.8^{D}	92.5^{i}	90.7^{i}	90.7^{i}	88.1^{i}	90.5^{D}
Mean	173^{A}	157^{B}	121^{C}	93.1^{D}	**132**	175^{A}	158^{B}	130^{C}	98.4^{D}	**140**	174^{A}	158^{B}	126^{C}	95.7^{D}	**133**
LSD ($p\leq0.05$)	Pb	3.49				Pb	2.72				Pb	1.98			
	Cd	3.49				Cd	2.72				Cd	1.98			
	Pb×Cd	6.99				Pb×Cd	5.44				Pb×Cd	3.97			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p\leq0.05$)

Table 4.39 Effect of heavy metals on total and available sulphur in soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total sulphur (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	475^{a}	428^{b}	402^{bcd}	392^{cd}	424^{A}	453^{a}	414^{b}	394^{bcd}	386^{bcd}	412^{A}	464^{a}	421^{b}	398^{bcde}	389^{cde}	418^{A}
Cd_5	452^{a}	406^{bcd}	398^{cd}	386^{d}	411^{B}	446^{a}	400^{bcd}	392^{bcd}	380^{d}	405^{AB}	449^{a}	403^{bcde}	395^{cde}	383^{de}	408^{B}
Cd_{15}	417^{bc}	410^{bcd}	393^{cd}	385^{d}	401^{B}	411^{bc}	404^{bcd}	387^{bcd}	379^{d}	395^{B}	414^{bc}	407^{bcd}	390^{cde}	382^{de}	398^{C}
Cd_{25}	387^{d}	385^{d}	385^{d}	382^{d}	385^{C}	381^{cd}	381^{cd}	378^{d}	376^{d}	379^{C}	384^{de}	383^{de}	382^{de}	379^{e}	382^{D}
Mean	433^{A}	407^{B}	395^{C}	386^{C}	**405**	423^{A}	400^{B}	388^{BC}	380^{C}	**398**	428^{A}	404^{B}	391^{BC}	383^{C}	**401**
LSD ($p \leq 0.05$)	Pb	8.34				Pb	8.74				Pb	7.81			
	Cd	8.34				Cd	8.74				Cd	7.81			
	Pb×Cd	16.7				Pb×Cd	17.4				Pb×Cd	15.6			
Available sulphur (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	26.3^{a}	19.5^{c}	12.4^{f}	9.27^{ijk}	16.9^{A}	24.5^{a}	20.9^{c}	14.9^{f}	11.8^{i}	18.0^{A}	25.4^{a}	20.2^{c}	13.7^{f}	10.5^{ij}	17.4^{A}
Cd_5	20.4^{b}	15.3^{d}	11.5^{g}	8.95^{jk}	14.0^{B}	22.9^{b}	17.8^{d}	14.0^{fg}	11.5^{i}	16.5^{B}	21.7^{b}	16.6^{d}	12.8^{g}	10.2^{ij}	15.3^{B}
Cd_{15}	14.3^{e}	10.6^{h}	9.85^{i}	8.66^{k}	10.9^{C}	16.8^{e}	13.1^{gh}	12.4^{hi}	11.2^{i}	13.4^{C}	15.6^{e}	11.9^{h}	11.1^{hi}	9.91^{j}	12.1^{C}
Cd_{25}	9.50	9.25^{ij}	8.97^{jk}	8.92^{jk}	9.2^{D}	12.0^{i}	11.8^{i}	11.8^{i}	11.4^{i}	11.7^{D}	10.8^{ij}	10.5^{ij}	10.4^{ij}	10.2^{ij}	10.4^{D}
Mean	17.6^{A}	13.7^{B}	10.7^{C}	9.0^{D}	**12.7**	19.1^{A}	15.9^{B}	13.3^{C}	11.5^{D}	**14.9**	18.3^{A}	14.8^{B}	12.0^{C}	10.2^{D}	**13.8**
LSD ($p \leq 0.05$)	Pb	0.196				Pb	0.351				Pb	0.289			
	Cd	0.196				Cd	0.351				Cd	0.289			
	Pb×Cd	0.392				Pb×Cd	0.701				Pb×Cd	0.579			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

g. Manganese (Mn)

The significant effect of different concentrations of Pb, Cd and their combinations on total and available manganese (Mn) of heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona* is depicted in Table 4.40. The significant decrease in total and available manganese content was observed with increasing Pb and Cd concentrations.

The heavy metal equilibrated soil (before planting) exhibited significant decline in both total and available Mn in comparison to control. Under Pb concentrations, the total and available Mn was maximum in control i.e. Pb_0 (394 mg/kg and 44.3 mg/kg respectively) that decreased significantly with increase in concentration from Pb_{100} (360 mg/kg and 39.8 mg/kg respectively) to Pb_{300} (294 mg/kg and 25.5 mg/kg). Similarly, with Cd concentrtions both total and available Mn content was maximum in control (401 mg/kg and 44.1 mg/kg respectively) which decreased significantly with increase in Cd concentration, thus recorded to be minimum with highest concentration i.e. Cd_{25} (291 mg/kg and 24.7 mg/kg respectively).

Similarly, after the uprooting of *Salix* and *Toona*, significant decrease in total and available Mn in soil was recorded in response to Pb and Cd concentrations, however, total and available Mn in heavy metal equilibrated soil remains unaffected after uprooting of *Salix* and *Toona* respectively.

h. Copper (Cu)

The data pertains to Table 4.41 depicts the effect of different concentrations of Pb and Cd on total and available copper (Cu) in heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona*. The significant decrease in total and available Cu was observed with increasing Pb and Cd concentrations in soils.

Before planting, heavy metal equilibrated soil showed significant decline in both total and available copper as compared to control. Under Pb concentrations, the total and available Cu was maximum in control i.e. Pb_0 (10.6 mg/kg and 1.6 mg/kg respectively) that decreased significantly with increase in Pb concentration, while minimum Cu (both total and available) was recorded under highest Pb concentration i.e. Pb_{300} (7.65 mg/kg and 0.92 mg/kg). Similarly, with Cd application both total and available Cu content was maximum in control (11.0 mg/kg and 1.70 mg/kg respectively) and minimum in Cd_{25} treated soil (7.01 mg/kg and 0.96 mg/kg respectively).

Similar trend was observed after uprooting of *Salix* and *Toona* that significant decrease in total and available Cu was recorded in response to Pb and Cd concentrations. On an average, total Cu in heavy metal equilibrated soil remains unaffected, but the available Cu content recorded with 15 to 18% decrease after uprooting of *Salix* and *Toona*.

Table 4.40 Effect of heavy metals on total and available manganese in soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total manganese (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	532^{a}	425^{b}	349^{d}	298^{e}	401^{A}	463^{a}	383^{b}	286^{g}	248^{h}	345^{A}	498^{a}	404^{b}	318^{d}	273^{e}	373^{A}
Cd_5	396^{c}	385^{c}	348^{d}	295^{e}	356^{B}	354^{c}	335^{d}	310^{ef}	256^{h}	314^{B}	375^{c}	360^{c}	329^{d}	276^{e}	335^{B}
Cd_{15}	352^{d}	338^{d}	329^{d}	293^{e}	328^{C}	304^{ef}	311^{e}	294^{fg}	258^{h}	292^{C}	328^{d}	325^{d}	311^{d}	276^{e}	310^{C}
Cd_{25}	296^{e}	290^{e}	289^{e}	289^{e}	291^{D}	255^{h}	265^{h}	259^{h}	250^{h}	257^{D}	275^{e}	277^{e}	274^{e}	269^{e}	274^{D}
Mean	394^{A}	360^{B}	329^{C}	294^{D}	**344**	344^{A}	324^{B}	287^{C}	253^{D}	**302**	369^{A}	342^{B}	308^{C}	273^{D}	**323**
LSD ($p \leq 0.05$)	Pb	7.53				Pb	5.60				Pb	7.05			
	Cd	7.53				Cd	5.60				Cd	7.05			
	Pb×Cd	15.1				Pb×Cd	11.2				Pb×Cd	14.1			
Available manganese (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	63.8^{a}	50.6^{b}	32.5^{e}	29.7^{ef}	44.1^{A}	60.3^{a}	51.3^{b}	34.0^{f}	31.0^{gh}	42.8^{A}	62.1^{a}	50.9^{b}	33.3^{e}	30.3^{f}	44.3^{A}
Cd_5	45.6^{c}	43.8^{c}	30.6^{ef}	26.4^{gh}	36.6^{B}	46.9^{c}	45.1^{d}	31.9^{g}	27.7^{i}	38.1^{B}	46.2^{c}	44.4^{c}	31.2^{ef}	27.0^{ghi}	35.2^{B}
Cd_{15}	39.5^{d}	38.8^{d}	28.7^{fg}	25.4^{gh}	33.1^{C}	40.8^{e}	40.1^{e}	29.9^{h}	26.7^{ij}	34.5^{C}	40.1^{d}	39.4^{d}	29.3^{fg}	26.0^{i}	34.1^{C}
Cd_{25}	28.3^{fg}	25.8^{h}	24.2^{gh}	20.5^{i}	24.7^{D}	29.6^{h}	27.1^{i}	25.5^{j}	21.8^{k}	26.1^{D}	28.9^{fgh}	26.4^{hi}	24.9^{i}	21.1^{j}	26.4^{D}
Mean	44.3^{A}	39.8^{B}	29.0^{C}	25.5^{D}	**34.6**	44.4^{A}	40.9^{B}	30.3^{C}	26.8^{D}	**35.6**	44.3^{A}	40.3^{B}	29.7^{C}	26.1^{D}	**35.1**
LSD ($p \leq 0.05$)	Pb	1.02				Pb	0.491				Pb	0.829			
	Cd	1.02				Cd	0.491				Cd	0.829			
	Pb×Cd	2.04				Pb×Cd	0.983				Pb×Cd	1.66			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

Table 4.41. Effect of heavy metals on total and available copper in soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total copper (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	14.7^{a}	11.6^{b}	9.53^{de}	8.26^{hi}	11.0^{A}	12.3^{a}	12.0^{a}	9.96^{cd}	8.69^{f}	10.7^{A}	13.5^{a}	11.8^{b}	9.75^{de}	8.48^{hi}	10.9^{A}
Cd_5	10.3^{c}	9.95^{cd}	8.93^{fg}	8.15^{hi}	9.34^{B}	10.8^{b}	10.4^{bc}	9.36^{de}	8.58^{f}	9.77^{B}	10.5^{c}	10.2^{cd}	9.15^{fg}	8.37^{hi}	9.55^{B}
Cd_{15}	9.51^{de}	9.24^{ef}	8.46^{gh}	7.95^{hi}	8.79^{C}	9.94^{cd}	9.67^{d}	8.89^{ef}	8.38^{f}	9.22^{C}	9.73^{de}	9.46^{ef}	8.68^{gh}	8.17^{hi}	9.01^{C}
Cd_{25}	7.85^{i}	7.09^{j}	6.83^{j}	6.25^{k}	7.01^{D}	8.28^{f}	7.52^{g}	7.26^{gh}	6.68^{h}	7.44^{D}	8.07^{i}	7.31^{j}	7.05^{j}	6.47^{k}	7.22^{D}
Mean	10.6^{A}	9.47^{B}	8.44^{C}	7.65^{D}	**9.04**	10.3^{A}	9.90^{B}	8.87^{C}	8.08^{D}	**9.29**	10.45^{A}	9.69^{B}	8.65^{C}	7.87^{D}	**9.16**
LSD ($p \leq 0.05$)	Pb	0.188				Pb	0.217				Pb	0.188			
	Cd	0.188				Cd	0.217				Cd	0.188			
	Pb×Cd	0.376				Pb×Cd	0.435				Pb×Cd	0.376			
Available copper (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	2.34^{a}	1.95^{c}	1.53^{e}	0.98^{hi}	1.70^{A}	2.13^{a}	1.74^{c}	1.32^{e}	0.77^{hi}	1.49^{A}	2.24^{a}	1.85^{c}	1.43^{e}	0.875^{hi}	1.60^{A}
Cd_5	2.08^{b}	1.85^{d}	1.51^{e}	0.92^{ij}	1.59^{B}	1.87^{b}	1.64^{d}	1.30^{e}	0.71^{ij}	1.38^{B}	1.98^{b}	1.75^{d}	1.41^{e}	0.815^{ij}	1.49^{B}
Cd_{15}	1.15^{f}	1.05^{gh}	0.95^{ij}	0.92^{ij}	1.02^{C}	0.94^{f}	0.84^{gh}	0.74^{ij}	0.71^{ij}	0.81^{C}	1.05^{f}	0.945^{gh}	0.845^{ij}	0.815^{ij}	0.90^{C}
Cd_{25}	1.08^{fg}	0.98^{hi}	0.92^{ij}	0.86^{j}	0.96^{D}	0.87^{fg}	0.77^{hi}	0.71^{ij}	0.65^{j}	0.75^{D}	0.975^{fg}	0.875^{hi}	0.815^{ij}	0.755^{j}	0.86^{D}
Mean	1.66^{A}	1.46^{B}	1.23^{C}	0.920^{D}	**1.32**	1.45^{A}	1.25^{B}	1.02^{C}	0.710^{D}	**1.11**	1.56^{A}	1.35^{B}	1.12^{C}	0.815^{D}	**1.23**
LSD ($p \leq 0.05$)	Pb	0.03				Pb	0.028				Pb	0.031			
	Cd	0.03				Cd	0.028				Cd	0.031			
	Pb×Cd	0.06				Pb×Cd	0.057				Pb×Cd	0.06			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

i. Iron (Fe)

Data presented in Table 4.42 reveals the negative effect of different concentrations of Pb, Cd and their combinations on total and available iron (Fe) content in heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona*.

The significant decline in both total and available Fe content was observed in heavy metal equilibrated soil (before planting) compared to control. Among Pb concentrations, the total and available Fe was maximum in control i.e. Pb_0 (9635 mg/kg and 162 mg/kg respectively) that decreased significantly with increase in concentration from Pb_{100} (8725 mg/kg and 77.4 mg/kg respectively) to Pb_{300} (5905 mg/kg and 27.2 mg/kg). Similarly, with Cd concentrtions both total and available Fe content was maximum in control (9570 mg/kg and 102 mg/kg respectively) which decreased significantly with increase in Cd concentration, thus recorded to be minimum with highest concentration i.e. Cd_{25} (6551 mg/kg and 30.2 mg/kg respectively).

Similarly, after the uprooting of *Salix* and *Toona* significant decrease in total and available Fe was recorded in response to Pb and Cd concentrations. On an average, both total and available Fe decreased (10-15%) after uprooting of both *Salix* and *Toona*.

j. Zinc (Zn)

The data pertains to Table 4.43 shows the significant effect of Pb, Cd and their combinations on both total and available zinc (Zn) content of heavy metal equilibrated soils (before planting) and after uprooting of *Salix* and *Toona*. Before planting, heavy metal equilibrated soil showed significant decline in both total and available Zn as compared to control. Under Pb concentrations, the total and available Zn content was maximum in control i.e. Pb_0 (36.4 mg/kg and 3.24 mg/kg respectively) that decreased significantly with increase in Pb concentration, while minimum Zn (both total and available) was recorded under highest Pb concentration i.e. Pb_{300} (27.1 mg/kg and 2.01 mg/kg). Similarly, with Cd application both total and available Zn content was maximum in control (37.8 mg/kg and 3.54 mg/kg respectively) and minimum in Cd_{25} treated soil (25.8 mg/kg and 1.67 mg/kg respectively).

Similar trend was observed after uprooting of *Salix* and *Toona* that significant decrease in total and available Zn content in response to Pb and Cd concentrations. Overall, total Zn content of heavy metal equilibrated soil remains unaffected after uprooting of *Salix* and *Toona*, whereas available Zn content was increased from 20% to 26% after uprooting of *Salix* and *Toona* as compared to heavy metal equilibrated soil.

Table 4.42. Effect of heavy metals on total and available iron in soils before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total iron (mg/kg)															
Before planting						**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	13248^{a}	10974^{b}	8085^{ef}	5972^{h}	9570^{A}	9953^{a}	9482^{b}	6593^{e}	4480^{g}	7627^{A}	11601^{a}	10228^{b}	7339^{ef}	5226^{h}	8598^{A}
Cd_5	9758^{c}	8598^{d}	8024^{f}	5964^{h}	8086^{B}	8266^{c}	7106^{d}	6532^{e}	4472^{g}	6594^{B}	9012^{c}	7852^{d}	7278^{ef}	5218^{h}	7340^{B}
Cd_{15}	8593^{d}	8485^{de}	7946^{f}	5857^{h}	7720^{C}	7101^{d}	6993^{d}	6454^{e}	4365^{g}	6228^{C}	7847^{d}	7739^{de}	7200^{f}	5111^{h}	6974^{C}
Cd_{25}	6942^{g}	6842^{g}	6592^{g}	5826^{h}	6551^{D}	5450^{f}	5350^{f}	5100^{f}	4334^{g}	5058^{D}	6196^{g}	6096^{g}	5846^{g}	5080^{h}	5805^{D}
Mean	9635^{A}	8725^{B}	7662^{C}	5905^{D}	**7982**	7693^{A}	7233^{B}	6170^{C}	4413^{D}	**6377**	8664^{A}	7979^{B}	6916^{C}	5159^{C}	**7179**
LSD ($p \leq 0.05$)	Pb	149.9				Pb	127.6				Pb	161.2			
	Cd	149.9				Cd	127.6				Cd	161.2			
	Pb×Cd	229.8				Pb×Cd	255.2				Pb×Cd	322.4			
Available iron (mg/kg)															
Before planting						**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	162.7^{a}	118.2^{b}	96.4^{d}	30.6^{h}	102^{A}	138.7^{a}	105.2^{b}	91.4^{c}	25.6^{g}	90.2^{A}	150.7^{a}	111.7^{b}	93.9^{d}	28.1^{h}	96.1^{A}
Cd_5	109^{c}	100.5^{d}	72.5^{e}	29.6^{hi}	78.0^{B}	104.2^{b}	95.5^{c}	67.5^{d}	24.6^{gh}	73.0^{B}	106.7^{c}	98.0^{d}	70.0^{e}	27.1^{h}	75.5^{B}
Cd_{15}	98.4^{d}	60.4^{f}	25.7^{ij}	25.8^{ij}	52.6^{C}	93.4^{c}	55.4^{e}	20.7^{hi}	20.8^{hi}	47.6^{C}	95.9^{d}	57.9^{f}	23.2^{hi}	23.3^{hi}	50.1^{C}
Cd_{25}	38.8^{g}	30.5^{h}	28.9^{hi}	22.6^{j}	30.2^{D}	33.8^{f}	25.5^{g}	23.9^{gh}	17.6^{i}	25.2^{D}	36.3^{g}	28.0^{h}	26.4^{h}	20.1^{i}	27.7^{D}
Mean	102.3^{A}	77.4^{B}	55.9^{C}	27.2^{D}	**65.7**	92.5^{A}	70.4^{B}	50.9^{C}	22.2^{D}	**59.0**	97.4^{A}	73.9^{B}	53.4^{C}	24.7^{D}	**62.3**
LSD ($p \leq 0.05$)	Pb	1.43				Pb	1.36				Pb	1.60			
	Cd	1.43				Cd	1.36				Cd	1.60			
	Pb×Cd	2.86				Pb×Cd	2.72				Pb×Cd	3.21			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

Table 4.43. Effect of heavy metals on total and available zinc in soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total zinc (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	48.8^{a}	42.6^{b}	30.7^{ef}	28.9^{fg}	37.7^{A}	45.1^{a}	39.3^{b}	27.4^{ef}	25.6^{fg}	34.3^{A}	47.0^{a}	41.0^{b}	29.0^{ef}	27.2^{fg}	36.0^{A}
Cd_5	38.5^{c}	35.6^{d}	29.4^{efg}	28.6^{fgh}	33.0^{B}	35.2^{c}	32.3^{d}	26.1^{efg}	25.3^{fgh}	29.7^{B}	36.8^{c}	33.9^{d}	27.7^{efg}	26.9^{fgh}	31.4^{B}
Cd_{15}	31.5^{e}	29.5^{efg}	27.8^{ghi}	26.3^{hij}	28.8^{C}	28.2^{e}	26.2^{efg}	24.5^{ghi}	23.0^{ij}	25.4^{C}	29.8^{e}	27.8^{efg}	26.1^{ghi}	24.6^{hij}	27.1^{C}
Cd_{25}	26.6^{hij}	26.3^{hij}	25.8^{ij}	24.7^{j}	25.8^{D}	23.3^{hij}	23.0^{ij}	22.5^{ij}	21.4^{j}	22.5^{D}	24.9^{hij}	24.6^{hij}	24.1^{ij}	23.0^{j}	24.2^{D}
Mean	36.4^{A}	33.5^{B}	28.4^{C}	27.1^{D}	**31.4**	32.9^{A}	30.2^{B}	25.1^{C}	23.8^{D}	**28.0**	34.6^{A}	31.8^{B}	26.8^{C}	25.5^{D}	**29.7**
LSD ($p \leq 0.05$)	Pb	0.726				Pb	0.669				Pb	0.724			
	Cd	0.726				Cd	0.669				Cd	0.724			
	Pb×Cd	1.453				Pb×Cd	1.34				Pb×Cd	1.45			
Available zinc (mg/kg)															
	Before planting					**After uprooting of *Salix***					**After uprooting of *Toona***				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	4.24^{a}	4.05^{b}	3.52^{c}	2.36^{g}	3.54^{A}	5.08^{a}	4.97^{a}	4.5^{b}	3.35^{f}	4.48^{A}	4.66^{a}	4.51^{ab}	4.01^{c}	2.86^{g}	4.01^{A}
Cd_5	3.95^{b}	3.27^{d}	2.96^{e}	2.46^{g}	3.16^{B}	4.94^{a}	4.26^{c}	3.95^{d}	3.45^{f}	4.15^{B}	4.45^{b}	3.77^{d}	3.46^{e}	2.96^{g}	3.72^{B}
Cd_{15}	2.68^{f}	2.46^{g}	2.04^{h}	1.98^{h}	2.29^{C}	3.67^{e}	3.45^{f}	3.03^{g}	2.97^{g}	3.28^{C}	3.18^{f}	2.96^{g}	2.54^{h}	2.48^{h}	2.83^{C}
Cd_{25}	2.07^{h}	1.97^{h}	1.38^{i}	1.25^{i}	1.67^{D}	3.06^{g}	2.96^{g}	2.37^{h}	2.24^{h}	2.66^{D}	2.57^{h}	2.47^{h}	1.88^{i}	1.75^{i}	2.24^{D}
Mean	3.24^{A}	2.94^{B}	2.48^{C}	2.01^{D}	**2.67**	4.19^{A}	3.91^{B}	3.46^{C}	3.00^{D}	**3.64**	3.71^{A}	3.42^{B}	2.97^{C}	2.51^{D}	**3.15**
LSD ($p \leq 0.05$)	Pb	0.061				Pb	0.055				Pb	0.07			
	Cd	0.061				Cd	0.055				Cd	0.07			
	Pb×Cd	0.122				Pb×Cd	0.110				Pb×Cd	0.14			

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

Hence, the present results conferred that Pb and Cd application in soil negatively affects the quantity of total and available forms of all studied macro (N, P, K, Ca, Mg, S) and micro (Mn, Cu, Fe, Zn) nutrients in the soil, however the drastic effect was observered in available nutrient content compared to total nutrient content in soil. Among all the nutrients, Pb and Cd concentrations adversaly affect the Mn, Fe and Zn availability to plants which further results into decrease in corresponding nutrient content in plants.

The preset results are in aggrement with Zhen *et al* (2019), who reported that heavy metal concentrations in soils alters the soil properties along with availability of essential nutrient content due to change in pH, organic matter and soil enzyme activities which are identified as key factors for shaping soil ecological functions. Heavy metal contamination reduces the plant growth by affecting soil microbial population which have significant role in regulating nutrient availability in soil (Wei *et al* 2020). Hamid *et al* (2020) reported that cadmium contamination in soil affects the availability of other nutrients (N, K, Mn, Cu, Fe, Zn), but the use of organic amendments (biochar and bio-wastes as soil additives) can decrease the Cd availability in soil and their associated toxic effect in plant-soil ecosystem.

At the termination of experiment i.e. after uprooting of *Salix* and *Toona*, 10 to 15% increase in some available nutrients (N, P, K, Ca, Zn and Mn) were recorded, this might be due to addition of organic matter and root exudates by plants. The rhizosphere is the primary route through which plants obtain their mineral nutrients. To control nutrient availability and detoxify unwanted metal contaminants in soil, plants produce a huge variety of metabolites into the rhizosphere. Generally, root exudates are complex mixture of organic acids, phytosiderophores, sugars, vitamins, amino acids and inorganic ions (such as HCO_3, OH, and H^+) along with gaseous molecules (CO_2, H_2) that have a significant direct or indirect impact on the nutrient bioavailabity in soil and uptake of mineral nutrients for plant development (Dakora and Phillips 2002). Chen *et al* (2017) reported that root exudates plays crucial role in bioavailability of soil, metal uptake, aaquisition and tolerance in plants grown on heavy metal contaminated soil. Bian *et al* (2021) reported that heavy metal contamination in soil negatively affects the nutrient composition in plant and soil system, which results in declined biomass in bamboo species, these results are in line with present study.

CHAPTER – V

SUMMARY

The present investigations entitled "Evaluation of morpho-physiological and metal accumulation potential of *Salix alba* L. and *Toona ciliata* M. Roemer grown on heavy metal contaminated soils" was performed to study the morphological, physiological and biochemical characteristics of *Salix* and *Toona* plants in response to different concentrations of lead (Pb), cadmium (Cd) and their combinations (Pb+Cd). Along with this, metal accumulation efficiency of both *Salix* and *Toona* was evaluated on the basis of their differential mechanism of metal uptake, translocation and bioaccumulation in different plant parts (root. stem and leaves).

Fresh cuttings of 15-20cm length of *Salix alba* were prepared from one year plants and were planted (one cutting/pot) in treated soil, whereas nursery of *Toona ciliata* was first raised in small polybags and transplanted when they attained the height of approx. 15cm in treated soils in the month of February in the year 2020 and 2021. The pot experiment was carried out with tested soil (5 kg/pot) and was arranged in factorial completely randomized design (CRD) for 16 treatment levels of Pb (100 mg/kg, 200 mg/kg and 300 mg/kg) and Cd (5 mg/kg, 15 mg/kg and 25 mg/kg) single as well as in combinations. For the preparation of heavy metal-enriched media, stock solutions of lead ($Pb(NO_3)_2$) and cadmium ($Cd(NO_3)_2$) were freshly prepared in the laboratory, and soil in pots treated with different concentration levels of Pb and Cd. The morpho-physiological and biochemical observations were recorded after three and six months.

The significant decrease in survival percentage of *Salix* was observed in response to different Pb and Cd concentrations, but the survival percentage of *Toona* plants remains unaffected even at higher Pb and Cd ($Pb_{300}Cd_{25}$) concentrations. However, *Salix* showed sufficient survival percentage (> 85%) with Pb_{200} and Cd_{15} concentrations, further increase in concentration leads to drastic reduction in survival percentage. The effect of Cd concentrations on survival percentage of *Salix* was recorded to be more than the Pb concentrations, but the effect of their combination concentrations was more pronounced in an order as Pb+Cd > Cd > Pb.

With increase in heavy metal (Pb and Cd) concentrations, significant reduction in growth and biomass traits i.e. plant height (cm), collar diameter (cm), root length (cm), root number, number of branches, number of leaves, fresh and dry weight of roots and shoots (g) was observed in both *Salix* and *Toona*, however the reduction was more prominent in *Salix* than *Toona*. The significant reduction in pigment (total chlorophyll and carotenoid) concentration of both *Salix* and *Toona* was observed due to Pb and Cd concentrations. Physiological and biochemical parameters such as proline, total soluble sugars, total soluble proteins and total antioxidants content showed enhanced accumulation in leaves due to

increased Pb and Cd concentratons treatments. Thus, the accumulation of organic osmolyte (proline) and metabolites (total soluble sugars and total soluble proteins) along with increased activity of antioxidant enzymes (peroxidase, catalase and superoxide dismutase) conferred the enhanced tolerance against heavy metal stress in both *Salix* and *Toona*.

Anatomical studies revealed that both tree species *Salix* and *Toona* showed different mechanism to cope up with heavy meta1 stress. In *Salix*, a marked decrease in stomatal pore size, stomatal density and trichome density were observed in heavy metal treated plants ($Pb_{300}Cd_{25}$) as compared to control. The rapid and preferential metal absorption by subsidiary cells followed by alterations in membrane permeability that resulted in a drop in cell turgor, which could be considered as the cause of smaller stomatal pores of plants under heavy metal stress. Additionally, increased concentrations of Pb and Cd modify ultrastructure of stomata, which would alter the normal stomatal functioning that affects photosynthetic rate and hence reduced biomass. The trichome density was also reduced under heavy metal stress in *Salix*, because Pb and Cd accumulation in leaves possess toxicity by inducing inhibitory signal that negatively affects the trichome development and further reduce the total trichome number on leaf epidermal surface. The decrease in trichome density might be due to chlorosis and necrotic spots that disrupts the normal development and functioning of epidermal layer in *Salix*, however, as such no toxicity symptom was observed in leaves of *Toona* under Pb and Cd stress.

Toona plants showed increased stomatal density and trichome density along with decreased stomatal pore size with $Pb_{300}Cd_{25}$ concentrations. Thus, the higher number of stomata with reduced stomatal pore size can be considered as adaptive mechanism of plant for biomass production under heavy metal stress conditions, as increased stomatal density maintains sufficient CO_2 flow for photosynthesis leading to less effect on biomass and decreased stomatal pore size to reduce excess water loss through transpiration. Along with this, *Toona* plants showed increased trichome density in response to heavy metals as compared to control which might be considered as plant adaptative mechanism, that maintain the reduced toxic ion concentration in internal leaf tissues as large amount of metals accumulate in trichomes, from where it can be easily removed without interfering plant metabolism.

The energy dispersive x-ray spectroscopy (EDS) predicts the Pb and Cd accumulation sites in different tissue of root and leaves of both *Salix* and *Toona*. EDS results of *Salix* roots concluded that Pb accumulation was higher in outer (epidermal and cortex) region, whereas Cd accumulation was highest in vascular region that is due to the mobile nature of Cd as it is easily transported through endodermal barrier. Since, the Cd loading in root vascular channel was higher than Pb, the EDS values of leaves showed the maximum distribution of Cd over Pb in whole leaf tissues. In *Toona,* EDS conferred higher Pb accumulation than Cd in

epidermal, cortex and vascular region of roots, similarly higher Pb accumulation over Cd was recorded in all leaf tissues. Among different plant parts, higher heavy metal accumulation was recorded in aerial plant parts (leaves) than roots and these results are supported with bioconcentration factors (BCF) and translocation factors (TF).

The phytoremediation efficiency evaluation factors such as bioconcentration factor (BCF), translocation factor (TF) and metal removal efficiency (MRE) were recorded for Pb and Cd concentrations in *Salix* and *Toona*. With increasing Pb and Cd concentration, the change in BCF and TF were observed in both species, but the significant decline was recorded in combinations (Pb+Cd) concentrations compared to their individual element application which might be due to heavy metals in soil as well as in plant tissues interfere with each other and suggests antagonistic relationship among them. The phytoremediation efficiency evaluation factor (BCF, TF and MRE) signifies that the both *Salix* and *Toona* have stronger enrichment ability for Cd than Pb, however, their partitioning and accumulation behaviour are different as *Salix* accumulates higher Pb and Cd in roots than stem and leaves (root> stem> leaves), whereas *Toona* showed maximum Pb and Cd accumulation in leaves than stem and roots in an order as leaves > stem > roots.

Salix showed BCF >1 (for roots only) and TF < 1, thus can be categorized as excluders, as they maintain high uptake of soil-root with small root to shoot translocation. Along with this, *Salix* shows sufficient metal removal efficiency (65%) up to Pb_{200} mg/kg and Cd_{15} mg/kg (individual as well as in combination), further increase in Pb and Cd concentration affect their uptake, translocation and accumulation capabilities as well as survival percentage. *Toona ciliata* showed BCF >1 and TF >1 which is suitable for phytoextraction and can be categorized as accumulator/hyper accumulator. *Toona* plants show sufficient metal removal efficiency (> 65%) as well as better survival percentage with higher Pb and Cd concentrations ($Pb_{300}Cd_{25}$ mg/kg). Heavy metal tolerance index was calculated on the basis of biomass, although both *Salix* and *Toona* showed strong tolerance (> 65%) against heavy metal stress, but the tolerance index was comparatively higher in *Toona* than *Salix*.

FTIR results of *Salix* and *Toona* confirmed that Pb and Cd interaction within plant tissues was mostly confined to carboxyl and amino functional groups, however, the binding relationship with amide, hydroxyl, phosphate and carboxyl groups were also evaluated. The interactions of functional groups in plants with metal cation exchange led to modifications in cellulose, lipids, carbohydrates and protein structural moieties resulting in disruption of plant growth and development patterns.

Heavy metals negatively affects the macro nutrient (N, P, K, Ca, Mg, S) and micro nutrient profile (Mn, Zn, Cu, Fe) of *Salix* and *Toona* plant parts (i.e. root, stem and leaves), which might be due to intereference of heavy metals in contaminated soil malfunctioned the ability of plants to absorb water and nutrients either through immobilization or decreased

uptake which results in nutrient deficiency in plants. The reduction in nutrient bioavailability was also recorded in heavy metal contaminated soils which is also responsible for decrease in plant nutrient content. Along with this, significant effect on soil properties suc as pH, electrical conductivity (EC), soil organic carbon (SOC) and cation exchange capacity (CEC) in heavy metal equilibrated soil (before planting) and after uprooting of *Salix* and *Toona*. The significantly increased pH and EC in response to heavy metal stress was observed which might be due to the $Pb(NO_3)_2$ and $Cd(NO_3)_2$ salts used for spiking. Along with this, significant increased soil organic carbon conferred that heavy metal contamination reduces the soil carbon utilization speed may be due to change in biotic composition in rhizosphere which further results accumulation of soil organic carbon and increased cation exchange capacity (CEC) of soil.

Conclusion

From the critical rummage of observations recorded for *Salix alba* and *Toona ciliata* displayed in the form of tables and graphs, it is hereby concluded that:

- *Toona* plants showed better survival percentage (>97%) than *Salix* (< 50%) at higher Pb and Cd ($Pb_{300}Cd_{25}$) concentrations, however, *Salix* showed sufficient survival percentage (> 85%) with Pb_{200} and Cd_{15} concentrations.
- The significant negative effect on growth and biomass of *Salix* and *Toona* was recorded under heavy metal stress (Pb+Cd > Cd > Pb), however the reduction was more pronounced in *Salix* than *Toona*.
- The accumulation of organic osmolytes and metabolites (proline, total soluble sugars, total soluble protein), and enzymatic antioxidants was recorded, which conferred the enhanced tolerance against heavy metal stress.
- Anatomical analysis (FE-SEM and EDS) revealed the alterations in ultra-structure of plant and confirmed the Pb and Cd accumulation sites in roots and leaves of both *Salix* and *Toona*.
- *Salix* showed BCF >1 (for roots only) and TF < 1, thus can be categorized as excluders, as they maintain high uptake of soil-root with small root to shoot translocation.
- *Toona ciliata* showed BCF >1 and TF >1 which is suitable for phytoextraction and can be categorized as hyper accumulator.
- Heavy metal tolerance index was calculated on the basis of biomass, although both *Salix* and *Toona* showed strong tolerance (> 65%) against heavy metal stress.

Thus, the present study reflects that both *Salix alba* and *Toona ciliata* had great phytoremediation potential for reclaiming Pb and Cd contaminated soils.

REFERENCES

Abbas T, Rizwan M, Ali S, Adrees M, Zia-ur-Rehman M, Qayyum M F, Ok Y S and Murtaza G (2017) Effect of biochar on alleviation of cadmium toxicity in wheat (*Triticum aestivum* L.) grown on Cd-contaminated saline soil. *Environ Sci Pollut Res* **25:**25668-80.

Adrees M, Ali S, Rizwan M, Zia-ur-Rehman M, Ibrahim M, Abbas F, Farid M, Qayyum M F and Irshad M K (2015) Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: A review *Ecotoxicol Environ Saf* **119:**186-97.

Afshan S, Ali S, Bharwana S A, Rizwan M, Farid M, Abbas F, Ibrahim M, Mehmood M A and Abbasi G H (2015) Citric acid enhances the phytoextraction of chromium, plant growth, and photosynthesis by alleviating the oxidative damages in *Brassica napus* L. *Environ Sci Pollut Res* **22:**11679–89.

Ahmad M S A, Ashraf M, Tabassam Q, Hussain M and Firdous H (2011) Lead (Pb)-induced regulation of growth, photosynthesis, and mineral nutrition in maize (*Zea mays* L.) plants at early growth stages. *Biol Trace Elem Res* **144:**1229–39.

Ahmadi O, Pandey J, HademMoghadam N, AsgariLajayer B and Ghorbanpour M (2020) Phytoremediation of contaminated soils using trees. In: Faisal M, Saquib Q and Alatar A A (ed) *Cellular and molecular phytotoxicity of heavy metals nanotechnology in the life sciences*. Pp. 191-226. Springer, Cham, Denmark.

Al-Ghouti M A, Li J, Salamh Y, Al-Laqtah N, Walker G and Ahmad M N M (2010) Adsorption mechanisms of removing heavy metals and dyes from aqueous solution using date pits solid adsorbent. *J Hazard Mater* **176:**510–20.

Ali B, Mwamba T M, Gill R A, Yang C, Ali S, Daud M K *et al* (2014). Improvement of element uptake and antioxidative defense in Brassica napus under lead stress by application of hydrogen sulfide. *Plant Growth Regul* **74:**261–73.

Ali H, Khan E and Sajad MA (2013) Phytoremediation of heavy metals—Concepts and applications. *Chemosphere* **91:**869–81.

Ali N, Masood S, Mukhtar T, Kamran M A, Rafique M, Munis M F H, Chaudhary H J (2015) Differential effects of cadmium and chromium on growth, photosynthetic activity, and metal uptake of *Linum usitatissimum* in association with *Glomus intraradices*. *Environ Monit Assess* **187:**311.

Alia N, Sardar K, Said M, Salma K, Sadia A, Sadaf S, Toqeer A and Miklas S (2015) Toxicity and bioaccumulation of heavy metals in spinach (*Spinacia oleracea*) grown in a controlled environment. *Int J Environ Res Public Health* **12:**7400-16.

Alsokari S S and Aldesuquy H S (2011) Synergistic effect of polyamines and waste water on leaf turgidity, heavy metals accumulation in relation to grain yield. *J Appl Sci Res*

7:376–84.

Anjum N A, Hasanuzzaman M, Hossain M A, Thangavel P, Roychoudhury A, Gill S S, Rodrigo M A M, Adam V, Fujita M and Kizek R (2015) Jacks of metal/metalloid chelation trade in plants—An overview. *Front Plant Sci* **6:**192.

Aprile F and Lorandi R (2012) Evaluation of cation exchange capacity (CEC) in tropical soils using four different analytical methods. *J Agric Sci* **4:**278-89.

Ashraf U, Kanu A S, Deng Q, Mo Z and Pan S *et al* (2017) Lead (Pb) toxicity; physio-biochemical mechanisms, grain yield, quality, and Pb distribution proportions in scented rice. *Front Plant Sci* **8:**259.

Aslam M, Aslam A, Sheraz M, Ali B, Ulhassan Z, Najeeb U, Zhou W and Gill R A (2021) Lead toxicity in cereals: Mechanistic insight into toxicity, mode of action, and management. *Front Plant Sci* **11:**587785.

Audet P and Charest C (2007) Heavy metal phytoremediation from a meta-analytical perspective. *Environmental Pollution* **147:**231-37.

Baker A J M (1981) Accumulators and excluders – strategies in the response of plants to heavy metals. *J Plant Nutr* **3:**643–54.

Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair M R and Sadeghi M (2021) Toxic mechanisms of five heavy metals: Mercury, lead, chromium, cadmium and arsenic. *Front Pharmacol* **12:**643972.

Bates L S, Waldren R P and Teare I D (1973) Rapid determination of free proline for stress studies. *Plant Soil* **39:**205-07.

Bhatia N P, Walsh I K B, Siegele R, Ashwath N and Baker A J M (2004) Studies on spatial distribution of nickel in leaves and stems of the metal hyperaccumulator *Stackhousia tryonii* using nuclear microprobe (micro-PIXE) and EDXS techniques. *Funct Plant Biol* **31:**1061–74.

Bhatti S S, Kumar V, Singh N, Vasudha S, Singh J, Katnoria J K and Nagpal AV (2016) Physico-chemical properties and heavy metal contents of soils and Kharif crops of Punjab, India. *Proc Environ Sci* 35: 801- 08.

Bian F, Zhong Z, Li C, Zhang X, Gu L, Huang Z, Gai Z and Huang Z (2021) Intercropping improves heavy metal phytoremediation efficiency through changing properties of rhizosphere soil in bamboo plantation. *J Hazard Mater* **416:**125898.

BIS (2012) Indian Standard for Drinking Water, Bureau of Indian Standards (IS-10500), New Delhi.

Bluskov S and Arocena JM (2005) Uptake, distribution, and speciation of chromium in *Brassica juncea*. *Int J Phytoremediation* **7:**153–65.

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T and Scheckel K (2014) Remediation of heavy metal(loid)s contaminated soils—to mobilize or to

immobilize? *J Hazard Mater* **266:**141–66.

Bower C A, Reitemeier R F and Fireman M (1952) Exchangeable cation analysis of saline and alkali soils. *Soil Sci* **73**: 251-61.

Bravin MN, Garnier C, Lenoble V, Gerard F, Dudal Y and Hinsinger P (2012) Root-induced changes in pH and dissolved organic matter binding capacity affect copper dynamic speciation in the rhizosphere. *Geochim Cosmochim Ac* **84:**256–68.

Briffa J, Sinagra E and Blundell R (2020) Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon* **6:**e04691.

Buendía-González L, Orozco-Villafuerte J, Cruz-Sosa F, Barrera-Díaz C E and Vernon-Carter E J (2010) Prosopis laevigata a potential chromium (VI) and cadmium (II) hyperaccumulator desert plant. *Bioresour Technol* **101:**5862–67.

Cailliatte R, Schikora A, Briat J F, Mari S and Curie C (2010) High affinity manganese uptake by the metal transporter nramp1 is essential for Arabidopsis Growth in Low Manganese Conditions. *Plant Cell* **22:**904–17.

Chance B and Mahley A C (1955) Assay of catalases and peroxidases. In: Colowick S P and Kaplan N O (ed) *Methods in enzymology*. Pp. 764–75. Academic Press, New York.

Chaney R L, Angle J S, McIntosh M S, Reeves R D, Li Y M, Brewer E P, Chen K Y, Roseberg R J, Perner H, Synkowski E C, Broadhurst C L, Wang S and Baker A J (2005) Using hyperaccumulator plants to phytoextract soil Ni and Cd. *Z Naturforsch C J Biosci* **60:**190–98.

Chaney R L, Broadhurst C L and Centofanti T (2010) Phytoremediation of soil trace elements In: Hooda P S (ed) *Trace elements in soils*. Pp 311-352. John Wiley & Sons Inc, United Kingdom.

Chen J P (2012) *Decontamination of heavy metals: processes, mechanisms, and applications*. Pp 89-108. CRC Press, Boca Raton, Florida, USA.

Chen Y, Wang Y and Yeh K (2017) Role of root exudates in metal acquisition and tolerance. *Curr Opin Plant Biol* **39:**66-72

Cheng F M, Zhao N C, Xu H M, Li Y, Zhang W F, Zhu Z W and Chen M X (2006) Cadmium and lead contamination in japonica rice grains and its variation among the different locations in southeast China. *Sci Total Environ* **359**:156–66.

Chibuike G U and Obiora S C (2014) Heavy metal polluted soils: Effect on plants and bioremediation methods. *Appl Environ Soil Sci* **21:**1-12.

Chowardhara B, Borgohain P, Saha B, Awasthi J P and Panda S K (2020) Differential oxidative stress responses in Brassica juncea (L.) Czern and Coss cultivars induced by cadmium at germination and early seedling stage. *Acta Physiol Plant* **42:**105.

Chun S C, Paramasivan M and Chandrasekaran M (2018) Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. *Front Microbiol* **9:**2525.

Cioica N, Tudora C, Iuga D, Deak G, Matei M, Nagy EM and Gyorgy Z (2019) A review on phytoremediation as an ecological method for in situ clean up of heavy metals contaminated soils *E3S Web of Conferences* **112**:03024.

Clabeaux B L, Navarro D A, Aga D S and Bisson M A (2013) Combined effects of cadmium and zinc on growth, tolerance, and metal accumulation in Chara australis and enhanced phytoextraction using EDTA. *Ecotoxicol Environ Saf* **98:**236–43.

CPCB (2009) Comprehensive environmental assessment of industrial clusters. Ecological impact assessment series: EIAS/5/2009-2010. Central pollution control board, Ministry of environment and forests, Govt. of India, New Delhi.

D'Souza L, Devi P, DivyaShridhar M and Naik C G (2008) Use of Fourier Transform Infrared (FTIR) spectroscopy to study cadmium induced changes in *Padina tetrastromatica* (Hauck). *Anal Chem Insights* **3:**11-17.

Dakora F D and Phillips D A (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. In: Adu-Gyamfi J J (ed) *Food security in nutrient-stressed environments: exploiting plants' genetic capabilities*. Pp. 201-13. Kluwer academic publishers, Netherlands.

Dalton T P, He L, Wang B, Miller M L, Stringer J L K F, Chang Z, Baxter C S and Neber D W (2005) Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. *Proc Natl Acad Sci USA.* **102:**3401–06.

De Carvalho K, de Campos M K F, Domingues D S, Pereira L F P and Vieira L G E (2013) The accumulation of endogenous proline induces changes in gene expression of several antioxidant enzymes in leaves of transgenic Swingle citrumelo. *Mol Biol Rep* **40:**3269–79.

Dhanwal P, Kumar A, Dudeja S, Chhokar V and Beniwal V (2017) Recent advances in phytoremediation technology. In: Kumar R (ed) *Advances in Environmental Biotechnology*. Pp. 227-41. Springer, Singapore.

Dietz A C and Schnoor J L (2001) Advances in phytoremediation: Environmental health perspectives. **109:**163-68.

Dixit R, Wasiullah M D, Pandiyan K, Singh U B, Sahu A, Shukla R, Singh B P, Rai J P, Sharma P K, Lake H and Paul D (2015) Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. *Sustainability* **7:** 2189–2212.

Dubois M, Gilles K A, Hamilton J K, Rebers P A and Smith F (1956) Colorimetric methods for determination of sugars and related substances. *Anal Chem* **28:**350-56.

Ehsan, S, Ali, S, Noureen S, Mahmood K, Farid M, Ishaque W, Shakoor M B and Rizwan M (2014) Citric acid assisted phytoremediation of cadmium by *Brassica napus* L. *Ecotoxicol Environ Saf* **106:**164–72.

El-Mahrouk E M, Eisa E A, Hegazi M A, Abdel-Gayed M E, Dewir Y H, El-Mahrouk M E and Naidoo Y (2019) Phytoremediation of cadmium-, copper-, and lead-contaminated soil by *Salix mucronata* (Synonym Salix safsaf). *Hortscience* **54:**1249–57.

Gajic´ G, Djurdjevic´ L, Kostic´ O, Jaric´ S, Mitrovic´ M and Pavlovic´ P (2018) Ecological potential of plants for phytoremediation and ecorestoration of fly ash deposits and mine wastes. *Front Environ Sci* **6:**124.

Gao M, Chang X, Yang Y and Song Z (2020) Foliar graphene oxide treatment increases photosynthetic capacity and reduces oxidative stress in cadmium-stressed lettuce. *Plant Physiol Biochem* **154:** 287–94.

Gerhardt K E, Gerwing P D and Greenberg B M (2017) Opinion: taking phytoremediation from proven technology to accepted practice. *Plant Sci* **256:**170–85.

Ghosh M and Singh S P (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. *Appl Ecol Environ Res* **3:**1–18.

Goh E J, Kim J, Kim W, Ha B, Kim S H, Kang S Y, Seo Y W and Kim D S (2014) Physiological changes and anti-oxidative responses of Arabidopsis plants after acute and chronic γ-irradiation. *Radiat Environ Biophys* **53:**677–93.

Gomes M P, Desoares E M and Maria N (2011) Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in *Brachiaria decumbens*. *Sci Agric(Piracicaba, Braz.)* **68:**566–73.

Gomez L, Contreras A, Bolonio D, Quintana J, Oñate-Sánchez L and Merino I (2019) Phytoremediation with trees. In: Cánovas F M *Advances in Botanical Research*. Pp. 281-321. Academic Press, London.

Gopal R and Rizvi A H (2008) Excess lead alters growth, metabolism and translocation of certain nutrients in radish. *Chemosphere* **70:**1539–44.

Govere E M (2021) Environmental phytoremediation and analytical technologies for heavy metal removal and assessment. In: Ricroch A, Chopra S and Kuntz M (ed) *Plant Biotechnology*. Pp. 203-13. Springer, Cham. Denmark.

Gratao P L, Monteiro C C, Rossi M L, Martinelli A P, Peres L E P, Medici L O, Lea P J and Azevedo R A (2009) Differential ultrastructural changes in tomato hormonal mutants exposed to cadmium. *Environ Exp Bot* **67:**387–94.

Griffiths P R and De Haseth J A (ed) (2007) *Fourier transform infrared spectrometry*. Vol 171, pp 158-162. John Wiley & Sons. Hoboken, New Jersey, USA

Guerra F, Gainza F, Pérez R and Zamudio F (2011) Phytoremediation of heavy metals using poplars (Populus spp): A glimpse of the plant responses to copper, cadmium and zinc stress. In: Golubev I A (ed) *Handbook of Phytoremediation*. Pp. 387-413. Nova science publishers Inc, New York.

Günthardt-Goerg M S, Vollenweider P and Schulin R (2022) Metal accumulation and

biomass production in young afforestations established on soil contaminated by heavy metals. *Plants* **11:**523.

Guo C, Hu J, Gao W, Gao P, Cao Z, Liu N, Wang X, Liu W, Zhao J, Dong J, Genin G M and Zhou L H (2022) Mechanosensation triggers enhanced heavy metal ion uptake by non-glandular trichomes. *J Hazard Mater* **15:**426-34.

Gupta D K, Huang H G, Nicoloso F T, Schetinger M R, Farias J G, Li T Q *et al* (2013) Effect of Hg, As and Pb on biomass production, photosynthetic rate, nutrients uptake and phytochelatin induction in *Pfaffia glomerata*. *Ecotoxicol* **22:**1403–12.

Gupta P and Bhatnagar A K (2015) Spatial distribution of arsenic in different leaf tissues and its effect on structure and development of stomata and trichomes in mung bean, Vigna radiata (L.)Wilczek. *Environ Exp Bot* **109:**12–22.

Gupta V K, Nayak A and Agarwal S (2015) Bioadsorbents for remediation of heavy metals: Current status and their future prospects. *Environ Eng Res* **20:**1–18.

Haider F U, Liqun C, Coulter J A, Cheema S A, Wu J, Zhang R, Wenjun M and Farooq M (2021) Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicol Environ Saf* **211:** 111887.

Hamid Y, Tanga L, Hussain B, Usman M, Lin Q, Rashid MS, He Z and Yang X (2020) Organic soil additives for the remediation of cadmium contaminated soils and their impact on the soil-plant system: A review. *Sci Total Environ* **707:**136121.

Hamim H, Miftahudin M and Setyaningsih L (2018) Cellular and ultrastructure alteration of plant roots in response to metal stress. In: Ratnadewi D and Hamim H (ed) *Plant growth and regulation-alterations to sustain unfavorable conditions*. Pp. 21-41. IntechOpen. London.

Hamzah A, Hapsari R I and Wisnubroto E I (2016) Phytoremediation of cadmium-contaminated agricultural land using indigenous plants. *Int J Environ Agric Res* **2:**8–14.

Hasan M, Cheng Y, Kanwar M K, Chu X Y, Ahammed G J and Qi Z Y (2017) Responses of plant proteins to heavy metal stress—a review. *Front Plant Sci* **8:**1492.

Hassan M U, Chattha M U, Khan I, Chattha M B, Aamer M, Nawaz M, Ali A, Ullah Khan M A and Khan T A (2019) Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities—a review. *Environ Sci Pollut Res* **26:**12673–88.

Hatamian M, Nejad A R, Kafi M, Souri M K and Shahbazi K (2020) Interaction of lead and cadmium on growth and leaf morphophysiological characteristics of European hackberry (*Celtis australis*) seedlings. *Chem Biol Technol Agric* **7:**9.

Hayat S, Khalique G, Irfan M, Wani A S, Tripathi B N and Ahmad A (2012) Physiological changes induced by chromium stress in plants: An overview. *Protoplasma* **249:**599–

611.

He J, Ren Y, Chen X and Chen H (2014) Protective roles of nitric oxide on seed germination and seedling growth of rice (Oryza sativa L.) under cadmium stress. *Ecotoxicol Environ Saf* **108:**114–19.

Hermle S, Vollenweider P, Günthardt-Goerg M S, McQuattie C J and Matyssek R (2007) Leaf responsiveness of *Populus tremula* and *Salix viminalis* to soil contaminated with heavy metals and acidic rainwater. *Tree Physiol* **27:**1517-31.

Hiscox J D and Israelstam G F (1979) A Method for extraction of chlorophyll from leaf tissue without maceration. *Can J Bot* **57:**1332–34.

Hooda V (2007) Phytoremediation of toxic metals from soil and waste water. *J Environ Biol* **28:**367–76.

Hossain M A, Piyatida P, da Silva J A T and Fujita M (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: Central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *J Bot* **3:**872-875.

Hou S, Zheng N, Tang L, Ji X and Li Y (2019) Effect of soil pH and organic matter content on heavy metals availability in maize (*Zea mays* L.) rhizospheric soil of non-ferrous metals smelting area. *Environ Monit Assess* **191:** 634.

Huang L, Li W C, Tam N F Y and Ye Z H (2019) Effects of root morphology and anatomy on cadmium uptake and translocation in rice (*Oryza sativa* L.). *J Environ Sci* **75:**296–306.

Islam M M, Hoque M A, Okuma E, Banu M N A, Shimoishi Y, Nakamura Y and Murata Y (2009) Exogenous proline and glycine betaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. *J Plant Physiol* **166:**1587–97.

Ismael M A, Elyamine A M, Moussa M G, Cai M, Zhao X and Hu C (2018) Cadmium in plants: uptake, toxicity, and its interactions with selenium fertilizers. *Metallomics* **11:** 255-77.

Ismail S, Khan F and Iqbal M Z (2013) Phytoremediation: assessing tolerance of tree species against heavy metal (Pb and Cd) toxicity. *Pak J Bot* **45:**2181-86.

Jackson M L (1973) *Soil Chemical Analysis*. Pp. 498. Prentice Hall of India Pvt Ltd, New Delhi.

Jacob J M, Karthik C, Saratale R G, Kumar S S, Prabakar D, Kadirvelu K *et al* (2018) Biological approaches to tackle heavy metal pollution: a survey of literature. *J Environ Manage* **217:**56–70.

Järup L (2003) Hazards of heavy metal contamination. *Br Med Bull* **68:**167–82.

Javed M T, Tanwir K, Akram M S, Shahid M, Niazi N K and Lindberg S M (2019)

Phytoremediation of cadmium-polluted water/sediment by aquatic macrophytes: Role of plant-induced pH changes. In: Prasad N V and Fujita M (ed) *Cadmium toxicity and tolerance in plants*. Pp. 495-529. Academic press, London.

Jinadasa N, Collins D, Holford P, Milham P J and Conroy J P (2016) Reactions to cadmium stress in a cadmium tolerant variety of cabbage (Brassica oleracea L.): is cadmium tolerance necessarily desirable in food crops? *Environ Sci Pollut Res* **23:**5296–5306.

Jing Y D, Zhen Li H E, Yang X E (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *J Zhejiang Univ Sci B* **8:**192–207.

Kabata-Pendias A (2010) *Trace elements in soils and plants* (4th ed.). Pp. 224-60. CRC Press, Boca Raton, Florida, USA.

Kabata-Pendias A and Pendias H (1984) *Trace Elements in soils and plants*. Pp. 269-78.CPRC Press, Boca Raton, Florida, USA.

Kacálková L, Tlustoš P and Száková J (2015) Phytoextraction of risk elements by willow and poplar trees. *Int J Phytoremediati*on **17:**414-21.

Kanwal A, Farhan M, Sharif F, Hayyat M U, Shahzad L and Ghafoor G Z (2020) Effect of industrial wastewater on wheat germination, growth, yield, nutrients and bioaccumulation of lead. *Sci Rep* **10:**11361

Karaca A, Cetin S C, Turgay O C and Kizilkaya R (2010) Effects of heavy metals on soil enzyme activities. In: Sherameti I and Varma A (ed) *Soil heavy metals. Soil Biology 19*. Pp 237-65. Springer-Verlag, Berlin Heidelberg, New York.

Kasim W A (2006) Changes induced by copper and cadmium stress in the anatomy and grain yield of Sorghum bicolor (L.) Moench. *Int J Agric Biol* **8:**123–28.

Kaur J, Bhat S A, Singh N, Bhatti S S, Kaur V and Katnoria J K (2022) Assessment of the heavy metal contamination of roadside soils alongside Buddha Nullah, Ludhiana, (Punjab) India. *Int J Environ Res Public Health* **19:**1596.

Kaur J, Bhatti S S, Bhat S A, Nagpal A K, Kaur V and Katnoria J K (2021) Evaluating potential ecological risks of heavy metals of textile effluents and soil samples in vicinity of textile industries. *Soil Syst* **5:** 63.

Khan F I, Husain T and Hejazi R (2004) An overview and analysis of site remediation technologies. *J Environ Manag* **71:**95–112.

Khan M, Azzawi T N B, Imran M, Hussain A, Mun B J, Pande A and Yun B (2021) Effects of lead (Pb)-induced oxidative stress on morphological and physio-biochemical properties of rice. *Biocell* **45:**1413-23.

Khosropour E, Attarod P, Shirvany A, Pypker T G, Bayramzadeh V, Hakimi L and Moeinaddini M (2019) Response of Platanus orientalis leaves to urban pollution by heavy metals. *J Forest Res* **30:** 1437–45.

Kieffer P, Planchon S, Oufir M, Ziebel J, Dommes J, Hoffmann L, Hausman J F and Renaut J

(2009) Combining proteomics and metabolite analyses to unravel cadmium stress-response in poplar leaves. *J Proteome Res* **8:** 400–17.

Krantev A, Yordanova R, Janda T, Szalas G and Papova L (2008) Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. *J Plant Physiol* **165:**920–31.

Krishan G, Sudarsan N, Ghosh N C, Kumar S, Singh S, Sharma A, Mittal S, Sidhu B S and Vashisth R (2021) Concentration of heavy metals in groundwater and heavy metal pollution index in Punjab. *e-Journal Geohydrol* **2:**24-33.

Król A, Mizerna K and Bożym M (2020) An assessment of pH-dependent release and mobility of heavy metals from metallurgical slag *J Hazard Mater* **384:** 121502.

Kuiper I, Lagendijk E L, Bloemberg G V, Lugtenberg B J J (2004) Rhizoremediation: a beneficial plant-microbe interaction. *Mol Plant-Microbe Interact* **17:**6–15.

Kumar S S, Kadier A, Malyan S K, Ahmad A and Bishnoi N R (2017) Phytoremediation and rhizoremediation: uptake, mobilization and sequestration of heavy metals by plants. In: Singh D P (ed) *Plant-Microbe interactions in agro-ecological perspectives*. Pp. 367-94. Springer, Singapore.

Kumar V, Sharma A, Kaur P, Sidhu G P S, Bali A S, Bhardwaj R, Thukral A K and Cerda A (2019) Pollution assessment of heavy metals in soils of India and ecological risk assessment: A state-of-the-art. *Chemosphere* **216:** 449-62.

Kundal M, Thakur S and Dhillon G P S (2020) Evaluation of growth performance of half sib progenies of Toona ciliata M. Roem under field conditions. *Genetika* **52:**651-60.

Kupper H, Lombi E, Zhao F J, Wieshammer G and McGrath S P (2001) Cellular compartmentation of Ni in the hyperaccumulators *Alyssum lesbiacum, A. bertoloni*i and *Thlaspi goesingense*. *J Exp Bot* **52:**2291–2300.

Kushwaha A, Hans N, Kumar S and Rani R A (2018) Critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. *Ecotoxicol Environ Saf* **147:**1035–45.

Lamhamdi M, El Galiou O, Bakrim A, Nóvoa-Muñoz J C, Arias-Estevez M, Aarab A and Lafont R (2013) Effect of lead stress on mineral content and growth of wheat (*Triticum aestivum*) and spinach (*Spinacia oleracea*) seedlings. *Saudi J Biol Sci* **20:**29–36.

Largo-Gosens A, Hernandez-Altamirano M, Garcıa-Calvo L, Alonso-Simon A, Alvarez J and Acebes J L (2014) Fourier transform mid infrared spectroscopy applications for monitoring the structural plasticity of plant cell walls. *Front Plant Sci* **5:**303–30.

Latif U, Farid M, Rizwan M, Ishaq H K Farid S, Ali S, El-Sheikh M A, Alyemeni M N and Wijaya L (2020) Physiological and biochemical response of Alternanthera bettzickiana (Regel) G. Nicholson under acetic acid assisted phytoextraction of lead.

Plants **9:**1084.

Lee S, Moon J S, Ko T S, Petros D, Goldsbrough P B and Korban S S (2003) Over expression of *Arabidopsis* phytochelatin synthase paradoxically leads to hypersensitivity to cadmium stress. *Plant Physiol* **131:**656–63.

Lenart A and Wolny-Koładka K (2013) The effect of heavy metal concentration and soil pH on the abundance of selected microbial groups within ArcelorMittal Poland steelworks in Cracow. *Bull Environ Contam Toxicol* **90:**85-90.

Li J, Yu J, Du D, Liu J, Lu H and Yan C (2019) Analysis of anatomical changes and cadmium distribution in *Aegiceras corniculatum* (L.) Blanco roots under cadmium stress. *Mar Pollut Bull* **149:**110536.

Li J, Yu J, Yan C, Du D, Liu J and Lu H (2018) Distribution correlations of cadmium to calcium, phosphorus, sodium and chloridion in mangrove *Aegiceras corniculatum* root tissues. *Mar Pollut Bull* **126:**179–83.

Li X, Zhou Q, Sun X and Ren W (2016) Effects of cadmium on uptake and translocation of nutrient elements in different welsh onion (*Allium fistulosum* L.) cultivars. *Food Chem* **194:**101–10.

Liang X, Zhang L, Natarajan S K and Becker D F (2013) Proline mechanisms of stress survival. *Antioxid Redox Signal* **19:**998–1011.

Lindsay W L and Norvell W A (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci Am J* **42:**421–28.

Liu D, Li S, Islam E, Chen J, Wu J, Ye Z, Peng D, Yan W and Lu K (2015) Lead accumulation and tolerance of Moso bamboo (*Phyllostachys pubescens*) seedlings: applications of phytoremediation. *J Zhejiang Univ Sci B (Biomed Biotechnol)* **16:**123-30.

Liu J, Cao C, Wong M, Zhang Z and Chai Y (2010) Variations between rice cultivars in iron and manganese plaque on roots and the relation with plant cadmium uptake. *J Environ Sci* **22:** 1067–72.

Liu J G, Liang J S, Li K Q, Zhang Z J, Yu B Y and Lu X L *et al* (2013) Correlations between cadmium and mineral nutrients in absorption and accumulation in various genotypes of rice under cadmium stress. *Chemosphere* **52:**1467–473.

Liu L W, Li W, Song W P and Guo M X (2018) Remediation techniques for heavy metal–contaminated soils: Principles and applicability. *Sci Total Environ* **633:**206–19.

Lone M I, He Z L, Stoffella P J and Yang X E (2008) Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. *J Zhejiang Univ–Sci B (Biomed Biotechnol)* **9:**210–20.

Lorenz N, Hintemann T, Kramarewa T, Katayama A, Yasuta T, Marschner P and Kandeler E (2006) Response of microbial activity and microbial community composition, in soils

to long-term arsenic and cadmium exposure. *Soil Biol Biochem* **38:**1430–37.

Lowry O H, Rosenbrough N J, Farr A L and Randall R J (1951) Protein measurements with folin phenol reagent. *J Biol Chem* **193:**263-75.

Lux A, Martinka M, Vaculik M and White P J (2011) Root responses to cadmium in the rhizosphere: a review. *J Exp Bot* **62:**21–37.

Maestri E, Marmiroli M, Visioli G and Marmiroli N (2010) Metal tolerance and hyperaccumulation: cost and trade-offs between traits and environment. *Environ Exp Bot* **68:**1-13.

Maksimović I, Kastori R, Krstić L and Luković J (2007) Steady presence of cadmium and nickel affects root anatomy, accumulation and distribution of essential ions in maize seedlings. *Biol Plant* **51:** 589–92.

Malandrino M, Abollino O, Giacomino A, Aceto M and Mentasti E (2006) Adsorption of heavy metals on vermiculite: Influence of pH and organic ligands. *J Colloid Interface Sci* **299:**537-46.

Malik J A, Wani A A, Wani K A and Bhat M A (2020) Role of white willow (*Salix alba* L.) for cleaning up the toxic metal pollution. In: Hakeem K R *et al* (ed) *Bioremediation and Biotechnology*. Pp 257-68. Springer Cham, Denmark.

Manzoor N, Ahmed T, Noman M, Shahid M, Nazir M M, Ali L, Alnusaire T S, Li B, Schulin R and Wang G (2021) Iron oxide nanoparticles ameliorated the cadmium and salinity stresses in wheat plants, facilitating photosynthetic pigments and restricting cadmium uptake. *Sci Total Environ* **769:**145221.

Marklund S and Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* **47:**469–74.

Marmiroli M, Pietrini F, Maestri E, Zacchini M, Marmiroli N and Massacci A (2011) Growth, physiological and molecular traits in Salicaceae trees investigated for phytoremediation of heavy metals and organics. *Tree Physiol* **31:**1319-34.

Marques A P, Rangel A O and Castro P M (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. *Crit Rev Env Sci Technol* **39:**622–54.

Marques A P G C, Rangel A O S S and Castro P M L (2009) Remediation of heavy metal contaminated soils: Phytoremediation as a potentially promising clean-up technology. *Crit Rev Environ Sci Tec* **39:**622-54.

Martins J P R, Vasconcelos L L, Braga P C S, Rossini F P, Conde LT, Rodrigues L C A, Falqueto A R and Gontijo A B P L (2020) Morphophysiological responses, bioaccumulation and tolerance of Alternanthera tenella Colla (Amaranthaceae) to excess copper under in vitro conditions. *Plant Cell Tissue Organ Cult* **143:**303–18.

Mesjasz-Przybyłowicz J, Nakonieczny M, Migula P, Augustyniak M, Tarnawska M, Reimold U *et al* (2004) Uptake of cadmium, lead nickel and zinc from soil and water solutions by the nickel hyperaccumulator Berkheyacoddii. *Acta Biol Cracoviensia Ser Bot* **46:**75–85.

Mishra S, Srivastava S, Tripathi R D, Kumar R, Seth C S and Gupta D K (2006) Lead detoxification by coontail (Ceratophyllum demersum L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere* **65:**1027-39.

Miyadate H, Adachi S, Hiraizumi A, Tezuka K, Nakazawa N, Kawamoto T, Katou K, Kodama I, Sakurai K, Takahashi H, Satoh-Nagasawa N, Watanabe A, Fujimura T and Akagi H (2011) OsHMA3, a P18-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *New Phytol* **189:**190–99.

Mleczek M, Goliński P, Krzesłowska M, Gąsecka M and Magdziak Z *et al* (2017) Phytoextraction of potentially toxic elements by six tree species growing on hazardous mining sludge. *Environ Sci Pollut Res* **24:** 22183–95.

Mleczek M, Rutkowski P, Rissmann I, Kaczmarek Z, Golinski P, Szentner K, Strażyńska K and Stachowiak A (2010) Biomass productivity and phytoremediation potential of Salix alba and Salix viminalis. *Biomass Bioenerg* **34:** 1410-18.

Moghimian N, Hosseini S M, Kooch Y and Darki B Z (2017) Impacts of changes in land use/cover on soil microbial and enzyme activities. *Catena* **157:**407–14.

Moradi L and Ehsanzadeh P (2015) Effects of Cd on photosynthesis and growth of safflower (Carthamus tinctorius L.) genotypes. *Photosynthetica* **53:**506–18.

Muradoglu F, Gundogdu M, Ercisli S, Encu T, Balta F, Jaafar H Z E, Zia–Ul–Haq M (2015) Cadmium toxicity affects chlorophyll a and b content, antioxidant enzyme activities and mineral nutrient accumulation in strawberry. *Biol Res* **48:**1–7.

Muszynska E, Labudda M, Rozanska E, Hanus-Fajerska E and Koszelnik-Leszek A (2019) Structural, physiological and genetic diversification of *Silene vulgaris* ecotypes from heavy metal-contaminated areas and their synchronous in vitro cultivation. *Planta* **249:**1761–78.

Nagajyoti P C, Lee K D and Sreekanth T V M (2018) Heavy metals, occurrence and toxicity for plants: A review. *Environ Chem Lett* **8:**199–216.

Nair P M G and Chung I M (2015) Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (Brassica juncea L.). *Ecotoxicol Environ Saf* **113:**302–13.

Nanjo Y, Skultety L, Uvackova L, Klubicova K, Hajduch M and Komatsu S (2011) Mass spectrometry-based analysis of proteomic changes in the root tips of flooded soybean seedlings. *J Proteome Res* **11:**372–85.

Nedunuri K V, Lowell C, Meade W, Vonderheide A P and Shann J R (2009) Management practices and phytoremediation by native grasses. *Int J Phytoremediation* **12:**200-14.

Nikolic N, Kojic D, Pilipovic A, Pajevic S, Krstic B, Borisev M and Orlovic S (2008) Responses of hybrid poplar to cadmium stress: Photosynthetic characteristics, cadmium and proline accumulation, and antioxidant enzyme activity. *Acta Biol Cracoviensia Ser Bot* **50:**95–103.

Öztürk M, Ashraf M, Aksoy A, Ahmad M S A and Hakeem K R (ed) (2015) *Plants, pollutants and remediation*. Pp 249-308. Springer, Germany.

Palliyath S and Puthur J T (2018) The modulation of various physiochemical changes in Bruguiera cylindrica (L.) Blume affected by high concentrations of NaCl. *Acta Physiol Plant* **40:**160.

Pandey R, Ansari N, Prasad R and Chavali M (2014) Pb(II) removal from aqueous solution by *Cucumis sativus* (Cucumber) peel: kinetic, equilibrium & thermodynamic study. *Am J Environ Protect* **2:** 51–58.

Patel V R, Patel P R and Kajal S S (2010) Antioxidant activity of some selected medicinal plants in western region of India. *Adv Biol Res* **4:**23-26.

Pathak S, Agarwal A V and Pandey V C (2020) Phytoremediation—a holistic approach for remediation of heavy metals and metalloids. In: Pandey V C and Singh V (ed) *Bioremediation of pollutants*. Pp. 3-14. Elsevier, Amsterdam, Netherlands.

Peng H, Wang Y, Tan T L and Chen Z (2020) Exploring the phytoremediation potential of water hyacinth by FTIR Spectroscopy and ICP-OES for treatment of heavy metal contaminated water. *Int J Phytoremediation* **22:**939-51.

Pilipović A, Zalesny R S, Rončević S, Nikolić N, Orlović S, Beljin J and Katanić M (2019) Growth, physiology, and phytoextraction potential of poplar and willow established in soils amended with heavy-metal contaminated, dredged river sediments. *J Environ Manage* **239:**352-65.

Pourrut B, Shahid M, Dumat C, Winterton P and Pinelli E (2011) Lead uptake, toxicity, and detoxification in plants. *Rev Environ Contam Toxicol* **213:**113–36.

Pulford I D and Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees : A review. *Int J Environ* **29:**529-40.

Radoglou K M and Jarvis P G (1990) Effects of CO_2 enrichment on four poplar clones. II. Leaf surface properties. *Ann Bot* **65:** 627–32.

Rafiq M T, Aziz R, Yang X, Xiao W, Stoffella P J, Saghir A, Azam M and Li T (2014) Phytoavailability of cadmium (Cd) to Pak choi (*Brassica chinensis* L.) grown in Chinese soils: a model to evaluate the impact of soil Cd pollution on potential dietary toxicity. *Plos one* **9:** e111461.

Rafique N and Tariq S R (2016) Distribution and source apportionment studies of heavy

metals in soil of cotton/wheat fields. *Environ Monit Assess* **188:**309.

Rasool M, Anwar-Ul-Haq M, Jan M, Akhtar J, Ibrahim M and Iqbal J (2020) Phytoremedial potential of maize (Zea mays L.) hybrids against cadmium (Cd) and lead (Pb) toxicity. *Pure Appl Biol (PAB)* **9:**1932–45.

Rathore S S, Shekhawat K and Dass A *et al* (2019) Phytoremediation mechanism in Indian Mustard (*Brassica juncea*) and its enhancement through agronomic interventions. *Proc Natl Acad Sci India, Sect B Biol Sci* **89:**419–27.

Rengel Z (2004) Heavy metals as essential nutrients. In: Prasad M N V and Hagemeyer J (ed) *Heavy metal stress in plants: Molecules to ecosystems*. Pp. 271-94. Springer-Verlag, Berlin, Heidelberg, New York.

Rivelli A R, Maria S D, Puschenreiter M and Gherbin P (2012) Accumulation of cadmium, zinc, and copper by *Helianthus Annuus* L.: Impact on plant growth and uptake of nutritional elements. *Int J Phytoremediation* **14:**320-34.

Riyazuddin R, Nisha N, Ejaz B, Khan M I R, Kumar M, Ramteke P W and Gupta R A (2022) Comprehensive review on the heavy metal toxicity and sequestration in plants. *Biomolecules* **12:** 43.

Riyazuddin R, Nisha N, Singh K, Verma R and Gupta R (2021) Involvement of dehydrin proteins in mitigating the negative effects of drought stress in plants. *Plant Cell Rep* **41:**519-33.

Rizwan M, Ali S, Abbas F, Adrees M, Zia-ur-Rehman M and Farid M *et al* (2017) Role of organic and inorganic amendments in alleviating heavy metal stress in oil seed crops. In: Ahmad P (ed) *Oilseed crops (yield and adaptations under environmental stress)*. Pp. 224–35. John Wiley & Sons Ltd., New Jersey, United States.

Rizwan M, Meunier J D, Davidian J C, Pokrovsky O S, Bovet N and Keller C (2016) Silicon alleviates Cd stress of wheat seedlings (Triticum turgidum L. cv. Claudio) grown in hydroponics. *Environ Sci Pollut Res* **23:**1414–27.

Rucińska-Sobkowiak R (2016) Water relations in plants subjected to heavy metal stresses. *Acta Physiol Plant* **38:** 257.

Rzymski P, Niedzielski P, Poniedziałek B and Klimaszyk P (2014) Bioaccumulation of selected metals in bivalves (Unionidae) and *Phragmites australis* inhabiting a municipal water reservoir. *Environ Monitor Asses* **186:** 3199–3212.

Sagardoy R, Vázquez S, Florez-Sarasa I D, Albacete A, Ribas-Carbó M, Flexas J, Abadía J and Morales F (2010) Stomatal and mesophyll conductances to CO_2 are the main limitations to photosynthesis in sugar beet (Beta vulgaris) plants grown with excess zinc. *New Phytol* **187:**145–58.

Sangeetha P, Venkatachalam P and Geetha N (2019) Exploring the phytomediation potential of *Calotropis gigantean* L. using a combined FTIR and principal component analysis.

In: Kumar M, Muthusamy A, Kumar V and Bhalla-Sarin N (ed) *In vitro plant breeding novel agronomic traits*. Pp. Springer, Singapore.

Sengar R S, Gautam M, Sengar K, Chaudhary R and Garg S (2010) Physiological and metabolic effect of mercury accumulation in higher plants system. *Toxicol Environ Chem* **92:**1265–81.

Seth C S, Misra V and Chauhan L K S (2012) Accumulation, detoxification, and genotoxicity of heavy metals in Indian mustard (Brassica juncea L.). *Int J Phytoremediation* **14:**1–13.

Shahid M, Pourrut B, Dumat C, Nadeem M, Aslam M and Pinelli E (2014) Heavy-metal-induced reactive oxygen species: Phytotoxicity and physicochemical changes in plants. *Rev Environ Contam Toxicol* **232:**1–44.

Shanker A K, Cervantes C, Loza-Tavera H and Avudainayagam S (2005) Chromium toxicity in plants. *Environ Int* **31:**739–53.

Shanker S and Sahni S K (2014) Hazardous metals and Minerals pollution in india: sources, toxicity and management. *Indian Natl Sci Acad* **9:**15-24.

Shannon L M, Kay E and Lew J Y (1966) Peroxidase isozymes from horseradish roots. I. Isolation and physical properties. *J Biol Chem* **241:**2166–72.

Sharma A, Kumar V, Shahzad B, Ramakrishnan M, Sidhu G P S, Bali A S, Handa N, Kapoor D, Yadav P and Khanna K (2019) Photosynthetic response of plants under different abiotic stresses: A review. *J Plant Growth Regul* **39:**509-31.

Sharma M S and Raju N S (2013) Correlation of heavy metal contamination with soil properties of industrial areas of Mysore, Karnataka, India by cluster analysis. *Int Res J Environ Sci* **2:**22-27.

Sharma P, Jha A B, Dubey R S and Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J Bot* **3:**26–34.

Sharma R and Dutta A (2017) A study of heavy metal pollution in groundwater of malwa region of Punjab, India: current status, pollution and its potential health risk. *Int J Eng Res Appl* **7:**81-91.

Sharma S S and Dietz K J (2009) The relationship between metal toxicity and cellular redox imbalance. *Trends Plant Sci* **14:**43–50.

Shi Q, Wang J, Zou J, Jiang Ze, Wang J, Wu H, Jiang W and Liu D (2015) Cadmium uptake and accumulation and its toxic effects on leaves in *Hordeum vulgare*. *Fresenius Environ Bull* **24:** 4504-11.

Shu X, Yin L, Zhang Q and Wang W (2012) Effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L. *Environ Sci Pollut Res* **19:**893–902.

Shukla O P, Juwarkar A, Singh S K., Khan S and Rai U N (2011) Growth response and metal accumulation capabilities of woody plants during the phytoremediation of tannery sludge. *Waste Manage* **31:**115–23.

Silva P and Matos M (2016) Assessment of the impact of aluminum on germination, early growth and free proline content in *Lactuca sativa* L. *Ecotoxicol Environ Saf* **131:**151–56.

Singh S, Parihar P, Singh R, Singh V P and Prasad S M (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. *Front Plant Sci* **6:**11-43.

Sivakumar P (2016) Phytoremediation of tannery waste polluted soil using Hyptissuaveolens (Lamiaceae). *Int J Pure App Biosci* **4:**265–72.

Siyar S, Sami S and Majeed A (2020) Heavy metal stress in plants: Effects on nutrients and water uptake. In: Faisal M, Saquib Q, Alatar A A and Al-Khedhairy A A (ed) *Cellular and molecular phytotoxicity of heavy metals. Nanotechnology in the life sciences*. Pp. 89-98. Springer Cham, Denmark.

Sofo A, Bochicchio R, Amato M, Rendina N, Vitti A, Nuzzaci M, Altamura M M, Falasca G, Della R F, Scopa A (2017) Plant architecture, auxin homeostasis and phenol content in Arabidopsis thaliana grown in cadmium-and zinc-enriched media. *J Plant Physiol* **216:**174–80.

Sofo A, Vitti A, Nuzzaci M, Tataranni G, Scopa A, Vangronsveld J, Remans T, Falasca G, Altamura M M and Degola F (2013) Correlation between hormonal homeostasis and morphogenic responses in Arabidopsis thaliana seedlings growing in a Cd/Cu/Zn multi-pollution context. *Physiol Plant* **149:**487–98.

Souza J F, Dolder H and Cortelazzo A L (2005) Effect of excess cadmium and zinc ions on roots and shoots of maize seedlings. *J Plant Nutr* **28:**1923–31.

Srinivasan M, Sahi S V, Paulo J C F and Venkatachalam P (2014) Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [Eichhornia crassipes (Mart.)]. *Botanical Studies* **55:**54.

Su N, Ling F, Xing A, Zhao H, Zhu Y, Wang Y, Deng X, Wang C, Xu X and Hu Z (2020) Lignin synthesis mediated by CCoAOMT enzymes is required for the tolerance against excess Cu in Oryza sativa. *Environ Exp Bot* **175:**104059.

Suman J, Uhlik O, Viktorova J and Macek T (2018) Phytoextraction of Heavy Metals: A Promising Tool for Clean-Up of Polluted Environment? *Front Plant Sci* **9:**1476.

Taamalli M, D'Alessandro A, Marrocco C, Gevi F, Timperio A M and Zolla L (2015) Proteomic and metabolic profiles of *Cakile maritima* Scop. Sea rocket grown in the presence of cadmium. *Molecular BioSystem* **11:**1096–1109.

Thakur S, Singh N B, Sharma J, Thakur S and Gupta R K (2015) Developing climate resilient

Salix clones through control breeding. *Ind J Genet Pl Br* **74:**572-77.

Thambavani D S and Prathipa V (2012) Quantitative assessment of soil metal pollution with principal component analysis, geo accumulation index and enrichment index. *Asian J Environ Sci* **7:**125–34.

Tripathi A K and Tripathi S (1999) Changes in some physiological and biochemical characters in Albizia lebbek as bio-indicators of heavy metal toxicity. *J Environ Biol* **20:**93–98.

USEPA (1999) Report on bioavailability of chemical wastes with respect to the potential for soil remediation. T28006: QT-DC-99-003260.

Usman K, Al-Ghouti M A and Abu-Dieye M H (2019) The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant *Tetraena qataranse. Sci Rep* **9:**5658.

Vaculík M, Konlechner C, Langer I, Adlassnig W, Puschenreiter M, Lux A and Hauser M T (2012) Root anatomy and element distribution vary between two *Salix caprea* isolates with different Cd accumulation capacities. *Environ Pollut* **163:**117-26.

Verma S and Dubey R S (2001) Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. *Biol plant* **44:**117-23.

Walkley A J and Black I A (1934) Estimation of soil organic carbon by the chromic acid titration method. *Soil Sci* **37:**29-38.

Wang A S, Angle J S and Chaney R L *et al* (2006) Soil pH effects on uptake of Cd and Zn by *Thlaspi caerulescens*. *Plant Soil* **281:**325–37.

Wang C, Lu J, Zhang S, Wang P, Hou J and Qian J (2011) Effects of Pb stress on nutrient uptake and secondary metabolism in submerged macrophyte *Vallisneria natans*. *Ecotoxicol Environ Saf* **74:**1297–1303.

Wani K A, Sofi Z M, Malik J A and Wani JA (2020) Phytoremediation of heavy metals using Salix (Willows) In: Bhat R, Hakeem K and Dervash M (ed) *Bioremediation and Biotechnology*. Pp 161-74. Springer Cham, Switzerland.

Wei B, Yu J, Cao Z, Meng M, Yang L and Chen Q (2020) The availability and accumulation of heavy metals in greenhouse soils associated with intensive fertilizer application. *Int J Environ Res Public Health* **17:**5359.

Weryszko-Chmielewska E and Chwil M (2005) Lead-Induced histological and ultrastructural changes in the leaves of Soybean (*Glycine max* (L.) Merr.). *Soil Sci Plant Nutr* **51(2):**203-12.

Wuana R A and Okieimen F E (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *Int Sch Res Notic* **11:**15-22.

Xu D, Shen Z and Dou C *et al* (2022) Effects of soil properties on heavy metal bioavailability

and accumulation in crop grains under different farmland use patterns. *Sci Rep* **12:** 9211.

Xu Z and Zhou G (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J Exp Bot* **59:**3317–25.

Yadav G, Srivastava P K, Singh V P and Prasad S M (2014) Light intensity alters the extent of arsenic toxicity in Helianthus annuus L. seedlings. *Biol Trace Elem Res* **158:**410–21.

Yadav S K (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *S Afr J Bot* **76:**167-79.

Yan A, Wang Y, Tan S N, Mohd Yusof M L, Ghosh S and Chen Z (2020) Phytoremediation: A promising approach for revegetation of heavy metal-polluted land. *Front Plant Sci* **11:**359.

Yang J X, Liu Y and Ye Z H (2012) Root-Induced changes of pH, Eh, Fe(II) and fractions of Pb and Zn in rhizosphere soils of four wetland plants with different radial oxygen losses. *Pedosphere* **22**:518–27.

Yang J, Li K, Zheng W, Zhang H, Cao X, Lan Y, Yang C and Li C (2015) Characterization of early transcriptional responses to cadmium in the root and leaf of Cd-resistant *Salix matsudana* Koidz. *BMC Genomics***16:**705.

Yoon J, Cao X, Zhou Q and Ma L Q (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida Site. *Sci Total Environ* **368:**456–64.

Yoshihara T, Hodoshima H and Miyano Y *et al* (2006) Cadmium inducible Fe deficiency responses observed from macro and molecular views in tobacco plants. *Plant Cell Rep* **25:**365–73.

Zhang H, Xu Z, Guo K, Huo Y, He G, Sun H, Guan Y, Xu N, Yang W and Sun G (2020) Toxic effects of heavy metal Cd and Zn on chlorophyll, carotenoid metabolism and photosynthetic function in tobacco leaves revealed by physiological and proteomics analysis. *Ecotoxicol Environ Saf* **202:**110856.

Zhang J, Yang N, Geng Y, Zhou J and Lei J (2019) Effects of the combined pollution of cadmium, lead and zinc on the phytoextraction efficiency of ryegrass (*Lolium perenne* L.). *RSC Advances* **9:**20603

Zhang M, Xian S and Xu J (2020) Heavy metal pollution in the East China Sea: A review. *Marine Pollution Bulletin* **159:**111473

Zhang X, Xia H, Li Z, Zhuang P and Gao B (2011) Identification of a new potential Cd-hyperaccumulator Solanum photeinocarpum by soil seed bank-metal concentration gradient method. *J Hazard Mater* **189:**414–19

Zhao F J, Ma J F, Meharg A A and McGrath S P (2009) Arsenic uptake and metabolism in plants. *New Phytologist* **181:**777–94.

Zhao H, Guan J, Liang Q *et al* (2021) Effects of cadmium stress on growth and physiological characteristics of *sassafras* seedlings. *Sci Rep* **11:** 9913.

Zhen Z, Wang S, Luo S, Ren L, Liang Y, Yang R, Li Y, Zhang Y, Deng S, Zou L, Lin Z and Zhang D (2019) Significant impacts of both total amount and availability of heavy metals on the functions and assembly of soil microbial communities in different land use patterns. *Front Microbiol* **10:**2293.

Zhong X, Chen Z, Li Y, Ding K, Liu W and Liu Y *et al* (2020) Factors influencing heavy metal availability and risk assessment of soils at typical metal mines in Eastern China. *J Hazard Mater* **400:**123289.

Zhou B, Yao W, Wang S, Wang X and Jiang T (2014) The metallothionein gene, TaMT3, from Tamarix androssowii confers Cd2+ tolerance in tobacco. *Int J Mol Sci* **15:**10398–409.

Zhu Y G, Kneer R and Tong Y P (2004) Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. *Trends Plant Sci* **9:**7–9.

Zulfiqar U, Farooq M, Hussain S, Maqsood M, Hussain M, Ishfaq M, Ahmad M and Anjum M Z (2019) Lead toxicity in plants: Impacts and remediation. *J Environ Manag* **250:**109557.

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Appendix i. Effect of heavy metals on lead (Pb) content of soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

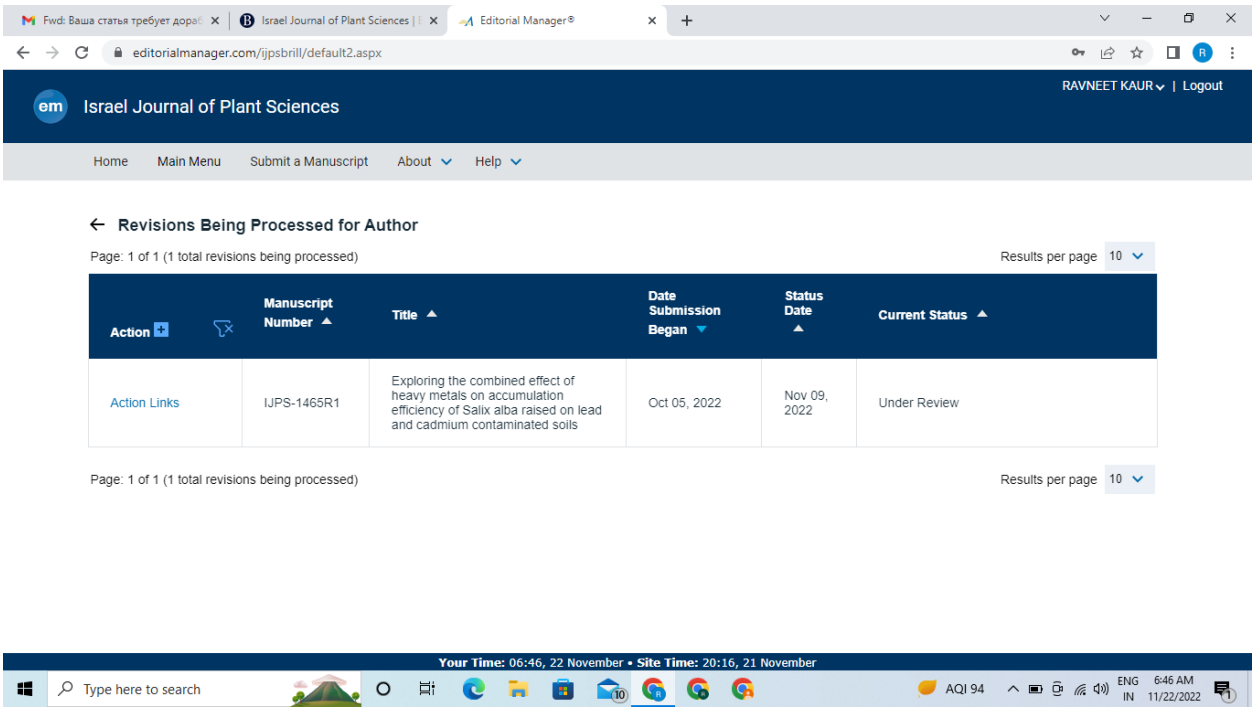
Total lead (mg/kg)																				
Before planting of *Salix*						**After uprooting of *Salix***					**Before planting of *Toona***					**After uprooting of *Toona***				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	17.1	113	221	341	172.8	11.25	37.2	75.40	127	62.7	15.2	110.2	212.8	320.3	164.6	8.43	32.9	74.3	112	56.9
Cd_5	21.2	119	217	336	173.0	14.56	44.5	96.8	193	87.1	16.4	109.4	214.6	316.7	164.3	9.95	39.7	78.9	126	63.6
Cd_{15}	15.9	115	216	331	169.6	11.24	55.0	120	201	97.0	14.2	113.5	210.6	314.3	163.1	8.75	43.6	81.3	132	66.4
Cd_{25}	21.5	107	218	339	171.3	15.68	64.0	141	242	116	14.8	105.1	215.3	320.4	163.9	10.6	48.4	85.8	139	71.0
Mean	18.9	113	218	336		13.2	50.2	108.4	191		15.15	109.5	213.3	317.9		9.43	41.2	80.1	127	
Available lead (mg/kg)																				
Before planting of *Salix*						**After uprooting of *Salix***					**Before planting of *Toona***					**After uprooting of *Toona***				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	0.91	53.0	89.9	209	88.1	0.88	2.47	6.87	20.97	7.80	0.98	60.3	90.4	189.9	85.4	0.08	2.39	5.68	18.9	6.76
Cd_5	0.84	51.0	82.0	197	82.6	0.79	2.14	6.64	19.47	7.26	0.89	58.9	85.4	192.4	84.4	0.079	2.84	6.64	19.5	7.26
Cd_{15}	0.67	50.5	78.9	192	80.4	0.75	2.06	6.57	17.94	6.83	0.78	57.8	85.4	195.6	84.9	0.075	3.24	7.22	20.9	7.86
Cd_{25}	0.08	48.6	75.4	186	77.6	0.74	1.96	6.46	16.4	6.39	0.82	57.4	85	190.5	83.4	0.074	3.45	7.57	22.0	8.27
Mean	0.63	50.8	81.5	196	82.2	0.79	2.16	6.64	18.70	7.07	0.875	58.6	86.55	192.1		0.08	2.98	6.78	20.3	

Appendix ii. Effect of heavy metals on cadmium (Cd) content of soil before planting and after uprooting of *Salix alba* and *Toona ciliata*

Total cadmium (mg/kg)																				
Before planting of *Salix*						After uprooting of *Salix*					Before planting of *Toona*					After uprooting of *Toona*				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	0.13	0.16	0.12	0.11	0.13	0.08	0.075	0.068	0.06	0.071	0.186	0.216	0.176	0.166	0.186	0.136	0.131	0.124	0.116	0.13
Cd_5	8.79	8.53	8.05	7.70	8.27	2.16	2.50	2.78	3.56	2.75	7.71	7.42	6.90	6.81	7.21	1.24	1.62	1.73	1.99	1.65
Cd_{15}	18.8	18.1	17.8	16.6	17.83	7.45	8.44	8.95	9.68	8.63	16.9	16.3	15.8	15.6	16.2	3.56	3.97	4.52	4.66	4.18
Cd_{25}	26.8	25.8	25.5	24.3	25.57	12.0	12.5	12.6	12.3	12.4	27.0	26.5	26.2	25.3	26.2	8.88	8.99	9.45	10.8	9.53
Mean	13.6	13.1	12.8	12.2	12.9	5.43	5.89	6.10	6.39	5.95	13.0	12.6	12.3	12.0		3.45	3.68	3.96	4.41	3.87
Available cadmium (mg/kg)																				
Before planting of *Salix*						After uprooting of *Salix*					Before planting of *Toona*					After uprooting of *Toona*				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	0.003	0.019	0.023	0.026	0.018	0.0012	0.0023	0.0019	0.0018	0.002	0.059	0.075	0.079	0.082	0.074	0.057	0.058	0.06	0.058	0.06
Cd_5	5.84	5.43	5.01	4.92	5.300	2.04	2.01	1.96	1.99	1.99	5.61	5.2	4.78	4.69	5.07	0.84	0.81	0.76	0.79	0.80
Cd_{15}	6.69	6.64	6.43	6.19	6.488	3.07	2.97	2.81	2.67	2.88	6.46	6.41	6.2	5.96	6.26	1.87	1.77	1.61	1.47	1.68
Cd_{25}	8.19	7.16	6.98	6.78	7.278	4.94	4.62	3.81	2.95	4.08	7.96	6.93	6.75	6.55	7.05	3.74	3.42	2.61	1.75	2.88
Mean	5.18	4.81	4.61	4.48	4.77	2.51	2.40	2.14	1.90	2.24	5.02	4.65	4.45	4.32		1.63	1.51	1.26	1.0	1.35

LIST OF PUBLISHED/ SUBMITTED RESEARCH PAPERS

Sr. No.	Title	Journal	Score	Remarks
1	Exploring the combined effect of heavy metals on accumulation efficiency of *Salix alba* raised on lead and cadmium contaminated soils	Israel Journal of Plant Sciences	NAAS 6.72	Under Review (Revision submitted)
2	An untapped phytoextraction efficiency of *Toona ciliata* for heavy metal contaminated soils through morphological and anatomical analysis	Acta Physiologiae Plantarum	NAAS 8.35	Submitted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Exploring the combined effect of heavy metals on accumulation efficiency of *Salix alba* raised on lead and cadmium contaminated soils

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Exploring the combined effect of heavy metals on accumulation efficiency of *Salix alba* raised on lead and cadmium contaminated soils

Abstract

The present study was carried out to study the effect of lead (Pb) and cadmium (Cd) treatments (single and combination) on growth and biomass production in *Salix alba*. The results illustrated that *Salix* can accumulate high level of Pb and Cd in different plant parts, with maximum accumulation in roots followed by stem and leaves in the order Cd >Pb >Cd+Pb. The phytoremediation evaluation factors such as bioconcentration factor (BCF) and translocation factor (TF) was higher for Cd over Pb in all plant parts, further the BCF for both Pb and Cd was maximum in root (BCF>1) followed by stem and leaves. Higher accumulation of Cd over the Pb was observed inside the plant tissues due to Cd mimics with other elements and gets transported through respective transporters. The combined treatment of Pb and Cd affected the bioaccumulation at every treatment level suggesting the negative effect among both elements. Higher survival rate (>85%) was recorded up to 200mgPb/kg and 15mgCd/kg, while further increase in metal concentration reduced the plant efficiency to remediate contaminated soils, hence results in declined survival rate. The FTIR analysis revealed that Pb and Cd accumulation in plants induced changes in carboxy, amino, hydroxyl and phosphate groups that ultimately caused alteration in physiological and biochemical processes of plant and thus provided an insight to the interaction, binding and accumulation of heavy metals. Thus, *S. alba* could be considered as an excluder plant as it accumulates enough amount of Pb and Cd in their roots; thereby minimizing the risk of contamination through leaf fall.

***Keywords*:** Phytoremediation, lead, cadmium, interaction, FTIR, *Salix*

Abbreviations

FTIR: Fourier Transformed Infrared Spectroscopy

ICP-MS: Inductively Coupled Plasma-Mass Spectroscopy

ATR: Attenuated Total Reflection

BCF: Bioconcentration factor

TF: Translocation factor

MR: Metal removal percent

Pb: Lead

Cd: Cadmium

Introduction

Heavy metals (HMs) are ubiquitous environmental pollutants owing to their toxic, bio accumulative nature and prolonged persistence in the environment. Globally, around 500 M ha of our land resources are facing the problem of soil contamination ended up with higher concentrations of heavy metals compared to the regulatory levels (Liu et al. 2018). Arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) are listed among the ten chemicals of major public concern by the WHO for their potential to be carcinogenic and inflict acute organ damage (Briffa et al. 2020). Among all the toxic heavy metals, Cd ranks highest in terms of damage to plant growth and human health. Moreover, its uptake and accumulation in plants poses a serious health threat to humans via the food chain (Haider et al. 2021). Exposure to Pb in the environmental and occupational settings continues to be a serious public health problem (WHO 1995; Balali-Mood, 2021). Pb and Cd as non-essential elements, can be readily taken up by plant roots and induce adverse effects on plant metabolism, growth and development (Hatamian et al. 2020).

Soil heavy metal pollution is not caused by single element, it is multi-element problem due to interaction of several heavy metal elements viz., lead, cadmium, zinc, arsenic, nickel, chromium etc. (Clabeaux et al. 2013). Research studies revealed that the action mechanism of combined heavy metal pollution is antagonistic and synergistic due to interaction between heavy metal elements (Willscher et al. 2017). Under these circumstances, synergistic and antagonistic interactions may be important, and predicted impact based on individual effects of each metal species is likely to be erroneous (Hatamian et al. 2020). Therefore, a clear understanding of interactive effects produced by combinations of metal ions at different concentrations is highly desirable.

Lead (Pb) and cadmium (Cd) are phytotoxic in nature as they induces alterations in plant at all physiological, biochemical and genetic aspects, which inhibits plant growth by suppressing respiration rate, photosynthetic rate and water nutrient uptake (Alia et al. 2015). Lead affect the uptake and translocation of magnesium (Mg) and iron (Fe) that is responsible for chlorophyll synthesis, hence it affect photosynthesis by inducing alteration in enzymatic activities inside the photosynthetic apparatus (Aslam et al. 2021). The phytotoxic nature of cadmium is due to its high mobility inside the plant that adversely affects the plant growth by reducing new cell production and root growth, also induce oxidative stress by inhibiting antioxidant enzyme activities (peroxidase, catalase, superoxide dismutase) (Zhao et al. 2021).

Plants are composed of macromolecules (i.e. carbohydrates, proteins, lipids and nucleic acids) with unique functional groups that interact with heavy metals (Usman et al. 2019). These functional groups react to certain infrared light frequencies and their interactions with the heavy metal can be investigated using Fourier Transformed Infrared Spectroscopy (FTIR) (Griffiths and Haseth, 2007) which

will reflect the presence of heavy metals in plant tissues using metal cation binding in treated plant samples.

Utilizing perennial trees for restoring the balance seems to be an effective in-situ remediation technology that utilizes the inherent natural physiological mechanisms of plants to degrade immobilized or selective uptake of contaminants from soil and water (Ahmadi et al. 2020). Phytoremediation of heavy metal polluted sites using trees may be preferred over annual plants on account of their ability to produce higher biomass, extensive root system and potential to translocate high quantities of metal in their biomass (Mleczek et al. 2017). It is a promising approach to substitute costly remediation technologies as well as has aesthetic advantage and long term applicability. The ideal plant species to remediate a heavy metal-contaminated site would be a high biomass producing trees that could both tolerate and accumulate the contaminants (Wani et al. 2020). However, meager quantitative information is available reflecting the effect of combined heavy metal pollution on uptake, translocation and bioaccumulation by *Salix alba*.

Arborescent willows are good candidates for phytoremediation owing to their perennial nature, high biomass under short rotation, ease of vegetative propagation coupled with high coppicing ability and absence of linkage with food chain (Thakur, 2015; Malik et al. 2020). *Salix* holds immense potential for the biological control of soil erosion and siltation, phyto-remediation of sewage and polluted water and nutrient cycling (Marmiroli et al. 2011; Mleczek et al. 2017). Remarkable genetic variability has been reported to exist among willow species/ clones and ecotype adapted to soil of varying level metal contaminants (Yang et al. 2015). However, a systematic research approach to explore the untapped potential in genus *Salix* clone (UHF799 i.e. *S. matsudana* x *S. alba* x *S. matsudana*) for its remediation potential is highly desirable. Holistic studies encompassing uptake, translocation and accumulation coupled with central thrust on validation of plant genotype and heavy metal interactions is decisive, because many physiological and morphological traits are expected to be linked by trade-off relationships where expression of one trait will govern the expression of another. Hence, an attempt has been made to study the phytoremediation efficiency of *Salix* raised on heavy metal contaminated soils so as to investigate the interactive effect of lead and cadmium on uptake, translocation and accumulation potential of plant.

Material and methods

Experimental design

The present investigation was conducted at Research Farm, Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana for two consecutive years 2020 and 2021. A total of 480 pots were filled with five kg of tested soil with Loamy sand soil texture having pH 8.06, EC (dS/m) 0.793, Available-NPK (183mg/kg, 50.9mg/kg, 148mg/kg respectively), total Pb (17.1mg/kg) total Cd (0.16mg/kg). The pots were arranged in completely randomized design (CRD) for 16 treatment levels of

lead and cadmium and three replications with a plot size of ten plants per replication. To prepare the heavy metal-enriched media, stock solutions of lead (in the form of Pb $(NO_3)_2$) and cadmium (in the form of $Cd(NO_3)_2$) were freshly prepared in the laboratory. The pots were spiked with different treatment levels of Pb (100 mg/kg, 200 mg/kg and 300 mg/kg) and Cd (5 mg/kg, 15 mg/kg and 25 mg/kg) individual as well as in combination (Pb+Cd). For uniform distribution of the treatment, the treated soil was equilibrated for about one month with mild irrigation as per the field capacity.

A total two hundred *Salix* clones were introduced in India by Dr. Y S Parmar University of Horticulture and Forestry (UHF), Solan, Himachal Pradesh from twenty countries. Out of these, forty two clones of *Salix* were procured and tested under nursery conditions in Punjab, out of which twenty eight clones were selected for field evaluation. Out of these, best clone *Salix alba* (UHF799) of parental line *S. matsudana* x *S. alba* x *S. matsudana* was selected to study their phytoremediation efficiency. Fresh cuttings of 15-20cm length of *Salix* were prepared from one year plants and were planted in (one cutting/pot) equilibrated soil. Observation on growth and biomass parameters were recorded on six months old plants. Thereafter, the plants were uprooted, washed with tap water, 0.1 N HCl water and distilled water. The washed root, stem and leaf samples were first air dried by keeping them in paper bags and then oven dried at 60^0C. After that these samples were ground and stored in paper bags for chemical analysis. Similarly, soil samples were taken from every pot after harvesting to study the heavy metal removal percent from phytoremediated soil.

Metal extraction and analysis

Plant sample of 0.5g powdered roots; stem and leaves were weighed and placed in acid-washed dried conical flasks. The powdered plant samples were mixed with 10ml di-acid (HNO_3 and $HClO_4$ in 3:1) and kept for overnight. Similarly, 2 g of air dried soil samples were taken in conical flask and 10 ml of aqua regia (HNO_3 and HCl) was added for digestion. On the following day, flasks containing samples were placed on hot plate and heated till whitish color and transparent solution obtained. When the digested samples cooled down, 50ml volume was made with distilled water and then solutions were filtered through Whatman no.1 filter paper. After making 25times more dilution, sample solutions were directly injected to Inductively Coupled Plasma Mass Spectrophotometer (Agilent based ICP-MS 7700, Germany) in Natural resource management (NRM) laboratory in the Department of Soil Science, Punjab Agricultural University, Ludhiana.

Fourier transformed infrared spectroscopy (FTIR) analysis was performed by using dried, fine powdered plant root, stem and leaf samples along with soils of control (Pb_0Cd_0) and highest treated plant ($Pb_{300}Cd_{25}$). These samples were analyzed at ATR (Attenuated Total Reflection) mode by using Raman spectrophotometer (Thermo Nicolet 6700 FTIR, Madison, WI USA) at electron microscopy and

nanoscience (EMN) laboratory, Department of soil science, Punjab Agricultural University, Ludhiana; data recorded within 400-4000 Cm^{-1} range.

Phytoremediation potential evaluation factors

Metal uptake, translocation and bioaccumulation potential of *S. alba* was evaluated by bioconcentration factor (BCF), translocation factor (TF), metal removal percent (MR%) and survival (%) by using following equations (Shukla et al. 2010; Zhang et al. 2019):

$$\text{BCFr} = \frac{\text{Concentration of HM in root (mg/kg)}}{\text{Concentration of HM in soil (mg/kg)}} \quad (1)$$

$$\text{BCFs} = \frac{\text{Concentration of HM in stem (mg/kg)}}{\text{Concentration of HM in soil (mg/kg)}} \quad (2)$$

$$\text{BCFl} = \frac{\text{Concentration of HM in leaf (mg/kg)}}{\text{Concentration of HM in soil (mg/kg)}} \quad (3)$$

$$\text{TF(r-l)} = \frac{\text{Concentration of HM in leaves (mg/kg)}}{\text{Concentration of HM in roots (mg/kg)}} \quad (4)$$

$$\text{MR \%} = 100 - \left\{ \frac{\text{Concentration of HM in phytoremediated soil (mg/kg)}}{\text{Concentration of HM in raw treated soil (mg/kg)}} \times 100 \right\} \quad (5)$$

$$\text{Survival (\%)} = \frac{\text{Number of plants survived}}{\text{Total number of plants}} \text{x} 100 \quad (6)$$

Where, BCFr, BCFs and BCFl are bioconcentration factor of *Salix* root, stem and leaves respectively, calculated on the basis of heavy metal availability in soil. The concentration of heavy metals in root, stem and leaves are given in Table 2 and concentration of respective available-Pb and Cd is represented in Appendix. TF(r-l) is translocation factor of *Salix* from root to stem to leaves. Further, depending on the BCF and TF values, the plant accumulation efficiency was determined as one of four groups: BCF >1: intensive; BCF=1–0.1: medium; BCF = 0.1–0.01 weak and BCF = 0.01–0.001: no accumulation; the similar criteria is followed for translocation factor (Kabata-Pendias and Pendias 1999).

The data was analyzed statistically using Tukey's HSD test. Two-way analysis of variance (ANOVA) was performed to confirm the variability among all treatments and to their interaction with the two metal elements using statistical analysis software (SAS) version 9.3 for Windows.

Results

Effect of lead and cadmium treatments on growth and biomass

The data pertaining to the table 1 reflects the growth performance of *Salix* in response to lead and cadmium treatments. With increasing concentrations of Pb and Cd in soil (as a single contaminant or

admixture of both) significant reduction in all growth and biomass attributes was observed, although non-significant differences were observed in combined treatments.

Maximum plant height (210 cm) was observed in control (Pb_0Cd_0) plants, while maximum reduction in plant height was observed in $Pb_{300}Cd_{25}$ (25.2%), followed by $Pb_{200}Cd_{25}$ (24.3%) and $Pb_{100}Cd_{25}$ (23.9%) treated plants. Similarly, maximum collar diameter (2.2 cm) was observed in control (Pb_0Cd_0) plants with maximum reduction in $Pb_{300}Cd_{25}$ (36.1%) treated plants followed by $Pb_{200}Cd_{25}$ (30.7%) and $Pb_{100}Cd_{15}$ (27.4%), respectively.

Plant biomass exhibited similar trend for all the treatments, depicting decrease in biomass production with increasing heavy metal concentrations. Maximum root and shoot dry weight (36.2 g, 57.2 g) was observed in control plants (Pb_0Cd_0), while maximum biomass reduction was recorded in $Pb_{300}Cd_{25}$(43.9%, 33.9%) followed by $Pb_{200}Cd_{25}$(42.8%, 28.4%), $Pb_{100}Cd_{15}$(40.5%, 25.8%) and $Pb_{300}Cd_{15}$(37.3%, 24.6%).

Conversely, higher concentrations of heavy metals had significantly negative effect on all studied traits with drastic reduction in growth and biomass characteristics. Further, it was also observed that effect of Cd was more prominent (either as a single or in combination) than Pb on growth and biomass attributes.

Accumulation of lead and cadmium

The data pertaining to the table 2 exhibited that increasing concentrations of Pb and Cd in soil (as a single contaminant or admixture of both) resulted in corresponding increase in accumulation in all plant parts in the following order: root >stem > leaves.

Significant increase in the concentration of Pb was observed in all plant parts, with exogenous increase in concentration of single element ($Pb_{100}Cd_0$, $Pb_{200}Cd_0$ and $Pb_{300}Cd_0$) in soils as compared to admixture with cadmium concentrations. Maximum Pb accumulation (225 mg/kg of root, 20.8 mg/kg of stem, 8.45 mg/kg of leaves) was observed in $Pb_{300}Cd_0$ treated plants followed by $Pb_{300}Cd_5$(216 mg/kg of root, 18.0 mg/kg of stem and 6.5mg/kg of leaves), $Pb_{300}Cd_{15}$ (209 mg/kg of root, 14.8 mg/kg of stem, 5.01 mg/kg of leaves) and $Pb_{300}Cd_{25}$(191 mg/kg of root, 12.4 mg/kg of stem, 4.05 mg/kg of leaves) treatments. Further, Pb accumulation with multiple concentrations of cadmium (Cd_5, Cd_{15} and Cd_{25}) showed 8.89%, 17.3% and 26.4% reduction in roots, 14.2%, 34.2% and 52.1% reduction in stem and 27.1%, 54.7% and 83.2% reduction in leaves, respectively.

Similarly, significant increase in the Cd accumulation was observed in all plant parts, with increase in concentration of single element (Pb_0Cd_5, Pb_0Cd_{15} and Pb_0Cd_{25}) in soils in comparison to admixture with lead concentrations. Maximum cadmium accumulation was found in Pb_0Cd_{25} treated plant parts viz., roots (18.4 mg/kg) > stem (14.7 mg/kg) > leaves (11.2 mg/kg) followed by $Pb_{100}Cd_{25}$, $Pb_{200}Cd_{25}$ and $Pb_{300}Cd_{25}$. However, percent reduction in cadmium accumulation observed with multiple

concentrations of lead i.e., 100mg/kg, 200mg/kg, 300mg/kg was 5.03%, 13.1% and 28.1% in roots, 9.1%, 27.0% and 31.7% in stem and 26.3%, 31.2% and 41.3% in leaves, respectively.

Effect of lead and cadmium on their BCF, TCF, survival rate and MR per cent of Salix

Bioconcentration factor (BCF) and translocation factor (TF) are the two important attributes that provides an insight towards the phytoremediation potential of a plant. The effects of lead and cadmium treatments on the bioconcentration factor of root, stem and leaves of *Salix* are depicted in Fig.1 (a,b). Among all plant parts, BCF values for both Pb and Cd under varying treatments was found highest in roots followed by stem and leaves (roots > stem > leaves). The BCF for Pb accumulation in roots was in range of 1-1.8 indicated that extensive Pb accumulation, whereas in stem (BCF= 0.20 to 0.25) and leaves (BCF= 0.05 to 0.10) denoted the medium and weak Pb accumulation respectively. Overall, the BCF values showed increased from Pb_{100} to Pb_{200} treatments, after that declining trend was recorded in all the plant parts with increase in metal concentration under varying levels and combinations. However, the decrease was more prominent in varied lead and cadmium treatment combinations as compared to the sole element.

Similarly, BCF values for Cd exhibited the higher Cd accumulation in roots (BCF= 0.8 to 2.5) followed by stem (BCF= 0.35 to 0.98) and leaves (BCF= 0.25 to 1.75). As BCF values for Cd in roots are more than one indicates the extensive Cd accumulation, further stem and leaves showed medium Cd accumulation. However, among both the metals the BCF values were confound maximum for cadmium over lead in all plant parts.

The translocation of lead and cadmium from root to stem and stem to leaves is calculated as translocation factor (Fig.1c), exhibited that with increase in metal concentration under varying levels and combinations. Further, the decrease in translocation was recorded in varied lead and cadmium treatment combinations as compared to the sole element. The translocation factor values (TF) for Pb concentrations in the range 0.025 to 0.085 indicated the weak Pb translocations. In case of Cd, the TF values were in range of 0.35 to 0.65, which showed medium translocation of cadmium inside plant tissues. However, among both the heavy metal elements, highest translocation values were observed for Cd as compared to Pb in all plant parts under varying treatment and combinations.

The effect of lead and cadmium treatments on survival rate of *Salix* is depicted in Fig. 1d. With increase in metal concentration significant decrease in metal survival percent was observed, whereas maximum reduction was recorded in combination treatments. The maximum survival 97.8 per cent was observed in control (Pb_0Cd_0), while minimum 46.7 per cent in highest treated plant ($Pb_{300}Cd_{25}$). *Salix* give best survival per cent (i.e. >85%) upto two treatment levels (Pb 100mg/kg and 200mg/kg; cadmium 5mg/kg and 15mg/kg) of lead and cadmium (single as well as in combination), after that survival rate was observed to declined.

The metal removal (MR) percent was determined by estimating the total Pb and Cd concentration in raw treated soil before planting and phytoremediated soils after uprooting plants at the termination of experiment, is exhibited in Table 3. Since, the MR values showed fluctuation with increasing Pb and Cd concentrations, but the MR values for Pb was in range of 27.1 to 67% and for Cd was in range of 38.4 to 75.4%. Thus, the data showed that *Salix* had an average 45 per cent metal removal efficiency under nursery conditions.

FTIR analysis

Heavy metal elements interact and bind with the functional groups of biomolecules present inside the plant tissues i.e. polysaccharides, proteins and nucleic acids which can be determined under infrared spectroscopy (Usman et al 2019). The region of functional group alterations in response to exposure of heavy metal was determined using control spectra and peaks were allocated using previous reports (Largo-Gosens et al. 2014; Pandey et al. 2014; Sivakumar 2016; Peng et al 2020).

All the spectra (Fig. 2) depicted six major bands corresponding to heavy metal interactions in plants as well as soil which were categorized in four major groups i.e. lipid region (3000-2000cm^{-1}), proteins (1800-1500cm^{-1}), carbohydrate (1500-1200cm^{-1}) and cell wall components (1000-600cm^{-1}). Selected peaks having variable wave number values in their respective regions for control and treated samples of soil and plant are represented in Table 4. The transmittance spectra values of treated samples were observed low in comparison to control spectra values, which might be due to changes in the vibrational frequency of several functional groups owing to their interaction with heavy metal ions accumulated in respective plant tissues.

The first marked variable absorption peak of soil spectra observed at 3473.5 cm^{-1} for control and 3473.3cm^{-1} for treated soils corresponds to the presence of functional groups of alcohol and phenols, which predict the change due to the vibrations of hydroxyl groups (Fig.2a). Similar type of band shift with different peak values was observed in roots (3605.4cm^{-1}, 3594.4cm^{-1}), stems (3627.3cm^{-1}, 3607.3cm^{-1}) and leaves (3620.3cm^{-1}, 3567.5cm^{-1}) that represents the change in functional groups of alcohol and phenol in cellulose structure owing to their interaction with Pb and Cd ions.

The significant difference between the second absorption peaks of control (Pb_0Cd_0) and treated ($Pb_{300}Cd_{25}$) samples of soils (2924.9cm^{-1},2857.2cm^{-1}), root (2917.3cm^{-1}, 2895.5cm^{-1}), stem (2931.9cm^{-1}, 2324.9cm^{-1}) and leaves (2890.6cm^{-1}, 2888.7cm^{-1}) denotes modifications in lipid regions containing glycerolipids, waxes and hydrocarbons with alteration in vibration frequency by strong narrow stretching of C-H and O-H bonds having carboxylic acids and alkanes as functional groups. The third band of plants root (2348.3 cm^{-1}, 2325.7cm^{-1}), stem (2354.8cm^{-1}, 2324.9cm^{-1}) and leaves (2345.0cm^{-1}, 2330.6cm^{-1}) spectra represents the carboxy-amino compounds and other compounds having alkene groups that showed strong sharp stretching of C=O. The third peak of soil samples (1645.7cm^{-1}, 1622.7cm^{-1}) and

fourth peaks of root (1640.7cm^{-1}, 1635.8cm^{-1}), stem (1734.2cm^{-1}, 1652.9cm^{-1}) and leaves (1652.5cm^{-1}, 1623.9cm^{-1}) showed the presence of protein amides in control and treated samples having strong broad stretching of C=O, N-H, C-H and C=C chemical bonds due to change in their vibrational frequency.

The fifth peak of plants root (1296.1cm^{-1}, 1244.9cm^{-1}) and stem (1239.8cm^{-1}, 1117.5cm^{-1}) showed common region of spectra representing narrow bend and variable stretching of C-O/C-C in lignin compounds, meanwhile leave sample showed sharp bend back in C-O, C-N corresponds to esters, ethers, alcohol and carboxylic acids containing carbohydrates. The sixth sharp peak of soil (754.6cm^{-1}, 707.1cm^{-1}) root (833.1cm^{-1}, 819.6cm^{-1}), stem (813.9cm^{-1}, 806.5cm^{-1}) and leaves (875.4cm^{-1}, 818.8cm^{-1}) denotes variable and sharp stretching of =C-H resulting in modifications in wave number for aromatic compounds, phosphate, primary and secondary amines and alkenes. The bend back peak of leaves sample in the respective region represents change in structure of carotenoids in plant.

Pb and Cd metal interaction within plant tissues in *Salix* was mostly confined to carboxyl and amino functional groups. The interactions of functional groups in plants with metal cation exchange led to modifications in cellulose, lipids, carbohydrates and protein structural moieties resulting in disruption of plant growth and development patterns.

Discussion

In the present investigation, the results revealed significant reduction in growth and biomass traits in response to different lead and cadmium concentrations as compared to the control plants. Similarly, El-Mahrouk et al. (2019) reported that Pb, Cd and Cu treatments negatively affected the plant growth in *S. mucronata*, as high concentration of metal ions in the soil media affects the elongation and meristem zone of root by altering the auxin distribution which inhibits the root growth as well as aerial plant parts. Cd toxicity reduces the uptake and translocation of mineral and nutrient ions that disrupts the normal plant metabolism and ultimately affects the plant morphology and physiology (Haider et al. 2021).

Salix accumulated significant level of lead and cadmium concentrations in its different plant parts following the order root>stem>leaves. The results are in accordance with previous research findings of Barkat (2011) that inferred that root tissues had a greater potential to accumulate metals than the aerial tissues owing to the fact that plants utilize rhizofiltration approach to remove heavy metals from contaminated site. Once entered in the root tissues, heavy metal ions binds with the cell wall components and triggers the production of chelating molecules such as organic acids, polysaccharide, metallothionein (MT), and phytochelatin (PC) (Ismail et al. 2013; Nas and Ali, 2018). PCs produced in the cytoplasm of root cells binds with the metal ions via their sulfhydryl and carboxyl groups and form complexes which are subsequently carried to the vacuoles, where they are sequestered and prevents the metal ions translocation to the aerial plant parts (Mendoza-Cozatl et al. 2011; Emamverdian et al. 2015). Presence of such interaction has been confirmed by the FTIR analysis.

The determination of bioconcentration factor (BCF) and translocation factor (TF) are crucial for developing a better understanding of the tolerance mechanism and survival strategy of plants on degraded sites (Baker, 1981). Plants with BCF <1 and TF higher than one as well as BCF and TF <1 are considered to possess phytostabilization potential and are known as excluders, as they maintain high uptake of soil-root with small root to shoot translocation. However, plants with BCF and TF >1 are suitable for phytoextraction and are termed as accumulators/hyper accumulators (Gajic et al 2018). Bioconcentration factor (BCF) defines the relationship between the concentration of chemical element in plant parts and the substrate and thus reflects the element accumulation (Yoon et al. 2006). The estimated BCF indicating that the majority of Pb and Cd accumulations in roots (BCF > 1), weak Pb accumulation (BCF=0.01 to 0.1) and medium Cd accumulation (BCF= 0.1 to 1) in both stem and leaves. As the BCF (root) values were more than one, hence, *S. alba* would be considered as a excluder plant.

TF value determines the efficiency of plants to translocate heavy metals from the root to the aerial plant parts. A plant is considered efficient in metal translocation when the TF value is higher than one indicating an efficient metal transport system (Gajic et al 2018). In present study, the translocation factor of Pb (TF = 0.01 to 0.1) and Cd (TF = 0.1 to 1) indicates the weak Pb and medium Cd translocation efficiency of *Salix*.

Further, it was observed that with increase in heavy metal concentration (either single element or in combination) in soil, the translocation factor was increased in plant parts. However, the TF exhibited more reduction during combined heavy metal pollution in comparison to the single element contamination which might be due to the heavy metals present in soil as well as plant tissues interfere with each other and other micronutrients for bioavailability and translocation either through xylem or phloem tissues using transporter proteins. Similar findings of Zhang et al. (2019) in ryegrass suggested that Pb, Cd and Zn in combination negatively affect the metal translocations in plants.

Among the two selected heavy metal elements, phytoremediation evaluation factors i.e. BCF and TF were recorded maximum for cadmium which signifies that the *S. alba* has stronger enrichment ability for cadmium than lead. The highest translocation and accumulation of Cd in plant tissues is due to its polarized nature and soft cationic character that justifies its high translocation potential to form stable complexes with the soft ligands such as amino and sulfhydryl groups (Dalton et al. 2005). Owing to its elemental characteristics and mode of uptake, Cd is readily bioavailable and quickly translocate from the roots to various regions of the plants. Cd tends to enter the plant through the essential elements (Ca, Fe, and Zn) absorption pathway, as evidenced that even guard cell Ca^{2+} channels are permeable to Cd^{2+} (Kumar et al. 2017). Although in *S. alba*, Pb preferentially accumulates in the roots and is poorly translocate to the shoot region because of the complicated metal transport system. The metal enters the root through apoplast via water streams in the inner endodermis area, where the transport regulation is

initiated (Kushwaha et al. 2018). The negatively charged components in the cell wall, such as pectin, either trap Pb ions during transit or held them in the endodermis by the casparian strip, or follow the symplastic transport to excrete the majority of the isolated Pb ions out of the plant tissues (Pourrut et al. 2011).

FTIR results confirmed the binding relationship of amide, hydroxyl, phosphate and carboxyl groups with Pb and Cd ions in *Salix*. The availability of binding sites determines the affinity of different plant tissues for specific metal ions. The ion exchange via carboxyl groups in the plant tissues is considered as a primary mechanism for phytoremediation (Usman et al. 2019). The transmittance value of metal treated soil and plants were recorded maximum as compared to control reflecting the strong binding of heavy metal ions with their respective functional groups. The strong functional group binding with the corresponding metal ion is desired in order to maintain the availability of heavy metals in soil (rhizosphere) and their accumulation in plants in order to prevent the heavy metal leaching and releasing back to the contaminated media (Sangeetha et al. 2019).

Peng et al. (2020) noticed similar trend of FTIR spectra for chlorophyll and carotenoid stretching in contaminated samples which were correlated with leaf chlorosis due to heavy metal toxicity. Findings of D'Souza et al. (2008) reported that the heavy metal ions interaction with the hydroxyl ions band shift in the range of 3627.3 to 3456.5cm^{-1} corresponds to metal and oxygen binding. Further, the results are in conformity with Al-Ghouti et al. (2010), who also reported that metal ion accumulation in plant biomass affects the general ligno-cellulosic content in tissues. The strong metal band shift in response to accumulation of heavy metals in plant depicts the behavior of chemical constituents and their reactions with heavy metal ions. Similarly, Usman et al. (2019) reported that metal accumulation in plant tissues cause in alteration in plant cell wall components which are responsible for metal ion exchange, interaction and binding, also limits the metal translocation to other tissues in *Tetraena qataranse*.

Conclusions

From the present studies, it is hereby concluded that *Salix* can be categorized as an excluder with maximum accumulation in the roots followed by stem and leaves in the order Cd >Pb >Cd+Pb. The combined (Pb+Cd) treatment has significant impact on the bioaccumulation and translocation in *Salix* suggesting the negative effect as compared to sole element application. Furthermore, FT-IR results confirmed the tolerance mechanism of *Salix* and detected modifications in the functional groups (carboxyl, amino, hydroxyl and sulfhydral) of plant tissues upon binding of heavy metals that activated the protective mechanism of plants to detoxify the harmful effects of lead and cadmium. Hence, *Salix* can be recommended as a potential species for stabilization of heavy metal (Pb and Cd) polluted sites.

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Author contributions

All authors have made substantial contributions: the conception and design of the study (RS), acquisition of data or analysis (RK) and interpretation of data (RK, SC), drafting the article (RK, ST), revising (RS, SC, SKC) it critically for important intellectual content final approval of the version to be submitted.

References

Ahmadi, O., Pandey, J., HademMoghadam, N., AsgariLajayer, B., Ghorbanpour, M. (2020) Phytoremediation of contaminated soils using trees, in: Faisal, M., Saquib, Q., Alatar, A.A. (Eds.), Cellular and molecular phytotoxicity of heavy metals. Nanotechnology in the life sciences. Springer, Cham. https://doi.org/10.1007/978-3-030-45975-8_21.

Al-Ghouti, M.A., Li, J., Salamh, Y., Al-Laqtah, N., Walker, G., Ahmad, M.N.M. (2010) Adsorption mechanisms of removing heavy metals and dyes from aqueous solution using date pits solid adsorbent. J. Hazard. Mater. 176, 510–520. https://doi.org/10.1016/j.jhazmat.2009.11.059

Alia, N., Sardar, K., Said, M., Salma, K., Sadia, A., Sadaf, S., Toqeer, A., Miklas, S. (2015) Toxicity and bioaccumulation of heavy metals in spinach (*Spinacia oleracea*) grown in a controlled environment. Int. J. Environ. Res. Public Health. 12, 7400-7416. https://doi.org/10.3390/ijerph120707400

Aslam, M., Aslam, A., Sheraz, M., Ali, B., Ulhassan, Z., Najeeb, U., Zhou, W., Gill, R.A. (2021) Lead toxicity in cereals: Mechanistic insight into toxicity, mode of action, and management. Front. Plant Sci. 11, 587785. https://doi.org/10.3389/fpls.2020.587785.

Baker, A. J. M. (1981) Accumulators and excluders – strategies in the response of plants to heavy metals. J. Plant Nutr. 3, 643–654. https://doi.org/10.1080/0190416810936286

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M.R., Sadeghi, M. (2021) Toxic mechanisms of five heavy metals: Mercury, lead, chromium, cadmium and arsenic. Front. Pharmacol. 12, 643972. https://doi.org/10.3389/fphar.2021.643972

Barakat, M.A. (2011) New trends in removing heavy metals from industrial wastewater. Arabian J. Chem. 4(4), 361–377. https://doi.org/doi:10.1016/j.arabjc.2010.07.019.

411 Briffa, J., Sinagra, E., Blundell, R. (2020) Heavy metal pollution in the environment and their
412 toxicological effects on humans. Heliyon. 6(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691.
413 Clabeaux, B.L., Navarro, D.A., Aga, D.S., Bisson, M.A. (2013) Combined effects of cadmium
414 and zinc on growth, tolerance, and metal accumulation in Chara australis and enhanced phytoextraction
415 using EDTA. Ecotoxicol. Environ. Saf. 98, 236–243. http://dx.doi.org/10.1016/j.ecoenv.2013.08.014
416 D'Souza, L., Devi, P., DivyaShridhar, M., Naik, C.G. (2008) Use of Fourier Transform Infrared
417 (FTIR) spectroscopy to study cadmium induced changes in *Padina tetrastromatica* (Hauck). Anal. Chem.
418 Insights. 3, 117739010800300001.
419 Dalton, T. P., He, L., Wang, B., Miller, M.L., Jin L. Stringer KF, Chang Z, Baxter CS, Neber
420 D.W. (2005) Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced
421 toxicity in the testis. Proc. Natl. Acad. Sci. USA. 102, 3401–3406.
422 https://doi.org/10.1073/pnas.0406085102
423 El-Mahrouk, E.M., Eisa, E.A., Hegazi, M.A., Abdel-Gayed, M.E., Dewir, Y.H., El-Mahrouk,
424 M.E., Naidoo, Y. (2019) Phytoremediation of cadmium-, copper-, and lead-contaminated soil by *Salix*
425 *mucronata* (Synonym Salix safsaf) Hortscience 54(7), 1249–1257.
426 https://doi.org/10.21273/HORTSCI14018-19
427 Emamverdian, A., Ding, Y., Mokhberdoran, F., Xie, Y. (2015) Heavy metal stress and some
428 mechanisms of plant defense response. Sci. World J. 756120. https://doi.org/10.1155/2015/756120.
429 Gajic´, G., Djurdjevic´, L., Kostic´, O., Jaric´, S., Mitrovic´, M., Pavlovic´, P. (2018) Ecological
430 potential of plants for phytoremediation and ecorestoration of fly ash deposits and mine wastes. Front.
431 Environ. Sci. 6, 124. https://doi.org/10.3389/fenvs.2018.00124
432 Griffiths, P. R., De Haseth, J. A (2007) Fourier transform infrared spectrometry. Vol. 171, John
433 Wiley & Sons. Hoboken, NJ, USA
434 Haider, F.U., Liqun, C., Coulter, J.A., Cheema, S.A., Wu, J., Zhang, R., Wenjun, M., Farooq, M.,
435 (2021) Cadmium toxicity in plants: Impacts and remediation strategies. Ecotoxicol. Environ. Saf. 211,
436 111887. https://doi.org/10.1016/j.ecoenv.2020.111887.
437 Hatamian, M., Nejad, A.R., Kafi, M., Souri, M.K., Shahbazi, K (2020) Interaction of lead and
438 cadmium on growth and leaf morphophysiological characteristics of European hackberry (*Celtis*
439 *australis*) seedlings. Chem. Biol. Technol. Agric. 7, 9. https://doi.org/10.1186/s40538-019-0173-0
440 Ismail, S., Khan, F., Iqbal, M.Z (2013) Phytoremediation: assessing tolerance of tree species
441 against heavy metal (Pb and Cd) toxicity. Pak. J. Bot. 45(6), 2181-2186.
442 Kabata-Pendias, A., Pendias, H. (1999) Biogeochemia pierwiastko'w s'ladowych.
443 Biogeochemistry of trace elements, 3rd ed. Wyd.Naukowe PWN, Warsaw (in Polish).

Kumar, S.S., Kadier, A., Malyan, S.K., Ahmad, A., Bishnoi, N.R. (2017) Phytoremediation and rhizoremediation: uptake, mobilization and sequestration of heavy metals by plants, in: Singh, D.P. (Ed) Plant-Microbe interactions in agro-ecological perspectives. Springer, pp. 367-394. https://doi.org/10.1007/978-981-10-6593-4_15

Kushwaha, A., Hans, N., Kumar, S., Rani, R.A. (2018) Critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. Ecotoxicol. Environ. Saf. 147, 1035–1045. https://doi.org/10.1016/j.ecoenv.2017.09.049

Largo-Gosens, A., Hernandez-Altamirano, M., Garcıa-Calvo, L., Alonso-Simon, A., Alvarez, J., Acebes, J.L. (2014) Fourier transform mid infrared spectroscopy applications for monitoring the structural plasticity of plant cell walls. Front. Plant. Sci. 5, 303–330. https://doi.org/10.3389/fpls.2014.00303.

Liu, L.W., Li, W., Song, W.P., Guo, M.X. (2018) Remediation techniques for heavy metal–contaminated soils: Principles and applicability. Sci. Total Environ. 633, 206–219. https://doi.org/10.1016/j.scitotenv.2018.03.161.

Malik, J.A., Wani, A.A., Wani, K.A., Bhat, M.A. (2020) Role of white willow (*Salix alba* L.) for cleaning up the toxic metal pollution, in: Hakeem, K. R., et al. (Eds.), Bioremediation and Biotechnology. pp.257-268. https://doi.org/10.1007/978-3-030-35691-0_12.

Marmiroli, M., Pietrini, F., Maestri, E., Zacchini, M., Marmiroli, N., Massacci, A. (2011) Growth, physiological and molecular traits in Salicaceae trees investigated for phytoremediation of heavy metals and organics. Tree Physiol. 31, 1319-1334. https://doi.org/10.1093/treephys/tpr090

Mendoza-Cozatl, D.G., Jobe, T.O., Hauser, F., Schroeder, J.I. (2011) Long-distance transport, vacuolar sequestration, tolerance, and transcriptional responses induced by cadmium and arsenic. Curr. Opin. Plant Biol. 14(5), 554–562. https://doi.org/10.1016/j.pbi.2011.07.004

Mleczek, M., Goliński, P., Krzesłowska, M., Gąsecka, M., Magdziak, Z. et al. (2017) Phytoextraction of potentially toxic elements by six tree species growing on hazardous mining sludge. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-017-9842-3

Nas, F.S., Ali, M. (2018) The effect of lead on plants in terms of growing and biochemical parameters: a review. MOJ. Eco. Environ. Sci. 3(4), 265-268. https://doi.org/10.15406/mojes.2018.03.00098

Pandey, R., Ansari, N., Prasad, R., Chavali, M. (2014) Pb(II) removal from aqueous solution by *Cucumis sativus* (Cucumber) peel: kinetic, equilibrium & thermodynamic study. Am. J. Environ. Protect. 2(3), 51–58.

476 Peng, H., Wang, Y., Tan, T.L., Chen, Z. (2020) Exploring the phytoremediation potential of
477 water hyacinth by FTIR Spectroscopy and ICP-OES for treatment of heavy metal contaminated water, Int.
478 J. Phytoremediation. https://doi.org/10.1080/15226514.2020.1774499
479 Pourrut, B., Shahid, M., Dumat, C., Winterton, P., Pinelli, E. (2011) Lead uptake, toxicity, and
480 detoxification in plants. Rev. Environ. Contam. Toxicol. 213, 113–136 (Springer, 2011).
481 https://doi.org/10.1007/978-1-4419-9860-6_4.
482 Sangeetha, P., Venkatachalam, P., Geetha, N. (2019) Exploring the phytomediation potential of
483 *Calotropis gigantean* L. using a combined FTIR and principal component analysis, in : Kumar, M.,
484 Muthusamy, A., Kumar, V., Bhalla-Sarin, N. (Eds.), In vitro plant breeding novel agronomic traits.
485 Springer, Singapore. https://doi.org/10.1007/978-981-32-9824-8_5
486 Shukla, O.P., Juwarkar, A. a, Singh, S.K., Khan, S., Rai, U.N. (2011) Growth response and metal
487 accumulation capabilities of woody plants during the phytoremediation of tannery sludge. Waste Manag.
488 31, 115–23. https://doi.org/10.1016/j.wasman.2010.08.022
489 Sivakumar, P. (2016) Phytoremediation of tannery waste polluted soil using Hyptissuaveolens
490 (Lamiaceae). Int. J. Pure. App. Biosci. 4(1), 265–272. https://doi.org/10.18782/2320-7051.2223.
491 Thakur, S., Singh, N.B., Sharma, J., Thakur, S., Gupta, R.K (2015) Developing climate resilient
492 *Salix* clones through control breeding. Indian J. Genet. Pl. Br. 74 (4), 572-577.
493 Usman, K., Al-Ghouti, M.A. and Abu-Dieye, M.H. (2019) The assessment of cadmium,
494 chromium, copper, and nickel tolerance and bioaccumulation by shrub plant *Tetraena qataranse* Sci. Rep.
495 9, 5658. https://doi.org/10.1038/s41598-019-42029-9
496 Wani, K.A., Sofi, Z.M., Malik, J.A., Wani, J.A. (2020) Phytoremediation of heavy metals using
497 Salix (Willows), in: Bhat, R., Hakeem, K., Dervash, M. (Eds.), Bioremediation and Biotechnology,
498 Springer: Cham, Switzerland, Vol 2, pp. 161–174.
499 Willscher, S., Jablonski, L., Fona, Z., Rahmi, R., Wittig, J. (2017) Phytoremediation experiments
500 with *Helianthus tuberosus* under different pH and heavy metal soil concentrations. Hydrometallurgy, 168,
501 153–158. https://doi.org/10.1016/j.hydromet.2016.10.016
502 Yan, A., Wang, Y., Tan, S.N., Mohd Yusof, M.L., Ghosh, S., Chen, Z. (2020) Phytoremediation:
503 A promising approach for revegetation of heavy metal-polluted land. Front. Plant Sci. 11, 359.
504 https://doi.org/10.3389/fpls.2020.00359
505 Yang, J., Li, K., Zheng, W., Zhang, H., Cao, X., Lan, Y., Yang, C., Li, C. (2015) Characterization
506 of early transcriptional responses to cadmium in the root and leaf of Cd-resistant *Salix matsudana*
507 Koidz. BMC Genomics. 16, 705.

Yoon, J., Cao, X., Zhou,Q., Ma, L.Q. (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida Site. Sci. Tot. Environ. 368, 456–464. https://doi.org/10.1016/j.scitotenv.2006.01.016

Zhang, J., Yang, N., Geng, Y., Zhou, J., Lei, J (2019) Effects of the combined pollution of cadmium, lead and zinc on the phytoextraction efficiency of ryegrass (*Lolium perenne* L.) RSC Adv. 9, 20603 https://doi.org/10.1039/c9ra01986c

Zhao, H., Guan, J., Liang, Q., et al. (2021) Effects of cadmium stress on growth and physiological characteristics of *sassafras* seedlings. Sci. Rep. 11, 9913. https://doi.org/10.1038/s41598-021-89322-0

Table 1. Effect of lead and cadmium treatments on growth and biomass of *Salix*

Plant height (cm)					
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	$210.^{a}$	184^{b} (12.4%)	181^{bc} (13.9%)	176^{bcd} (16.2%)	188^{A}
Cd_5	182^{bc} (13.4%)	180^{bc} (14.1%)	176bcd (15.9%)	174^{bcdef} (17.1%)	178^{B}
Cd_{15}	174^{bcdef} (17.4%)	172^{bcdef} (18.3%)	168^{bcdef} (20.1%)	166^{cdef} (20.8%)	170^{C}
Cd_{25}	168^{bcdef} (20.10)	160^{def} (23.9%)	159^{ef} (24.3%)	157^{f} (25.2%)	161^{D}
Mean	183^{A}	174^{B}	171^{B}	168^{B}	

Collar diameter (cm)					
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	2.22^{a}	2.18^{ab} (1.83%)	2.12^{bcd} (4.59%)	2.06^{cde} (7.09%)	2.43^{A}
Cd_5	2.13^{bc} (4.12%)	2.05^{de} (7.61%)	2.04^{e} (8.10%)	2.03^{e} (8.43%)	2.06^{B}
Cd_{15}	1.84^{f} (16.9%)	1.83^{f} (17.5%)	1.81^{f} (18.3%)	1.80^{f} (18.8%)	1.82^{C}
Cd_{25}	1.71^{g} (22.7%)	1.61^{h} (27.4%)	1.53^{i} (30.7%)	1.41^{j} (36.1%)	1.56^{D}
Mean	1.97^{A}	1.92^{B}	1.87^{C}	1.56^{D}	

Root dry weight (g)					
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	36.25^{a}	33.95^{a} (6.34%)	30.43^{bc} (16.06%)	27.18^{def} (25.03%)	31.95^{A}
Cd_5	31.20^{b} (13.93%)	29.10^{bcd} (19.72%)	27.98^{cde} (22.83%)	26.0^{efg} (28.27%)	28.57^{B}
Cd_{15}	26.15^{efg} (27.86%)	25.05^{fgh} (30.89%)	23.68^{ghi} (34.68%)	22.72^{hij} (37.31%)	24.40^{C}
Cd_{25}	22.45^{hij} (38.06%)	21.75^{ij} (40.0%)	20.85^{ij} (42.48%)	20.33^{j} (43.93%)	21.34^{D}
Mean	29.01^{A}	27.46^{B}	25.73^{C}	24.06^{D}	26.57

Shoot dry weight (g)					
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	57.3^{a}	55.1^{ab} (3.79%)	52.8^{b} (7.7%)	48.2^{c} (15.76%)	53.3^{A}
Cd_5	52.0^{b} (9.13%)	48.3^{c} (15.6%)	46.5^{cde} (18.8%)	45.1^{cdef} (21.3%)	47.9^{B}
Cd_{15}	47.9^{cd} (16.3%)	46.6^{cde} (18.6%)	44.7^{def} (21.9%)	43.1^{fg} (24.6%)	45.6^{C}
Cd_{25}	44.4^{ef} (22.4%)	42.5^{fg} (25.8%)	40.9^{g} (28.4%)	37.8^{h} (33.9%)	41.4^{D}
Mean	50.4^{A}	48.1^{B}	46.2^{C}	43.6^{D}	

Mean values in the same column having different superscript are significantly different ($p<0.05$)
Values in parenthesis indicates reduction percent w.r.t. control

Table 2. Effect of heavy metal treatments on accumulation of lead (Pb) and cadmium (Cd) in *Salix* plant parts

LEAD ACCUMULATION															
	Root (mg/kg)					**Stem (mg/kg)**					**Leaves (mg/kg)**				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	7.80^{m} ± 0.10	90.4^{i} ± 0.41	175^{e} ± 0.52	225^{a} ± 0.57	125^{A}	2.80^{j} ±0.06	11.1^{fg} ±0.15	16.0^{bc} ±0.94	20.8^{a} ±0.55	12.6^{A}	0.8^{ef} ±0.04	4.56^{bdc} ±0.09	6.54^{ab} ±0.06	8.45^{a} ± 0.12	5.08^{B}
Cd_5	6.95^{m} ± 0.14	85.9^{j} ± 0.34	149^{f} ± 0.47	216^{b} ± 0.54	115^{B}	2.41^{j} ± 0.02	10.1^{gh} ±0.74	13.6^{de} ±0.82	18.0^{b} ±0.51	11.0^{AB}	0.53^{ef} ±0.15	3.82^{dc} ±0.16	4.98^{bdc} ±0.14	6.51^{bac} ± 0.10	3.95^{AB}
Cd_{15}	6.45^{m} ± 0.20	74.7^{k} ± 0.17	137^{g} ± 0.31	209^{c} ± 0.39	107^{B}	2.20^{j} ±0.04	8.56^{h} ±0.09	12.1^{efg} ±0.15	14.8^{cd} ±0.19	9.42^{BC}	0.39^{f} ±0.02	3.18^{de} ±0.13	4.30^{bdc} ±0.16	5.01^{bdc} ±0.25	3.23^{A}
Cd_{25}	5.95^{m} ± 0.05	68.5^{l} ± 0.24	135^{h} ± 0.35	191^{d} ± 0.38	100^{D}	1.50^{j} ±0.06	7.55^{i} ±0.16	11.3^{fg} ±0.20	12.4^{ef} ±0.34	8.18^{C}	0.31^{f} ±0.08	2.71^{def} ±0.14	3.82^{cdc} ±0.16	4.05^{bcd} ±0.11	2.71^{A}
Mean	6.79^{D}	79.9^{C}	149^{B}	210^{A}		2.23^{D}	9.33^{C}	13.3^{B}	16.5^{A}		0.51^{A}	3.57^{B}	4.90^{BC}	6.00^{C}	
CADMIUM ACCUMULATION															
	Root (mg/kg)					**Stem (mg/kg)**					**Leaves (mg/kg)**				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	0.02^{l} ±0.00	0.025^{l} ±0.00	0.03^{l} ±0.00	0.04^{l} ±0.00	0.029^{D}	0.01^{j} ±0.00	0.02^{j} ±0.00	0.025^{j} ±0.01	0.036^{j} ±06	0.023^{D}	0.009^{h} ±0.01	0.01^{h} ±0.00	0.02^{h} ±0.00	0.01^{h} ±0.00	0.01^{D}
Cd_5	5.08^{i} ±0.04	4.80^{ij} ±0.08	4.45^{ik} ±0.05	4.04^{k} ±0.09	4.59^{C}	2.84^{h} ±0.12	2.65^{hi} ±0.15	2.41^{hi} ±0.14	2.26^{i} ±0.19	2.54^{C}	2.03^{g} ±0.02	1.91^{g} ±0.04	1.72^{g} ±0.06	1.59^{g} ±0.05	1.81^{C}
Cd_{15}	13.5^{e} ±0.14	12.9^{f} ±0.11	11.6^{g} ±0.10	9.32^{h} ±0.12	11.8^{B}	7.82^{e} ±0.21	5.94^{f} ±0.22	5.14^{g} ±0.26	4.81^{g} ±0.28	5.92^{B}	6.08^{d} ±0.13	4.60^{e} ±0.11	3.89^{f} ±0.08	3.45^{f} ±0.03	4.50^{B}
Cd_{25}	18.4^{a} ±0.15	17.5^{b} ±0.14	16.1^{c} ±0.16	14.2^{d} ±0.12	16.6^{A}	14.7^{a} ±0.36	14.1^{b} ±0.41	12.8^{c} ±0.56	11.3^{d} ±0.62	13.2^{A}	11.2^{a} ±0.14	8.80^{b} ±0.17	7.81^{c} ±0.13	6.51^{d} ±0.18	8.58^{A}
Mean	9.26^{A}	8.84^{B}	8.06^{C}	6.91^{D}		6.33^{A}	5.64^{B}	5.08^{C}	4.59^{D}		4.83^{A}	3.83^{B}	3.36^{C}	2.89^{D}	

Mean values in the same column having different superscript are significantly different ($p<0.05$); ± represents standard error

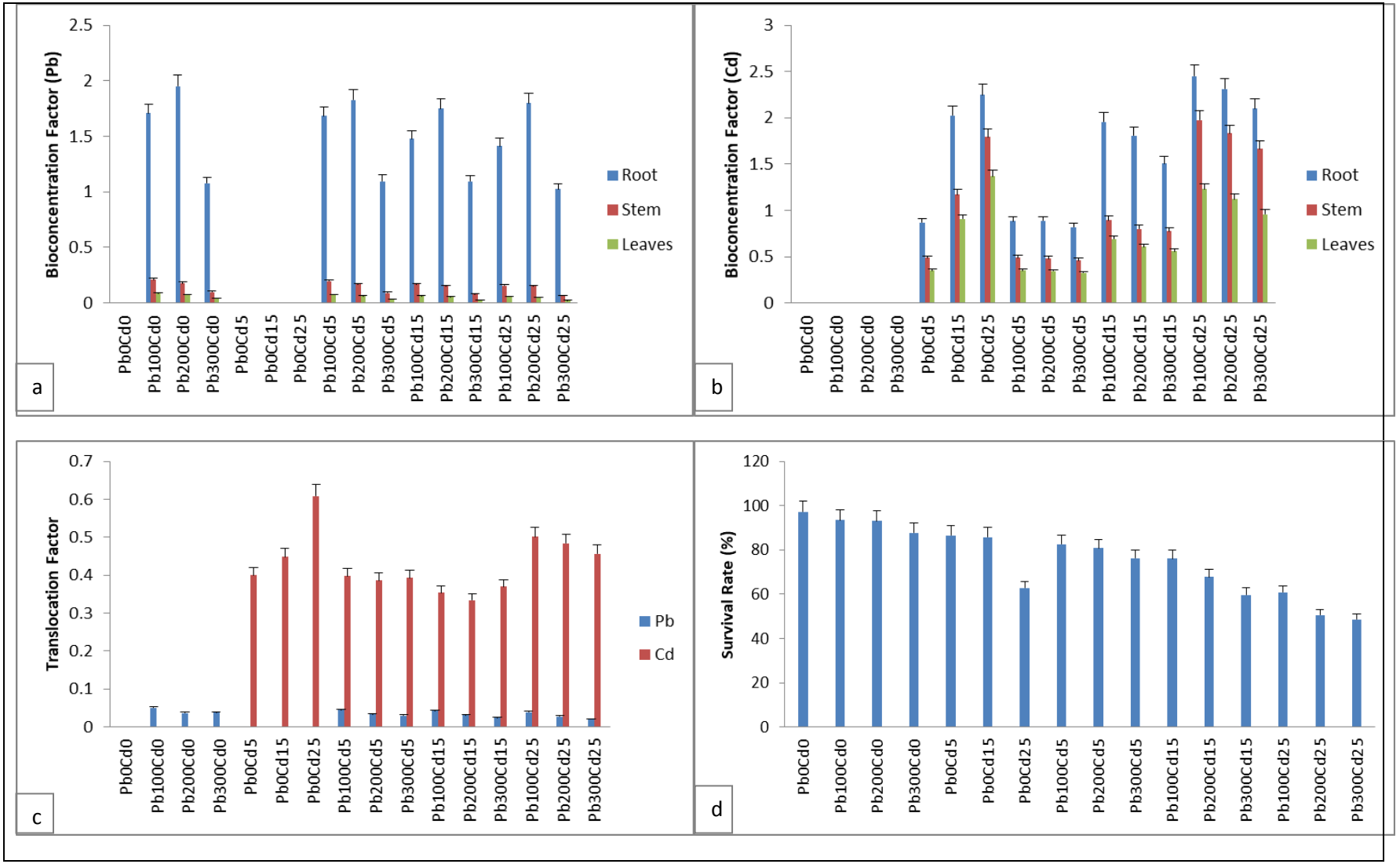
Table 3. The efficiency of *Salix* for metal removal (%) as influenced by different lead (Pb) and cadmium (Cd) treatments

Total Lead (mg/kg)															
Treated soil (before planting)						**Phytoremediated soil (after uprooting)**					**Lead metal removal (%)**				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	17.1^{hi}	113^{f}	221^{d}	341^{a}	172.8^{A}	11.25^{m}	37.2^{l}	75.4^{h}	127^{e}	62.7^{D}	34.2^{h}	67.0^{a}	65.8^{a}	62.7^{b}	57.5^{A}
Cd_5	21.2^{h}	119^{e}	217^{d}	336^{b}	173.0^{A}	14.56^{m}	44.5^{k}	96.8^{g}	193^{c}	87.1^{C}	31.3^{i}	62.4^{b}	55.2^{c}	42.6^{ef}	47.9^{B}
Cd_{15}	15.9^{i}	115^{ef}	216^{d}	331^{c}	169.6^{B}	11.24^{m}	55.0^{j}	120^{f}	201^{b}	96.9^{B}	29.3^{ij}	52.3^{d}	44.3^{e}	39.1^{g}	41.3^{C}
Cd_{25}	21.5^{h}	107^{g}	218^{d}	339^{ab}	171.3^{A}	15.68^{m}	64.0^{i}	141^{d}	242^{a}	115^{A}	27.1^{j}	40.1^{fg}	35.7^{h}	28.6^{ij}	32.9^{D}
Mean	18.9^{D}	113^{C}	218^{B}	336^{A}		13.2^{D}	50.2^{C}	108.4^{B}	191^{A}		30.5^{D}	55.4^{A}	50.3^{B}	43.3^{C}	
Total Cadmium (mg/kg)															
Treated soil (before planting)						**Phytoremediated soil (after uprooting)**					**Cadmium metal removal (%)**				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	0.13^{i}	0.16^{i}	0.12^{i}	0.11^{i}	0.13^{D}	0.08^{j}	0.075^{j}	0.068^{j}	0.06^{j}	0.071^{D}	38.4^{j}	53.1^{efg}	43.3^{hi}	55.4^{h}	45.1^{C}
Cd_5	8.79^{g}	8.53^{g}	8.05^{h}	7.70^{h}	8.27^{C}	2.16^{i}	2.50^{hi}	2.78^{h}	3.56^{g}	2.75^{C}	75.4^{a}	70.6^{b}	65.4^{c}	53.7^{ef}	66.3^{A}
Cd_{15}	18.8^{d}	18.1^{e}	17.8^{e}	16.6^{f}	17.8^{B}	7.45^{f}	8.44^{e}	8.95^{d}	9.68^{c}	8.63^{B}	60.2^{d}	53.4^{efg}	49.8^{g}	41.8^{ij}	51.3^{B}
Cd_{25}	26.8^{a}	25.8^{b}	25.5^{b}	24.3^{c}	25.6^{A}	12.0^{b}	12.5^{a}	12.6^{a}	12.3^{ab}	12.4^{A}	54.9^{e}	51.3^{efg}	50.4^{fg}	49.6^{g}	51.6^{B}
Mean	13.6^{A}	13.1^{B}	12.8^{C}	12.2^{D}		5.43^{D}	5.89^{C}	6.10^{B}	6.39^{A}		57.3^{A}	57.1^{A}	52.3^{B}	47.6^{C}	

Mean values in the same column having different superscript are significantly different ($p<0.05$)

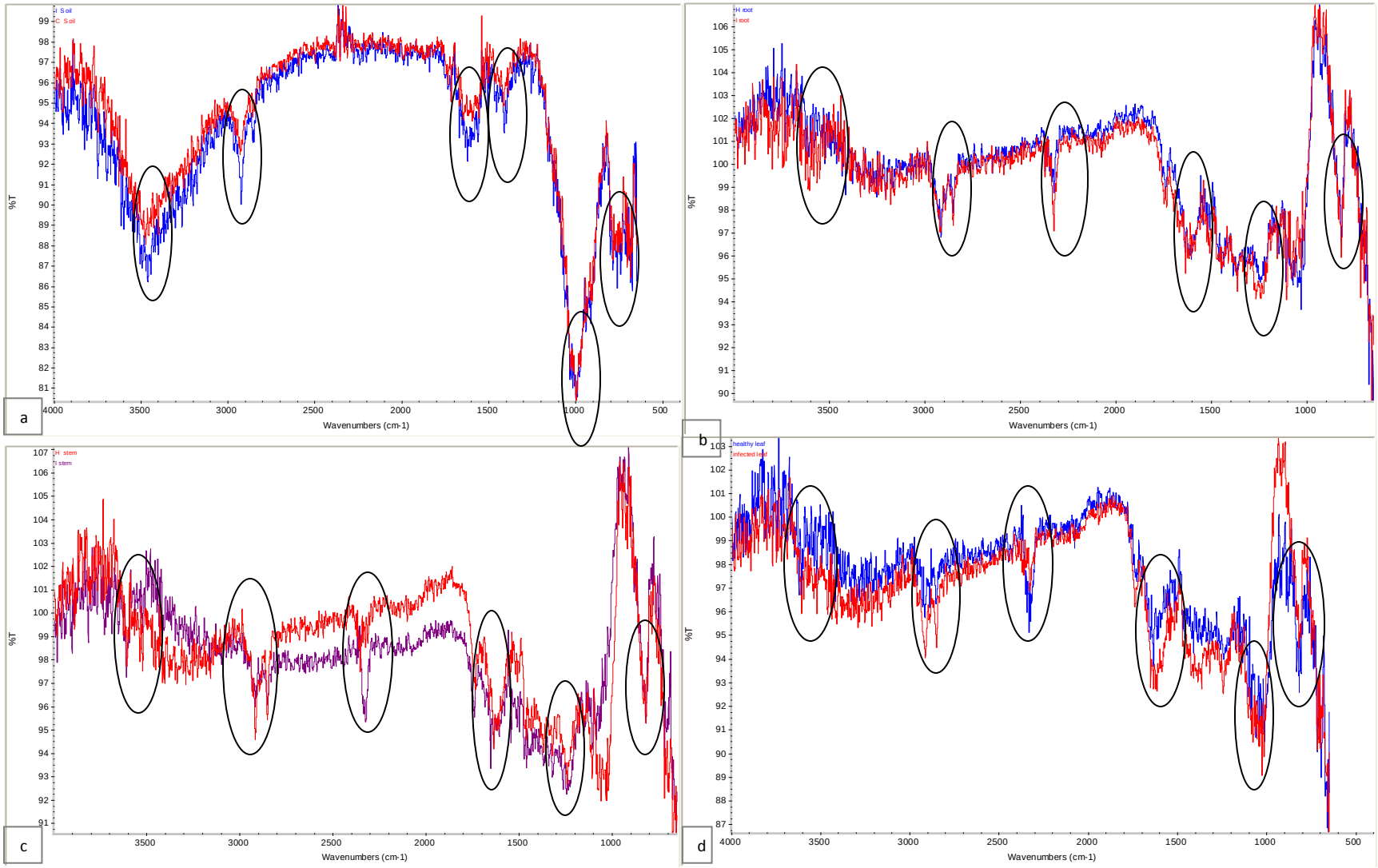
Table 4. Mean peak values of selected FTIR spectral regions for control (Pb_0Cd_0) and treated ($Pb_{300}Cd_{25}$) samples of soil and *Salix* plant parts

Sr No.	FTIR Soil					FTIR Root				
	Pb_0Cd_0 Wavenumber(Cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber(Cm^{-1})	Chemical assignment	Functional group	Compound	Pb_0Cd_0 Wavenumber(Cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber(Cm^{-1})	Chemical assignment	Functional group	Compound
1	3473.5	3473.3	O-H	Alcohols Phenols	Phenolic compounds, Cellulose	3605.4	3594.4	O-H	Alcohols Phenols	Phenolic compounds, Cellulose
2	2924.9	2857.2	S, C-H, O-H	Alkanes Carboxylic acid	**Lipid region, hydrocarbon**	2917.3	2895.5	S, C-H, O-H	Alkanes Carboxylic acid	Glycerolipid, wax, hydrocarbon
3	1645.7	1622.7	C=C, C-O, N-H, C=O	Alkenes, Aromatics, Amines, Benzene	**Amides of protein, Proline**	2348.3	2325.7	C=O, P-H	Alkenes, Phosphine	Carboxy-amino compounds, phosphates
4	1418.7	1412.2	C-H, C-C, C-O, N=O	Alkanes, Aromatics	**Polysaccharides**	1604.7	1635.8	C=C, C-C, N-H, C=O	Alkenes, Benzene, Aromatics, Amides	Amides of protein
5	997.4	997.3	C-H, C-O-P, S=O, =C-H	Alcohols, Aliphatic amines, Alkenes	**Phosphates, Sulphoxides**	1296.1	1266.7	C-O/C-C	Methoxy, Esters, Amines, Carbonyl, Carboxylic acid	Lignin, ligno-cellulose
6	754.6	707.1	C-O-P, =C-H, C-N	Alkynes, Benzene, Amines	**Polysaccharides, Xyloglucans**	833.1	819.6	N-H	Alkyl halides, Benzene, Amines	Chlorides
Sr No.	**FTIR Stem**					**FTIR Leaf**				
	Pb_0Cd_0 Wavenumber(Cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber(Cm^{-1})	Chemical assignment	Functional group	Compound	Pb_0Cd_0 Wavenumber(Cm^{-1})	$Pb_{300}Cd_{25}$ Wavenumber(Cm^{-1})	Chemical assignment	Functional group	Compound
1	3627.3	3607.3	O-H	Phenols, Alcohols	Phenolic compounds, Cellulose	3620.3 3606.6 3330.0	3567.5 3576.9 3362.3	O-H	Phenols	Phenolic compounds, Cellulose
2	2931.9	2324.9	S, C-H, O-H	Alkenes Carboxylic acid	Glycerolipid, wax, hydrocarbon	2916.7 2890.6 2652.9	2915.3 2888.7	S, C-H, O-H	Alkanes Carboxylic acid	Chlorophyll, Glycerolipid, wax, hydrocarbon
3	2354.8	2324.9	O-H, N-H	Alkenes, Amines	Carboxy-amino compounds	2345.0 2333.6	2330.6	O-H, N-H	Alkenes	Carboxy-amino compounds
4	1734.2	1652.9	C = C, C-C, C=O, N-H	Alkenes, Amines, Saturated aldehydes	Amides of protein	1652.5 1656.7	1623.9 1616.0	C=C, C-C, N-H	Alkenes, Aromatics, Amines	Amides of proteins
5	1239.8	1117.5	C-O/C-C, C-N	Methoxy, carbonyl, carboxylic acid	Lignin, ligno-cellulose	1033.7 1027.9	1014.0 1034.1	C-O	Esters, ethers, alcohols, carboxylic acids	Carbohydrate
6	813.9	806.5	=C-H, C-N	Alkenes, Benzene, Amine	Cellulose, Cell wall components	875.4 925.6	818.8 807.0	=C-H, C-N, C-C	Alkenes, Benzene	Carotenoids Cellulose, Chlorides



--Error bar represents standard error

Fig. 1. Bioconcentration factor (BCF), translocation factor (TF) and survival percentage of *Salix* as influenced by different concentrations of Pb and Cd



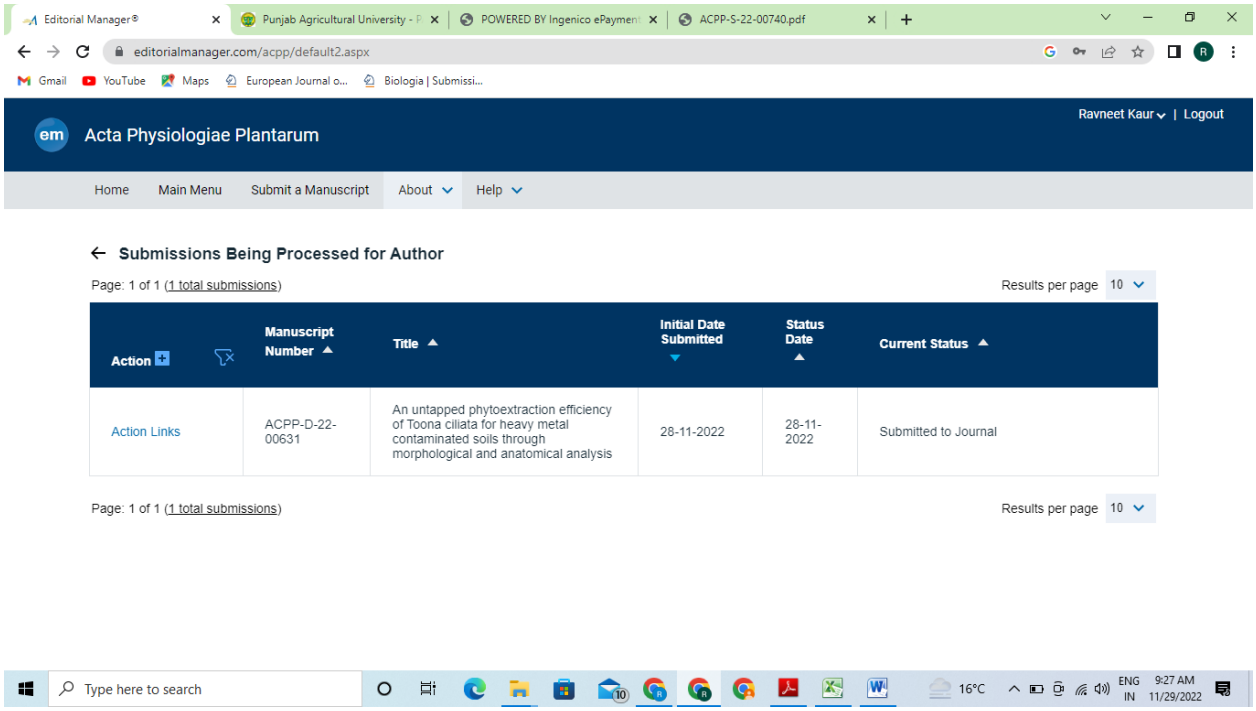
Black circles showing selected peaks

Fig. 2. FTIR spectra for soil and plant parts of *Salix*

(a) Soil control (Pb_0Cd_0, red line) vs treated ($Pb_{300}Cd_{25}$, blue line)
(b) Stems control (Pb_0Cd_0, red line) vs treated ($Pb_{300}Cd_{25}$, blue line)
(b)Roots control (Pb_0Cd_0, blue line) vs treated ($Pb_{300}Cd_{25}$, red line)
(d) Leaves control (Pb_0Cd_0, blue line) vs treated ($Pb_{300}Cd_{25}$, red line)

A1. **Available lead and cadmium concentration in soil after application of different lead and cadmium treatments**

	Available lead (mg/kg)					Available lead (mg/kg)				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	0.88	2.47	6.87	20.97	7.80	0.0012	0.0023	0.0019	0.0018	0.002
Cd_5	0.79	2.14	6.64	19.47	7.26	2.04	2.01	1.96	1.99	1.99
Cd_{15}	0.75	2.06	6.57	17.94	6.83	3.07	2.97	2.81	2.67	2.88
Cd_{25}	0.74	1.96	6.46	16.4	6.39	4.94	4.62	3.81	2.95	4.08
Mean	0.79	2.16	6.64	18.70	7.07	2.51	2.40	2.14	1.90	2.24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An untapped phytoextraction efficiency of Toona ciliata for heavy metal contaminated soils through morphological and anatomical analysis

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An Untapped phytoextraction efficiency of *Toona ciliata* for heavy metal contaminated soils through morphological and anatomical analysis

Abstract

Phytoremediation is a cost-effective and environmental friendly approach to reclaim heavy metal contaminated soils. The phytoremediation using tree species has been preferred over annual crops as they produce higher biomass and accumulate heavy metals for prolonged period. *Toona ciliata* M. Roemer is a large perennial tree, known to produce high biomass in short rotation, can be used for phytoremediation experiments due to their alternative use in versatile timber production and absence of linkage with food chain. Thus, present investigations were conducted to untapped phytoextraction efficiency of *T. ciliata* using morphological and anatomical analysis during two constitutive years 2020-21. *T. ciliata* raised on soils treated with different concentration of Pb, Cd and their combinations for six months, the results illustrated that plants can accumulate higher concentration of metals in shoots than roots having bioconcentration factor (BCF) and translocation factor (TF) more than one, which conferred that plants have efficient metal translocation and accumulation capability. Further, heavy metal tolerance index and survival percentage (>85%) denotes the plant's ability to tolerate heavy metals up to 300mgPb/kg and 25mgCd/kg. Anatomical changes induced due to heavy metals includes reduced root area as well as vascular bundle area as compared to control, also reduced stomatal pore size and increased stomatal index and trichome density suggesting the plant's adaptive response under heavy metal stress. Along with this, differential Pb and Cd accumulatory tissues of both leaves and roots were marked and confirmed by using Field emission scanning electron microscopy and energy dispersive x-ray spectroscopy (FESEM-EDS) which reflects that plant had strong enrichment ability for Pb accumulation in roots and more Cd translocation, accumulation and sequestration in aerial tissues. Hence, the present study reflects that *Toona ciliata* had great phytoextraction efficiency for reclaiming Pb and Cd contaminated soils.

Keywords: Tree, Phytoextraction, Accumulation, Lead, Cadmium, Anatomy

Introduction

Soil heavy metal pollution is an amalgamation of interaction between various heavy metal elements viz., lead, cadmium, zinc, arsenic, nickel, chromium etc. (Clabeaux et al. 2013). Among all the toxic heavy metals, Cd and Pb are considered as most harmful and have potential to negatively affect the plant growth and development (Kanwal et al. 2020; Maestri et al. 2010). Soils of Punjab, India are getting worse due to rapid industrialization and population growth. The toxic chemicals added by them includes heavy metals are affecting soil, crops and ultimately human health (Kaur et al. 2022). Globally, around 500 M ha of our land resources are facing the problem of soil contamination (Liu et al. 2018). In Punjab, the problem is prominent where industrial effluents get mixed with sewerage water making the condition worst. This affects crop productivity and soil microbial activity, hence affecting soil and water quality which make it difficult to meet the sustainable development goals of United Nations (Krishan et al. 2021). Therefore, the remediation of these heavy metal polluted soils becomes prerequisite.

Phytoremediation is an ecofriendly, cost-effective approach that involves use of plants for the extraction, immobilization and degradation of elemental pollutants from the polluted sites (Yan et al. 2020). Utilizing perennial plants for restoring the balance seems to be an effective in-situ remediation technology that utilizes the inherent physiological mechanisms of plants to degrade, immobilize or selectively uptake of contaminants from polluted soil and water interface (Ahmadi et al. 2020). Mleczek et al. (2017) suggested that the tree species should be preferred over annuals on account of their ability to produce higher biomass, extensive root system and potential to sequester high quantities of metal for a longer time span. The accumulation of heavy metals in plants involves series of processes including metal bioavailability, root uptake, xylem loading, root-shoot translocation, cellular compartmentalization and sequestration (Kumar et al. 2017). Thus, in order to understand the plant tolerance mechanisms, it is crucial to investigate the response and adaptability of plant anatomical structures in response to heavy metal pollution.

Under heavy metal stress, almost all the plant tissues show alteration in their morphology, ultra structure along with their associated physiological mechanisms (Aslam *et al* 2021). The alterations in plant morphology and anatomy in response to adverse environmental conditions indicates the tolerance and adaptability of plant against abiotic stress (Huang et al. 2019). Heavy metal toxicity induces ultra-structural changes in all plant parts, viz. root, stem and leaves which is responsible for modification in their normal functioning. Plant root system plays a crucial role to accumulate heavy metals and alters their bioavailability, further responsible for reclamation of contaminated soils and restores the soil fertility (Muszynska et al. 2019). In case of poplar, jatropha (Shu *et al* 2012), maize (Hayat *et al* 2012), wheat and rice (He *et al* 2014), it has been reported that heavy metals transported from contaminated soils via roots to aerial parts and their accumulation in plant cells interfere directly with the cellular metabolism of shoots, resulting in a reduction in height.

The stomatal index is an important biological indicators for plants which varies among species growing on heavy metal treated soils, as the stomatal number per unit area of *Beta vulgaris* (Sagardoy *et al* 2010) and *Sorghum vulgaris* (Kasim 2006) was shown to decrease under Zn and Cd-Cu stress, whereas increased number of stomata has been recorded in the presence of Pb and As in several plants, such as *Helianthus annuus* and *Vigna radiata*, at the early stages of metal toxicity, which was followed by the formation of fused and deformed stomata in *Vigna radiata* (Gupta *et al* 2015). Furthermore, as seen in *Brachiaria decumbens* under Cu stress, heavy metal exposure causes reduction in parenchymatous tissues and xylem vasculature, as well as the production of relatively smaller mesophyll tissue (Gomes *et al* 2011). All these heavy metal toxicity induced alterations in the stomata, xylem vessels, parenchymatous, and mesophyll cells impair the plant-water relationship, resulting in decreased leaf growth (Riyazuddin *et al* 2022).

Toona ciliata M. Roemer is a fast growing and large deciduous tree belongs to family Meliaceae, known to produce high biomass and its versatile timber used for building houses and ships, musical instruments, furniture and carvings and greater adaptability in sub-tropical regions in Punjab, India (Kundal et al. 2020). Thus, we hypothesize that *T. ciliata* plants can be used for phytoremediation studies although plants have some medicinal and antioxidant properties, but their alternate use in timber production will prevent the phytoremediated heavy metal entry into food chain. Since, the very limited studies on phytoremediation using *Toona ciliata* has been reported (Wang et al 2022, Sharma and Singh 2018), but their phytoextraction efficiency on the basis of uptake, translocation and accumulation using phytoremediation efficiency evaluation factors such as bioconcentration factor (BCF) and translocation factor (TF) along with morphological and anatomical analysis have been unexplored yet. Hence, an attempt has been made to untap the phytoremediation potential of *T. ciliata* for heavy metal contaminated soils, by exploring phytoremediation efficiency factors along with morphological and anatomical trait analysis of plants grown in heavy metal contaminated soils under nursery conditions.

Material and methods

Experimental design

The present investigation was carried out at Research Farm, Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana for two consecutive years 2020 and 2021. A total of 480 pots were filled with five kg of tested soil (Table 1) and were arranged in completely randomized design (CRD) for 16 treatment levels of Pb and Cd and three replications with a plot size of ten plants per replication. Three concentration levels of Pb (100mg/kg, 200mg/kg and 300mg/kg) and Cd (5mg/kg, 15mg/kg and 25mg/kg) were used single as well as in combination to study their combined effect. These sixteen treatment levels were assigned as follows:

T1: Pb_0Cd_0	T2: $Pb_{100}Cd_0$	T3: $Pb_{200}Cd_0$	T4: $Pb_{300}Cd_0$
T5: Pb_0Cd_5	T6: $Pb_{100}Cd_5$	T7: $Pb_{200}Cd_5$	T8: $Pb_{300}Cd_5$
T9: Pb_0Cd_{15}	T10: $Pb_{100}Cd_{15}$	T11: $Pb_{200}Cd_{15}$	T12: $Pb_{300}Cd_{15}$
T13: Pb_0Cd_{25}	T14: $Pb_{100}Cd_{25}$	T15: $Pb_{200}Cd_{25}$	T16: $Pb_{300}Cd_{25}$

The pots filled with sandy loam soil from study area were spiked with different treatment levels of Pb and Cd using $Pb(NO_3)_2$ and $Cd(NO_3)_2$. For uniform distribution of the treatment, the treated soil was equilibrated for about one month with mild irrigation as per the field capacity. The nursery of *Toona ciliata* was transplanted to pots containing heavy metals treated soils, after attained the height of approx. 15cm in the month of February. Observation on morphological and biomass parameters were recorded on six months old plants. Thereafter, the plants were uprooted, washed with tap water followed by 0.1 N HCl water and distilled water. The washed roots and shoots were first air dried by keeping them in paper bags and then oven dried at 60^0C. After that these samples were ground and stored in paper bags for chemical analysis. Similarly, soil samples were taken from every pot after harvesting to study the heavy metal removal percent from phytoremediated soil.

Metal extraction and analysis

Plant sample of 0.5g powered roots and shoots were weighed and placed in acid-washed dried conical flasks. The powdered plant samples were mixed with 10ml di-acid (HNO_3 and $HClO_4$ in 3:1) and kept for overnight. Similarly, 2 g of air dried soil samples were taken in conical flask and 10 ml of aqua regia (HNO_3 and HCl) was added for digestion. On the following day, flasks containing samples were placed on hot plate and heated till whitish color and transparent solution obtained. After digestion, appropriate dilutions were made, filtered through Whatman no.1 filter paper and sample solutions were directly injected to Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS) in Natural resource management (NRM) laboratory in the Department of Soil Science, Punjab Agricultural University, Ludhiana.

Phytoremediation potential evaluation factors

Metal uptake, translocation and bioaccumulation potential of *T. ciliata* was evaluated by bioconcentration factor (BCF) and translocation factor (TF) by using following equations 1 and 2 (Shukla et al. 2010; Zhang et al. 2019)

$$BCF = \frac{\text{Concentration of HM in root/stem/leaves}}{\text{Concentration of HM in soil}} \quad (1)$$

$$TF = \frac{\text{Concentration of HM in shoot}}{\text{Concentration of HM in root}} \quad (2)$$

Further, depending on the BCF, BAC and TF values, the plant accumulation efficiency was determined as one of four groups: BCF >1: intensive; BCF=1–0.1: medium; BCF = 0.1–0.01 weak and BCF = 0.01–0.001: no accumulation; the similar criteria is followed for BAC and TF (Kabata-Pendias and Pendias 2010).

Morphological and biomass attributes

After 6 months of planting, five plants from each replication were selected and observations were recorded for various growth and biomass attributes, such as plant height, collar diameter, root number, root length, root and shoot dry weight. Biomass tolerance index and plant survival percentage was calculated by using equation 3 and 4 (Chen et al. 2017):

$$\text{Tolerance index (\%)} = \frac{\text{Biomass of HM treated plants}}{\text{Biomass of control plants}} \times 100 \quad (3)$$

$$\text{Survival (\%)} = \frac{\text{No. of plants survived}}{\text{Total no. of plants}} \times 100 \quad (4)$$

Anatomical analysis

The anatomical analysis was performed by using Lieca Bright Field Research Microscope, Field Emission Scanning Electron Microscope (FE-SEM) and Energy Dispersive X-ray Spectroscopy (EDS). The fresh, acid-water washed roots and leaves of control (Pb_0Cd_0) and highest treated plant ($Pb_{300}Cd_{25}$) were selected for anatomical analysis. The impression approach was used to determine leaf stomatal density, which was expressed as the number of stomata per unit leaf area (Radoglou and Jarvis 1990). The adaxial surface of the leaf was cleaned using a degreased cotton ball and smeared with Quick fix in the mid-area between the central vein and the leaf edge, for approximately 5 min. The thin film was peeled off from the leaf surface, mounted on a glass slide, immediately covered with a cover slip. The number of stomata (s) and epidermal cells (e) for each film strip were counted under a Leica Bright Field Research Microscope (10X). The leaf stomatal index was estimated using the formula [s/(e + s)]×100. The stomatal pore size was measured between the junctions of the guard cells at each end of the stoma that may indicate the maximum potential opening of the stomatal pore (Xu and Zhou 2008). Similarly, number of trichomes per unit area represents the trichome density and was observed on adaxial surfaces of the control and treated plants.

The ultra-structural changes induced due to Pb and Cd stress in roots and leaves of *T. ciliata* were determined by FESEM and elemental distribution patterns in different plant tissues were analyzed by EDS at Sophisticated Analytical Instrumental Facility (SAIF), Punjab University, Chandigarh.

The data obtained for various growth, biomass, physiological and biochemical parameters were statistically analyzed using one-way ANOVA with Tukey's honestly significant difference test among treatment means and *p* value <0.05 was considered statistically significant using SAS windows version 9.3.

Results

Accumulation of Pb and Cd in different plant parts (root, stem and leaves) of *Toona ciliata*

Lead and cadmium concentrations were estimated in different plant parts (i.e. root, stem and leaves) of *T. ciliata*. The data presented in Table 1 exhibits that lead (Pb) and cadmium accumulation (Cd) increased with increasing concentrations of Pb (individual as well as in combination with Cd). Pb and Cd accumulation in plant parts were higher under sole application of Pb and Cd as compared to their combinations (Pb+Cd). The maximum Pb accumulation was recorded with higher Pb application i.e. $Pb_{300}Cd_0$ in root, stem and leaves i.e. 90.5 mg/kg 92.1 mg/kg and 139.8 mg/kg, respectively followed by $Pb_{300}Cd_5$ (84.5 mg/kg of root, 90.2 mg/kg of stem and 132.5mg/kg of leaves), $Pb_{300}Cd_{15}$ (79.3 mg/kg of root, 84.7 mg/kg of stem, 128.9 mg/kg of leaves) and $Pb_{300}Cd_{25}$

(72.3 mg/kg of root, 76.9 mg/kg of stem, 125.7 mg/kg of leaves). Among different plant parts the accumulation of Pb followed the order as leaves > stem > roots.

Similarly, the significant increase in Cd accumulation was recorded in all plant parts with increase in Cd concentration. The minimum mean Cd accumulation was observed in control i.e., Cd_0 (0.02 to 0.03 mg/kg) that increased significantly with increase in Cd concentration. Highest concentration of the Cd resulted in maximum accumulation of Cd i.e., Cd_{25} (12.1 mg/kg in roots, 12.8 mg/kg in stem and 16.9 mg/kg in leaves). Cd accumulation was higher with sole Cd application (Cd_5, Cd_{15} and Cd_{25}) as compared to their combination with Pb concentrations (Pb+Cd) in different plant parts and followed the order as leaves > stem > roots. The decreasing Pb and Cd accumulation in combination as compared to sole element application indicates their negative effect among both metals.

Phytoremediation efficiency evaluation factors for *Toona ciliata*

Bioconcentration factor (BCF) and translocation factor (TF) are the two important parameters that provides an insight towards the phytoremediation potential of a plant. The bioconcentration factors were evaluated on the basis of availability of heavy metals in soil (Appendix) and its accumulation in plant parts (Table 1).

Bioconcentration factor for both Pb and Cd varied with in response to heavy metal concentrations in soil (Fig. 1). On an average, the BCF for Pb was more than one in all plants i.e. root (BCF=1.5-2.6), stem (BCF=1.4-2.4) and leaves (0.99-1.5). Among Cd concentrations, the BCF was in the range of 0.5-1.8 in roots, 0.40-1.25 in stem and 0.45-0.95 in leaves. Among both Pb and Cd concentrations, BCF was comparatively higher for Pb than Cd in all plant parts, which reflects that *T. ciliata* plants had strong enrichment ability for Pb concentrations as compared to Cd.

The effect of heavy metals on translocation factors (TF) from root to shoots of *Toona* are expressed in Fig. 1. The TF values also showed variation among different concentration of Pb and Cd, however, translocation factor for both Pb (TF=1.5-2.0) and Cd (TF=1.20-1.45) was more than one (i.e. TF >1), which indicates that *T. ciliata* had efficient translocation mechanism for both Pb and Cd.

Morphological (growth and biomass) traits of *Toona ciliata* as affected by Pb and Cd

Effect of lead and cadmium on plant height, collar diameter, root length, root number, fresh and dry weight of both root and shoots were recorded (Table 2). *T. ciliata* showed significant decrease in plant height under different Pb and Cd concentrations. Higher plant height was recorded under control (Pb_0Cd_0, 268.7cm) that was at par with $Pb_{200}Cd_0$ (268 cm), $Pb_{100}Cd_0$ (267.7cm) and Pb_0Cd_5 (266.7cm) concentrations, these values decreased significantly with increase in heavy metal concentrations, hence, minimum plant height was recorded with highest heavy metal concentration (254.7cm), however, the reduction was only five per cent. Similarly, significant decrease in collar diameter with increasing concentration of Pb, Cd and their combinations was recorded. The maximum collar diameter was observed in control (2.57cm) and minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (2.22cm) with maximum reduction (13.5 %). Plant roots being first organ exposed to contaminated soil confers the variation

in their number along with their morphological alterations induced due to heavy metal stress. With increasing concentrations of Pb and Cd in soil, significant decrease in root number and root length was recorded in *T. ciliata*. Among Pb, Cd and their combinations, maximum root length was recorded in control (Pb_0Cd_0, 48.3 cm), which decreased significantly with increase in heavy metal concentration, hence minimum with highest combination concentration i.e. $Pb_{300}Cd_{25}$ (28.7 cm) with 40% reduction as compared to control. Similarly, maximum number of roots were recorded in control (15.7), that was recorded to be minimum with highest concentration i.e. $Pb_{300}Cd_{25}$ (7.33) and showed 53 % reduction with respect to control.

T. ciliata plants also showed significant reduction in biomass attributes viz., fresh weight and dry weight of roots and shoots, which was recorded maximum in control and decreased significantly with increase in Pb and Cd concentrations; however 20 to 30 per cent reduction in biomass was observed even at higher concentration ($Pb_{300}Cd_{25}$). Thus, the reduction in shoot biomass can be correlated with reduced number of branches and number of leaves which conferred that Pb and Cd concentrations adversely affect the shoot system. Similarly, the decline in root biomass might be due to decrease in root length and root number of plants grown on Pb and Cd contaminated soil.

Conversely, it is concluded from morphological parameters that higher concentrations of heavy metals had significant negative effect on all studied traits along with reduction in biomass characteristics of *T. ciliata* under nursery conditions. The adverse effect on morphological traits was more in combination (Pb+Cd) treatments as compared to single element application.

Survival percentage and tolerance index

As heavy metals negatively affect the plant growth that is also manifested in the survival percentage of plants grown on heavy metal contaminated soils. The maximum survival percentage (100%) was recorded in control (Pb_0Cd_0), the effect of different Pb and Cd concentrations on survival percentage was same, i.e. they do not affect survival percentage even at higher concentrations (Pb_{300} and Cd_{25}). Moreover, with highest combination concentration i.e. $Pb_{300}Cd_{25}$ the sufficient survival percentage (93.3%) was recorded (Fig. 2). On the basis of biomass, *T. ciliata* showed higher tolerance index (80 to 95%) with individual application of Pb and Cd than in combinations (70 to 80%). Thus, the decreased tolerance was more pronounced with combinations (Pb+Cd) compared to their individual element concentrations in an order Pb > Cd > Pb+Cd.

Anatomical alterations of *T. ciliata* grown under Pb and Cd contaminated soil

The morphological parameters of *T. ciliata* revealed negative effect with highest concentration of Pb and Cd combination i.e. $Pb_{300}Cd_{25}$. Hence, this concentration ($Pb_{300}Cd_{25}$) was selected for anatomical studies to study the Pb and Cd induced ultra-structural changes in plants as compared to control by using light microscopy and field emission scanning electron microscopy (FESEM). Along with this, Pb and Cd accumulation sites are predicted in different tissues of root and leaves of *T. ciliata* using energy dispersive x-ray spectroscopy (EDS).

The root system is the first organ exposed to polluted soil and is known to modify its anatomical characteristics in order to confer the plant adaptation under heavy metal stress. The cross-section of *T. ciliata* roots observed under field emission scanning electron microscopy (FESEM) was circular in shape in control plants, whereas the heavy metal treated roots showed wavy outline due to destruction of some epidermal and cortical cells under Pb and Cd induced toxicity (Fig. 3). This destruction is further responsible for reduced cortical region and reduced total root area (1.18 mm^2) as compared to control (1.24 mm^2) (Table 3). The vascular region consisted of pericycle, phloem and xylem tissues and outer endodermis. Due to Pb and Cd induced toxicity, structural changes in xylem vessels which includes enlarged or collapsed intercellular spaces in plants treated with $Pb_{300}Cd_{25}$ as compared to control. Further, the reduced root vascular bundle area was observed in $Pb_{300}Cd_{25}$ treated plants (0.65 mm^2) compared to control plants (0.68mm^2) as mentioned in Table 3.

In *Toona*, light microscopy and FESEM measurements (Fig. 4) conferred that the stomatal pore size decreased with $Pb_{300}Cd_{25}$ (18.24 µm) concentration as compared to control (21.86µm) as shown in Table 3. However, the stomatal density increased at higher concentration of Pb and Cd ($Pb_{300}Cd_{25}$; 165.4 no./mm^2) as compared to control (142.8 no./mm^2). Similarly, the higher stomatal index was observed in $Pb_{300}Cd_{25}$ (18.1%) treated plants as compared to control (13.8%). Trichome density on adaxial surface of *Toona* leaves were calculated and observed increase in total number of trichomes in response to $Pb_{300}Cd_{25}$ concentration (109.5 no./mm^2) as compared to control (85.2 no./mm^2) (Fig. 5). Overall, stomatal frequency, stomatal index and trichome density increased, whereas the stomatal pore size decreased in the *Toona ciliata* grown in Pb and Cd contaminated soils. In the plant leaves, trichomes are known to accumulate higher heavy metal toxic ions than other cells which prevent the interference of heavy metal in metabolism, thus the increased number of trichomes due to heavy metal toxicity considered as activation of plant tolerance mechanism against stress.

Pb and Cd distribution patterns in roots and leaves of *T. ciliata*

Energy dispersive x-ray spectroscopy (EDS) performed to expound the Pb and Cd accumulation sites in *T. ciliata* root and leaves. The EDS results of $Pb_{300}Cd_{25}$ treated plant roots indicates that higher concentration of Pb (0.98 wt. %) than Cd (0.13 wt.%) in outer (epidermal and cortical) tissues, similarly in vascular region the higher Pb accumulation (0.80 wt.%) over Cd (0.60 wt.%) was recorded (Fig 6). Similarly, the EDS values in plant leaves grown in $Pb_{300}Cd_{25}$ contaminated soils denoted that Pb accumulation was comparatively higher than the Cd (Fig.7). EDS values of epidermal surface of leaves showed that higher accumulation of Pb (4.10 wt.%) over Cd (1.52 wt.%). Similarly, the cortical and vascular region of $Pb_{300}Cd_{25}$ treated leaves showed the higher concentration of Pb (2.39 wt.%) over Cd (1.22 wt. %). Thus, EDS results of *T. ciliata* conferred higher Pb accumulation than Cd in epidermal, cortex and vascular region of roots, similarly higher Pb accumulation over Cd was recorded in all leaf tissues which is also observed in Fig.1 depicting higher BCF values for Pb than Cd. Among different plant parts, higher Pb and Cd accumulation was recorded in aerial plant parts (leaves) than roots and these results are in favor with BCF and TF values (Fig. 1). Hence, The FESEM and EDS analysis confirmed the uptake and accumulation of

Pb and Cd ions in different tissues of *T. ciliata* grown in Pb and Cd contaminated soils. These heavy metals are responsible for all morphological and ultra-structural alterations under heavy metal stress.

Discussion

The determination of bioconcentration factor (BCF) and translocation factor (TF) are crucial for developing a better understanding of the tolerance mechanism and survival strategy of plants on degraded sites. Heavy metal (Pb and Cd) accumulation was recorded more during single element contamination as compared to combination, as the heavy metals present in soil as well as plant tissues interfere with each other as well as other micronutrients for bioavailability and translocation either through xylem or phloem tissues using transporter proteins. Similar findings of Zhang et al. (2019) in ryegrass suggested that Pb, Cd and Zn combination concentrations in soil adversely affects the metal translocation in plants.

In the present study, phytoremediation evaluation factors of *T. ciliata* reveals that plants showed maximum Pb and Cd accumulation in their aerial plant parts than roots having BCF >1 and TF>1. According to Baker (1981), plants with BCF > 1 and TF >1 as well as BCF >1 and TF < 1 are considered to possess phytostabilization potential. *T. ciliata* showed BCF >1 and TF >1 which is suitable for phytoextraction and can be categorized as accumulators/hyper accumulators as per the criteria described by Gajic et al. (2018). *Toona* plants show better survival percentage along with sufficient tolerance index with higher Pb and Cd concentrations ($Pb_{300}Cd_{25}$ mg/kg). Among the two selected heavy metal elements, phytoremediation evaluation factors i.e. BCF and TF signifies that *T. ciliata* has stronger enrichment ability for Cd than Pb, however, their partitioning and accumulation behaviour were almost same having more accumulation in aerial plant parts than roots. The higher translocation and accumulation of Cd in plant tissues is due to its polarized nature and soft cationic character that justifies its high translocation potential to form stable complexes with the soft ligands such as amino and sulfhydryl groups (Dalton et al. 2005). Owing to its elemental characteristics and mode of uptake, Cd is readily bioavailable and quickly translocated from the roots to various regions of the plants. Cd tends to enter the plant through the essential elements (Ca, Fe, and Zn) absorption pathway, as evidenced that even guard cell Ca^{2+} channels are permeable to Cd^{2+} (Kumar et al. 2017). Pourrut *et al* (2011) and Kushwaha et al. (2018) reported that the negatively charged components in the cell wall, such as pectin, either trap Pb ions during transit or held them in the endodermis by the casparian strip, or follow the symplastic transport to excrete the majority of the isolated Pb ions out of the plant tissues.

Plant height, collar diameter, biomass are the primary determinants of plant growth, Pb and Cd being non-essential elements inhibits plant growth at higher concentration (Haider et al. 2021). In the present study, the reduction in morphological and biomass attributes were recorded in response to different Pb and Cd concentrations. Similarly, the reduction in plant height due to Cd toxicity in *Poplar* species and Pb toxicity in *Jatropha curcas* is reported by Kieffer et al. (2019) and Shu et al. (2012) respectively. Guerrea et al. (2011) reported that heavy metals such as Pb, Cd and Zn negatively affect the plant height and collar diameter of different *Poplar* species raised under

controlled conditions. The significant reduction in root length and root number was recorded in *T. ciliata* grown on heavy metal contaminated soil. These results are confirmed by El-Mahrouk et al. (2019), they reported that Pb, Cd and Cu treatments negatively affected the plant growth of *Salix mucronata* because the high concentration of metal ions in the soil media affects the elongation and meristem zone of root by altering the auxin distribution which inhibits the root growth by influencing root number, root length and root density as well as aerial plant parts. Similarly, Cd toxicity is also reported to reduce the uptake and translocation of mineral and nutrient ions by disturbing the normal root growth patterns which further deteriorate the plant metabolism and ultimately affects the plant morphology and biomass (Haider et al. 2021).

Overall, Pb and Cd treatments reduce the biomass with 25 % reduction in *Toona* even at higher concentrations. These results are in accordance with Günthardt-Goerg et al. (2022) who reported the average 23% reduction in biomass of trees such as *Betula*, *Populus*, *Salix* and *Picea abies* raised under long term heavy metal polluted site. Mleczek et al. (2010) reported that toxic heavy metal such as Pb, Cd and Hg reduce the biomass production ability of *Salix viminalis* and *Salix alba* var. Chermesina with an average reduction of 104 to 6.81Kg for each shrub. Under heavy metal stress, decrease in plant biomass may be linked to disrupted metabolic activity due to lower uptake of critical nutrients (Kacalkova et al. 2015). Plant survival rate is a critical metric for determining how well a species has adapted to particular environmental conditions and it also influence average biomass production (Chibuike and Obiora 2014). In the present study, the sufficient survival percentage (>90%) of *Toona ciliata* was recorded even at higher concentrations with least reduction in biomass.

Roots of Pb and Cd-treated plants undergo visible alterations, such as reduced root number, root length and biomass of *T. ciliata*. Similar Cd induced modifications were also reported by Vaculík et al. (2012) in *Salix caprea.* Cd-induced differences in the proportions of specific root tissues (rhizodermis cortex vascular bundles) or in the size and shape of individual cells have been noticed by Lux et al. (2011). These changes highlight the reduced potential of roots to absorb water and mineral leading to a disturbance of the main physiological processes of photosynthesis and transpiration in leaves (Huang et al. 2019). It is evidenced from current investigations that heavy metal accumulation in plant tissues stimulates the suberization and lignification of the root cells that further increase the cell thickness and collapse intercellular spaces, thus prevents the toxic ion movement inside plant tissues of *T. ciliata*. Hamim et al. (2018) reported that decreased root area and vascular region results in diminished conductive potential of the phloem and xylem tissues as inductive response to heavy metal stress, which is also in favour with present study.

The anatomical studies reveal that *T. ciliata* showed increased stomatal density and trichome density along with decreased stomatal pore size at higher concentrations of Pb and Cd. Our results in accordance with Hermle et al. (2007), who reported that the influence of Pb and Cd on stomatal opening was due to the reduced turgor of the subsidiary cells in *Populus tremula*. This may be due to rapid and preferential absorption of metals by subsidiary cells followed by changes in membrane permeability causing decrease in cell turgor, ultimately observed with

reduced pore size. Rucińska-Sobkowiak (2016) reported that water balance disturbance is an early stress-induced event; *Arabidopsis thaliana* had efficient adaptive mechanism to survive under heavy metal stress conditions by increased stomatal density to maintain sufficient CO_2 flow without affecting photosynthesis and by reduced stomatal pore size to reduce excess water loss through transpiration. Based on the bibliographic data (Weryszko-Chmielewska and Chwil 2005), it is assumed that high density of trichomes is one of the plant adaptation mechanisms that reduce the content of the toxic metal in internal leaf tissues, because high concentration of metal accumulates in trichomes, from where it can be easily removed and not interfered with plant metabolism. Similarly, Guo et al. (2022) reported that trichomes served as Cd^{2+} accumulation site in *Arabidopsis thaliana*, thereby it helps the plant to cope with heavy metal stress and detoxify the soil. Thus, the trichomes are also believed to prime plant defenses against biotic and abiotic stress. The EDS results of *T. ciliata* confirmed that aerial plant parts (leaves) had more Pb and Cd enrichment ability than roots which suggests that plant efficient translocation and detoxification mechanism.

Conclusion

From the present studies, it is hereby concluded that *T. ciliata* can be categorized as hyperaccumulator with maximum accumulation in shoots than roots having BCF >1 and TF >1. The combined treatment of Pb and Cd has significant impact on the bioaccumulation and translocation in *T. ciliata* suggesting their negative effect among them. Anatomical alterations such as decreased root area along with decreased stomatal pore size; and increased stomatal index and trichome density suggested plant's adaptive response under heavy metal stress. Field emission scanning electron microscopy and energy dispersive x-ray spectroscopy (FESEM-EDS) results confirmed the Pb and Cd accumulation sites in the leaves and root tissues of plants. Hence, in accordance with our findings *T. ciliata* can be recommended as a potential species for stabilization of heavy metal (Pb and Cd) polluted sites. The study further suggests to enhance the phytoremediation potential of *T. ciliata* at molecular level.

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Data availability Statement

The data used to support findings of this study is included within the article

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Conflict of Interests

The authors declare that they have no conflict of interest.

Author contributions

All authors have made substantial contributions: the conception and design of the study (RS), acquisition of data or analysis (RK) and interpretation of data (RK, SC), drafting the article (RK, ST), revising (RS, SC, SKC) it critically for important intellectual content final approval of the version to be submitted.

References

Ahmadi O, Pandey J, HademMoghadam N, AsgariLajayer B, Ghorbanpour M (2020) Phytoremediation of contaminated soils using trees. In: Faisal M, Saquib Q and Alatar AA (ed) Cellular and molecular phytotoxicity of heavy metals nanotechnology in the life sciences. Pp. 191-226. Springer, Cham, Denmark.

Aslam M, Aslam A, Sheraz M, Ali B, Ulhassan Z, Najeeb U, Zhou W and Gill R A (2021) Lead toxicity in cereals: Mechanistic insight into toxicity, mode of action, and management. *Front Plant Sci* **11:**587785.

Baker AJM (1981) Accumulators and excluders – strategies in the response of plants to heavy metals. J Plant Nutr 3:643–54.

Chen Y, Wang Y and Yeh K (2017) Role of root exudates in metal acquisition and tolerance. Curr Opin Plant Biol 39:66-72.

Chibuike GU and Obiora SC (2014) Heavy metal polluted soils: Effect on plants and bioremediation methods. Appl Environ Soil Sci 21:1-12.

Clabeaux BL, Navarro DA, Aga DS, Bisson MA (2013) Combined effects of cadmium and zinc on growth, tolerance, and metal accumulation in Chara australis and enhanced phytoextraction using EDTA. Ecotoxicol Environ Saf 98:236–43.

Dalton TP, He L, Wang B, Miller ML, Stringer JLKF, Chang Z, Baxter CS, Neber DW (2005) Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. Proc Natl Acad Sci USA 102:3401–06.

El-Mahrouk EM, Eisa EA, Hegazi MA, Abdel-Gayed ME, Dewir YH, El-Mahrouk ME, Naidoo Y (2019) Phytoremediation of cadmium-, copper-, and lead-contaminated soil by *Salix mucronata* (Synonym Salix safsaf). Hortscience 54:1249–57.

Gajic´ G, Djurdjevic´ L, Kostic´ O, Jaric´ S, Mitrovic´ M, Pavlovic´ P (2018) Ecological potential of plants for phytoremediation and ecorestoration of fly ash deposits and mine wastes. Front Environ Sci 6:124.

Gomes MP, Desoares EM, Maria N (2011) Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in *Brachiaria decumbens*. Sci Agric (Piracicaba, Braz.) 68:566–73.

Guerra F, Gainza F, Pérez R, Zamudio F (2011) Phytoremediation of heavy metals using poplars (Populus spp): A glimpse of the plant responses to copper, cadmium and zinc stress. In: Golubev I A (ed) Handbook of Phytoremediation. Pp. 387-413. Nova science publishers Inc, New York.

Günthardt-Goerg MS, Vollenweider P, Schulin R (2022) Metal accumulation and biomass production in young afforestations established on soil contaminated by heavy metals. Plants 11:523.

Guo C, Hu J, Gao W, Gao P, Cao Z, Liu N, Wang X, Liu W, Zhao J, Dong J, Genin GM, Zhou LH (2022) Mechanosensation triggers enhanced heavy metal ion uptake by non-glandular trichomes. J Hazard Mater 15:426-34.

Gupta V K, Nayak A and Agarwal S (2015) Bioadsorbents for remediation of heavy metals: Current status and their future prospects. *Environ Eng Res* **20:**1–18.

Haider FU, Liqun C, Coulter JA, Cheema SA, Wu J, Zhang R, Wenjun M, Farooq M (2021) Cadmium toxicity in plants: Impacts and remediation strategies. Ecotoxicol Environ Saf 211:111887.

Hamim H, Miftahudin M and Setyaningsih L (2018) Cellular and ultrastructure alteration of plant roots in response to metal stress. In: Ratnadewi D and Hamim H (ed) Plant growth and regulation-alterations to sustain unfavorable conditions. Pp. 21-41. IntechOpen. London.

Hayat S, Khalique G, Irfan M, Wani A S, Tripathi B N and Ahmad A (2012) Physiological changes induced by chromium stress in plants: An overview. *Protoplasma* **249:**599–611.

He J, Ren Y, Chen X and Chen H (2014) Protective roles of nitric oxide on seed germination and seedling growth of rice (Oryza sativa L.) under cadmium stress. *Ecotoxicol Environ Saf* **108:**114–19.

Hermle S, Vollenweider P, Günthardt-Goerg MS, McQuattie CJ and Matyssek R (2007) Leaf responsiveness of *Populus tremula* and *Salix viminalis* to soil contaminated with heavy metals and acidic rainwater. Tree Physiol 27:1517-31.

Huang L, Li WC, Tam NFY, Ye ZH (2019) Effects of root morphology and anatomy on cadmium uptake and translocation in rice (*Oryza sativa* L.). J Environ Sci 75:296–306.

Kabata-Pendias A (2010) Trace elements in soils and plants (4th ed.). Pp. 224-60. CRC Press, Boca Raton, Florida, USA.

Kacálková L, Tlustoš P, Száková J (2015) Phytoextraction of risk elements by willow and poplar trees. Int J Phytoremediation 17:414-21.

Kanwal A, Farhan M, Sharif F, Hayyat MU, Shahzad L, Ghafoor GZ (2020) Effect of industrial wastewater on wheat germination, growth, yield, nutrients and bioaccumulation of lead. Sci Rep 10:11361

Kasim W A (2006) Changes induced by copper and cadmium stress in the anatomy and grain yield of Sorghum bicolor (L.) Moench. *Int J Agric Biol* **8:**123–28.

Kaur J, Bhat S A, Singh N, Bhatti S S, Kaur V and Katnoria J K (2022) Assessment of the heavy metal contamination of roadside soils alongside Buddha Nullah, Ludhiana, (Punjab) India. *Int J Environ Res Public Health* **19:**1596.

Kieffer P, Planchon S, Oufir M, Ziebel J, Dommes J, Hoffmann L, Hausman JF, Renaut J (2009) Combining proteomics and metabolite analyses to unravel cadmium stress-response in poplar leaves. J Proteome Res 8:400–17.

Krishan G, Sudarsan N, Ghosh N C, Kumar S, Singh S, Sharma A, Mittal S, Sidhu B S and Vashisth R (2021)

Concentration of heavy metals in groundwater and heavy metal pollution index in Punjab. *e-Journal Geohydrol* **2:**24-33.

Kumar SS, Kadier A, Malyan SK, Ahmad A, Bishnoi NR (2017) Phytoremediation and rhizoremediation: uptake, mobilization and sequestration of heavy metals by plants. In: Singh D P (ed) Plant-Microbe interactions in agro-ecological perspectives. Pp. 367-94. Springer, Singapore.

Kundal M, Thakur S and Dhillon G P S (2020) Evaluation of growth performance of half sib progenies of Toona ciliata M. Roem under field conditions. *Genetika* **52:**651-60.

Kundal M, Thakur S, Dhillon GPS (2020) Evaluation of growth performance of half sib progenies of Toona ciliata M. Roem under field conditions. Genetika 52:651-60.

Kushwaha A, Hans N, Kumar S, Rani RA (2018) Critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. Ecotoxicol Environ Saf 147:1035–45.

Liu LW, Li W, Song WP, Guo MX (2018) Remediation techniques for heavy metal–contaminated soils: Principles and applicability. Sci Total Environ 633:206–19.

Lux A, Martinka M, Vaculik M, White PJ (2011) Root responses to cadmium in the rhizosphere: a review. J Exp Bot 62:21–37.

Maestri E, Marmiroli M, Visioli G, Marmiroli N (2010) Metal tolerance and hyperaccumulation: cost and trade-offs between traits and environment. Environ Exp Bot 68:1-13.

Mleczek M, Goliński P, Krzesłowska M, Gąsecka M, Magdziak Z et al. (2017) Phytoextraction of potentially toxic elements by six tree species growing on hazardous mining sludge. Environ Sci Pollut Res 24: 22183–95.

Muszynska E, Labudda M, Rozanska E, Hanus-Fajerska E, Koszelnik-Leszek A (2019) Structural, physiological and genetic diversification of *Silene vulgaris* ecotypes from heavy metal-contaminated areas and their synchronous in vitro cultivation. Planta 249:1761–78.

Pourrut B, Shahid M, Dumat C, Winterton P, Pinelli E (2011) Lead uptake, toxicity, and detoxification in plants. Rev Environ Contam Toxicol 213:113–36.

Radoglou KM, Jarvis PG (1990) Effects of CO_2 enrichment on four poplar clones. II. Leaf surface properties. Ann Bot 65: 627–32.

Riyazuddin R, Nisha N, Ejaz B, Khan M I R, Kumar M, Ramteke P W and Gupta R A (2022) Comprehensive review on the heavy metal toxicity and sequestration in plants. *Biomolecules* **12:** 43.

Rucińska-Sobkowiak R (2016) Water relations in plants subjected to heavy metal stresses. Acta Physiol Plant 38:257.

Sagardoy R, Vázquez S, Florez-Sarasa I D, Albacete A, Ribas-Carbó M, Flexas J, Abadía J and Morales F (2010) Stomatal and mesophyll conductances to CO_2 are the main limitations to photosynthesis in sugar beet (Beta vulgaris) plants grown with excess zinc. *New Phytol* **187:**145–58.

Sharma N and Singh M. 2018. Estimation of Heavy Metal Accumulation in Seeds of Leucaena leucocephala, Toona ciliata and Dalbergia sissoo Collected from Different Sites in Baddi Barotiwala and Nalagarh (BBN) Industrial Area of Himachal Pradesh. Int.J.Curr.Microbiol.App.Sci. 7(09): 2261-2267. doi: https://doi.org/10.20546/ijcmas.2018.709.280

Shu X, Yin L, Zhang Q, Wang W (2012) Effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L. Environ Sci Pollut Res 19:893–902.

Shukla OP, Juwarkar A, Singh SK., Khan S, Rai UN (2011) Growth response and metal accumulation capabilities of woody plants during the phytoremediation of tannery sludge. Waste Manage 31:115–23.

Vaculík M, Konlechner C, Langer I, Adlassnig W, Puschenreiter M, Lux A, Hauser MT (2012) Root anatomy and element distribution vary between two *Salix caprea* isolates with different Cd accumulation capacities. Environ Pollut 163:117-26.

Wang Q, Sun Q, Wang W, Liu X, Song L and Hou L (2022) Effects of Different Native Plants on Soil Remediation and Microbial Diversity in Jiulong Iron Tailings Area, Jiangxi. Forests 13: 1106. https:// doi.org/10.3390/f13071106

Weryszko-Chmielewska E, Chwil M (2005) Lead-Induced histological and ultrastructural changes in the leaves of Soybean (Glycine max (L.) Merr.). Soil Sci Plant Nutr 51(2):203-12.

Xu Z, Zhou G (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. J Exp Bot 59:3317–25.

Yan A, Wang Y, Tan SN, Mohd Yusof ML, Ghosh S, Chen Z (2020) Phytoremediation: A promising approach for revegetation of heavy metal-polluted land. Front Plant Sci 11:359.

Zhang J, Yang N, Geng Y, Zhou J, Lei J (2019) Effects of the combined pollution of cadmium, lead and zinc on the phytoextraction efficiency of ryegrass (*Lolium perenne* L.). RSC Advances 9:20603.

Table 1: Lead and cadmium accumulation in different plant parts of *Toona ciliata*

Lead accumulation															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	3.55^{l}	38.7^{h}	56.7^{e}	90.5^{a}	47.4^{A}	3.67^{j}	40.8^{g}	59.8^{d}	92.1^{a}	49.1^{A}	5.34^{j}	62.9^{g}	89.4^{d}	139.8^{a}	74.4^{A}
Cd_5	3.67^{l}	33.5^{i}	50.4^{f}	84.5^{b}	43.0^{B}	4.11^{j}	39.7^{g}	54.3^{e}	90.2^{a}	47.1^{B}	6.82^{j}	58.4^{g}	85.4^{de}	132.5^{b}	70.8^{B}
Cd_{15}	3.63^{l}	29.8^{j}	45.6^{g}	79.3^{c}	39.6^{C}	4.21^{j}	32.9^{h}	50.6^{e}	84.7^{b}	43.1^{C}	5.89^{j}	52.5^{h}	82.5^{ef}	128.9^{bc}	67.4^{C}
Cd_{25}	4.20^{l}	22.9^{k}	40.2^{h}	72.3^{d}	34.9^{D}	4.89^{j}	27.7^{i}	46.3^{f}	76.9^{c}	38.9^{D}	5.22^{j}	46.4^{i}	79.6^{f}	125.7^{c}	64.2^{D}
Mean	3.77^{D}	31.2^{C}	48.2^{B}	81.7^{A}		4.22^{D}	35.3^{C}	52.8^{B}	86.0^{A}		5.82^{D}	55.1^{C}	84.2^{B}	131.7^{A}	
Cadmium accumulation															
Root						**Stem**					**Leaves**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	0.01^{h}	0.01^{h}	0.02^{h}	0.02^{h}	0.02^{D}	0.01^{f}	0.02^{f}	0.025^{f}	0.036^{f}	0.023^{D}	0.02^{i}	0.025^{i}	0.029^{i}	0.03^{i}	0.03^{D}
Cd_5	4.89^{g}	4.74^{g}	4.68^{g}	4.62^{g}	4.73^{C}	5.32^{e}	4.93^{e}	4.82^{e}	4.75^{e}	4.96^{C}	5.89^{g}	5.42^{h}	5.23^{h}	4.99^{h}	5.38^{C}
Cd_{15}	10.3^{d}	9.68^{e}	9.21^{ef}	8.79^{f}	9.50^{B}	11.2^{c}	9.84^{d}	9.58^{d}	9.25^{d}	9.92^{B}	14.1^{d}	13.7^{e}	12.8^{f}	12.8^{f}	13.4^{B}
Cd_{25}	12.6^{a}	12.3^{ab}	11.9^{bc}	11.4^{c}	12.1^{A}	13.2^{a}	13.5^{a}	12.7^{ab}	12.3^{b}	12.8^{A}	18.3^{a}	17.6^{b}	15.9^{c}	15.6^{c}	16.9^{A}
Mean	6.95^{A}	6.68^{B}	6.45^{C}	6.21^{D}		7.38^{A}	6.95^{B}	6.78^{C}	6.58^{D}		9.58^{A}	9.19^{B}	8.49^{C}	8.36^{D}	

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

Table 2: Effect of lead and cadmium treatments on morphological traits of *Toona ciliata*

Plant height (cm)						**Collar diameter (cm)**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	268.7^{a}	267.7^{a}	268.0^{a}	265.3^{bc}	267.4^{A}	2.57^{a}	2.56^{a}	2.54^{ab}	2.54^{ab}	2.55^{A}
Cd_5	266.7^{a}	265.7^{ab}	266.0^{ab}	266.3^{a}	266.2^{A}	2.53^{ab}	2.53^{ab}	2.52^{abc}	2.50^{abcd}	2.52^{B}
Cd_{15}	268.0^{a}	268.3^{a}	268.0^{a}	264.3^{ab}	267.2^{A}	2.49^{abcd}	2.46^{bcde}	2.44^{cde}	2.43^{de}	2.45^{C}
Cd_{25}	265.0^{ab}	263.7^{ab}	261.0^{b}	254.7^{c}	261.1^{B}	2.40^{ef}	2.34^{f}	2.23^{g}	2.22^{g}	2.29^{D}
Mean	267.1^{A}	266.3^{A}	265.7^{A}	262.7^{B}		2.49^{A}	2.47^{A}	2.43^{B}	2.42^{B}	
Root length (cm)						**Root number**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	48.3^{a}	46.0^{ab}	45.3^{b}	41.0^{c}	45.2^{A}	15.7^{a}	14.7^{ab}	12.3^{cd}	12.0^{cde}	13.7^{A}
Cd_5	39.7^{cd}	38.3^{de}	34.7^{fg}	31.7^{hi}	36.1^{B}	13.7^{bc}	12.7^{cd}	11.7^{de}	11.3^{de}	12.3^{B}
Cd_{15}	37.0^{ef}	34.0^{gh}	31.0^{ij}	29.7^{ij}	32.9^{C}	10.3^{ef}	9.67^{fg}	8.67^{gh}	8.33^{gh}	9.25^{C}
Cd_{25}	31.0^{ij}	30.0^{ij}	29.0^{ij}	28.7^{j}	29.6^{D}	7.67^{h}	7.67^{h}	7.67^{h}	7.33^{c}	7.58^{D}
Mean	39.0^{A}	37.08^{B}	35.00^{C}	32.75^{d}		11.8^{A}	11.2^{B}	10.1^{C}	9.75^{C}	
Fresh weight of root (g)						**Dry weight of root (g)**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	60.4^{a}	58.4^{a}	54.7^{b}	53.3^{b}	56.7^{A}	22.0^{ab}	21.8^{ab}	20.6^{abc}	20.3^{bcd}	21.2^{A}
Cd_5	54.0^{b}	50.7^{c}	47.8^{cd}	44.7^{ef}	49.3^{B}	22.5^{a}	20.8^{abc}	18.9^{cde}	17.5^{e}	19.9^{B}
Cd_{15}	49.9^{c}	48.7^{c}	45.8^{de}	44.7^{ef}	47.3^{C}	20.4^{abcd}	18.9^{cde}	18.2^{e}	16.9^{e}	18.6^{C}
Cd_{25}	49.6^{c}	43.8^{efg}	42.1^{fg}	41.1^{g}	44.2^{D}	18.5^{de}	17.3^{e}	17.3^{e}	16.8^{e}	17.5^{D}
Mean	53.5^{A}	50.4^{B}	47.6^{C}	45.9^{D}		20.8^{A}	19.7^{B}	18.7^{C}	17.8^{D}	
Fresh weight of shoot (g)						**Dry weight of shoot (g)**				
Treatments	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean	Pb_0	Pb_{100}	Pb_{200}	Pb_{300}	Mean
Cd_0	140.6^{a}	129.2^{bc}	125.8^{cd}	117.5^{ef}	128.3^{A}	65.3^{a}	64.5^{a}	64.4^{a}	62.5^{ab}	64.2^{A}
Cd_5	133.3^{b}	126.2^{cd}	122.0^{de}	114.7^{fg}	124.0^{B}	60.0^{bc}	57.7^{cd}	66.8^{cd}	55.0^{de}	57.4^{B}
Cd_{15}	115.6^{f}	109.8^{gh}	105.6^{hi}	95.33^{l}	106.6^{C}	57.9^{cd}	55.4^{de}	52.9^{e}	47.4^{f}	53.4^{C}
Cd_{25}	104.3^{hij}	102.6^{ijk}	98.67^{jkl}	97.67^{kl}	100.8^{D}	52.5^{e}	47.8^{f}	46.9^{f}	45.2^{f}	48.1^{D}
Mean	123.4^{A}	116.9^{B}	113.1^{C}	106.3^{D}		58.9^{A}	56.4^{B}	55.3^{B}	52.5^{C}	

Small alphabets in the same column having different superscript represents interaction among combination treatments, capital alphabets represents mean significant difference ($p \leq 0.05$)

Table 3: Anatomical parameters of *Toona ciliata* as influenced by lead and cadmium treatments

S. No.	Parameters	*Toona ciliata*	
		Control (Pb_0Cd_0)	Treated ($Pb_{300}Cd_{25}$)
1.	Stomatal pore size (µm)	21.9	18.2
2.	Stomatal density (no./mm^2)	143	165
3.	Stomatal index (%)	13.8	18.1
4.	Trichome density (no./mm^2)	85.2	109
5.	Total root area (mm^2)	1.24	1.18
6.	Root vascular area(mm^2)	0.68	0.65

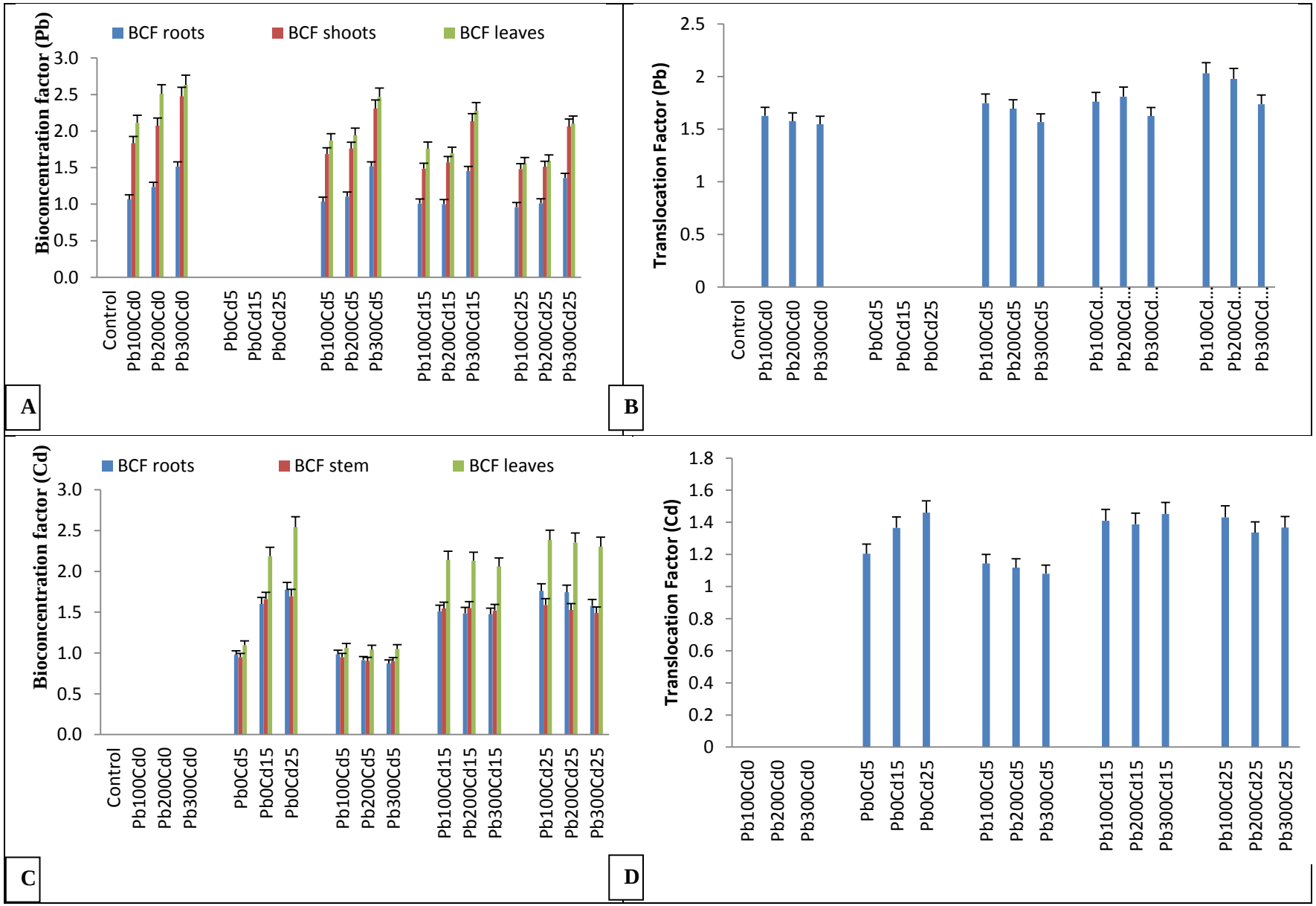

Fig 1: Phytoremediation efficiency evaluation factors in *Toona ciliata*

A: Bioconcentration factor (BCF) of Pb; B: Translocation factor (TF) of Pb

C: Bioconcentration factor (BCF) of Cd; D: Translocation factor (TF) of Cd

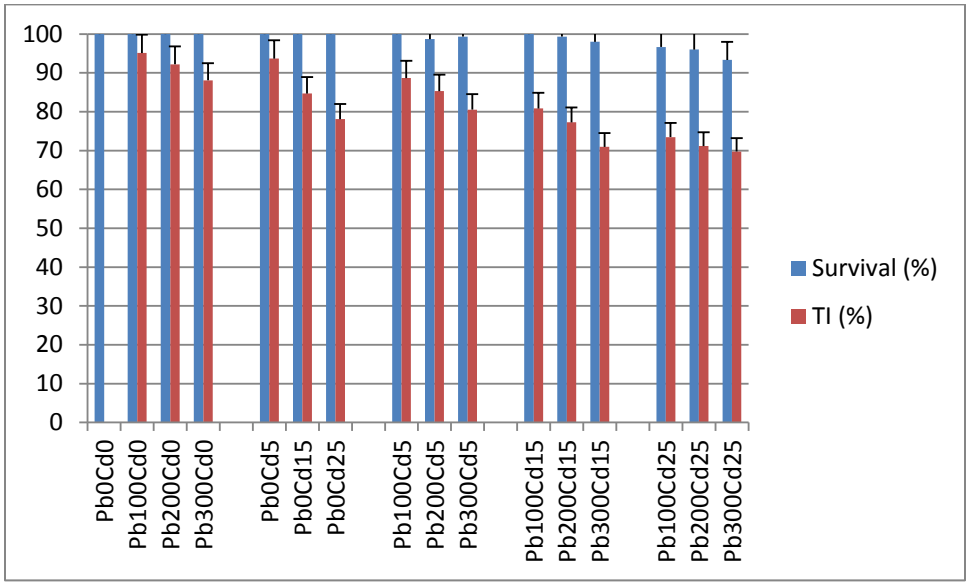

Fig. 2: Survival percentage and tolerance index of *Toona ciliata* as affected by heavy metal stress

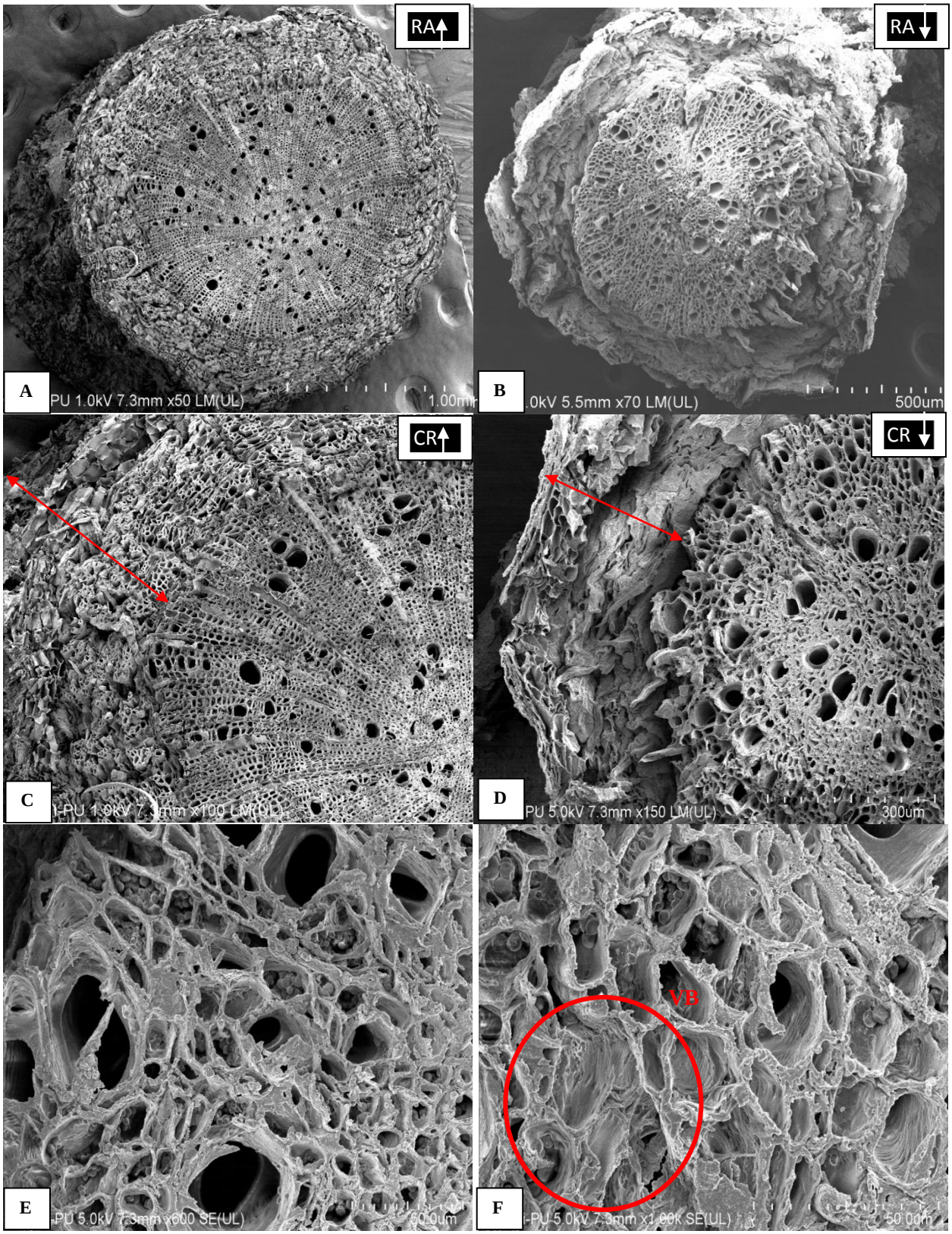

Fig.3: Field emission scanning electron micrograph (FESEM) of root cross-sections of *Toona ciliata* showing structural alterations induced due to Pb and Cd toxicity (A, C, E Control Pb_0Cd_0; B, D, F Heavy metal treated $Pb_{300}Cd_{25}$)
(RA= Root area, CR=Cortical region, Xy=xylem vessels, P = Pith cells)

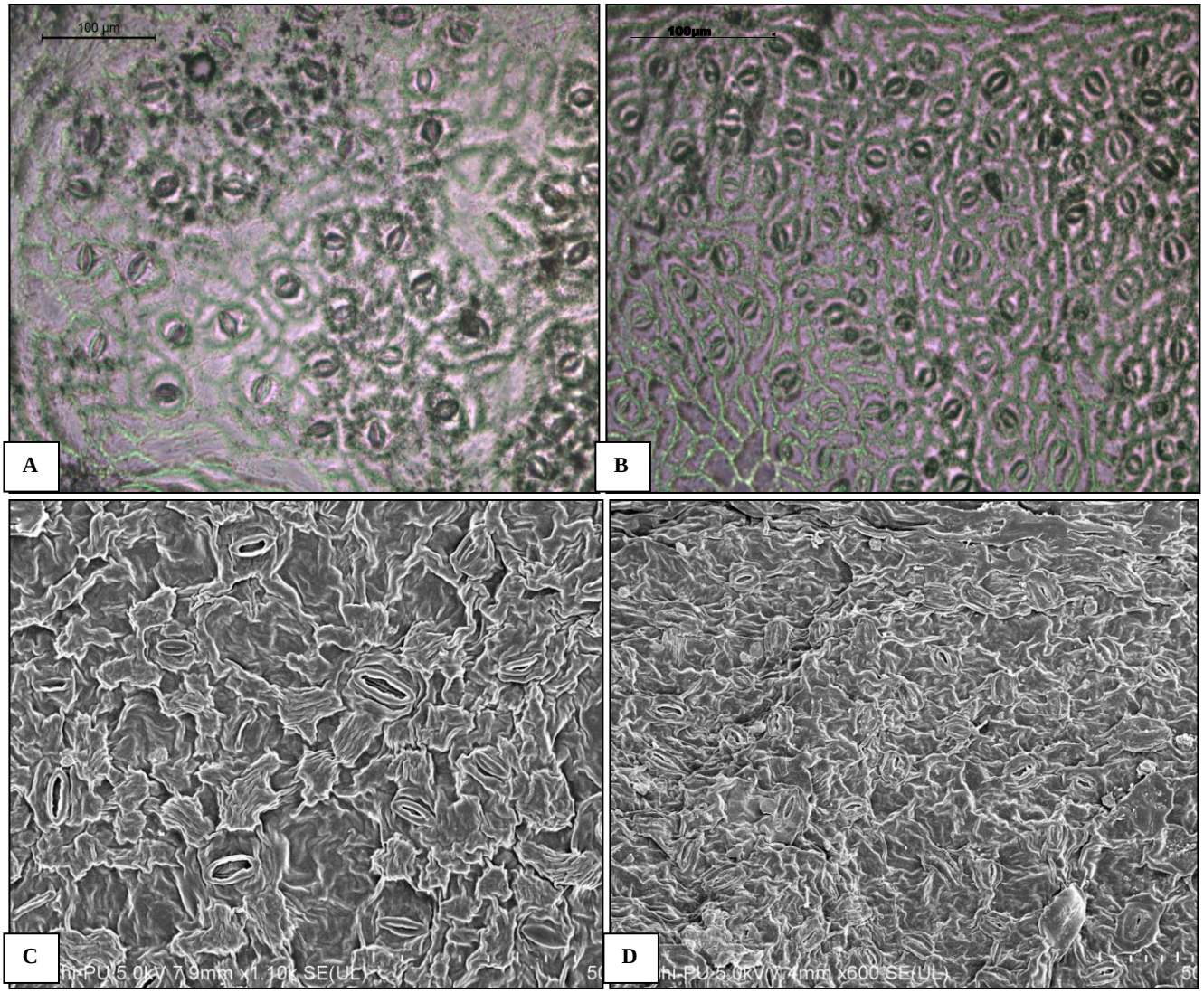

Fig. 4: Light microscopy and Field emission scanning electron micrograph (FESEM) showing stomatal alterations in leaves of *Toona ciliata* induced due to Pb and Cd toxicity

(A, C control (Pb_0Cd_0); B, D heavy metal treated $Pb_{300}Cd_{25}$)

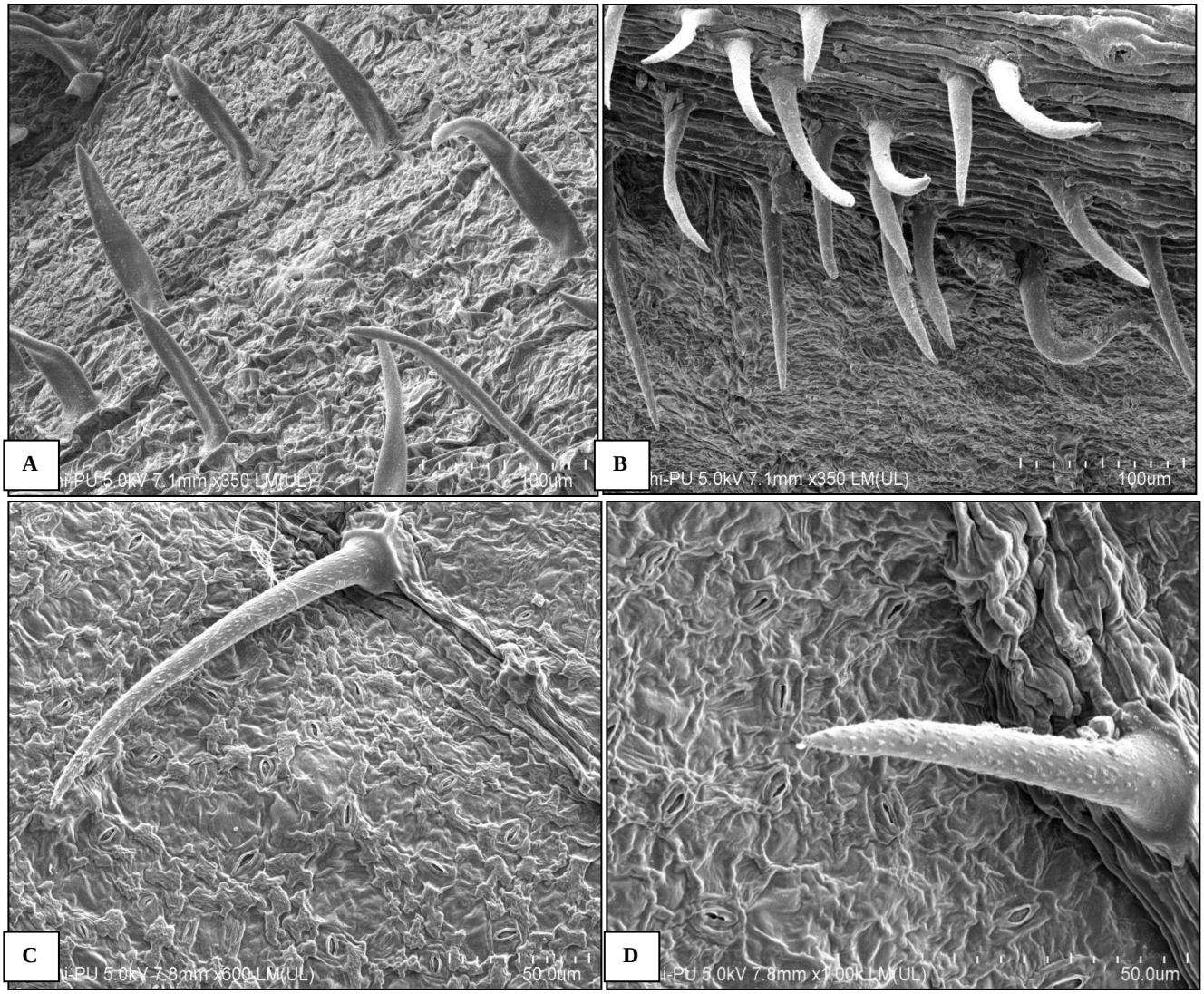

Fig. 5: Field emission scanning electron micrographs (FESEM) showing structural alterations in trichomes in leaves of *Toona ciliata* induced due to Pb and Cd toxicity

(A, C control Pb_0Cd_0; B, D heavy metal treated $Pb_{300}Cd_{25}$)

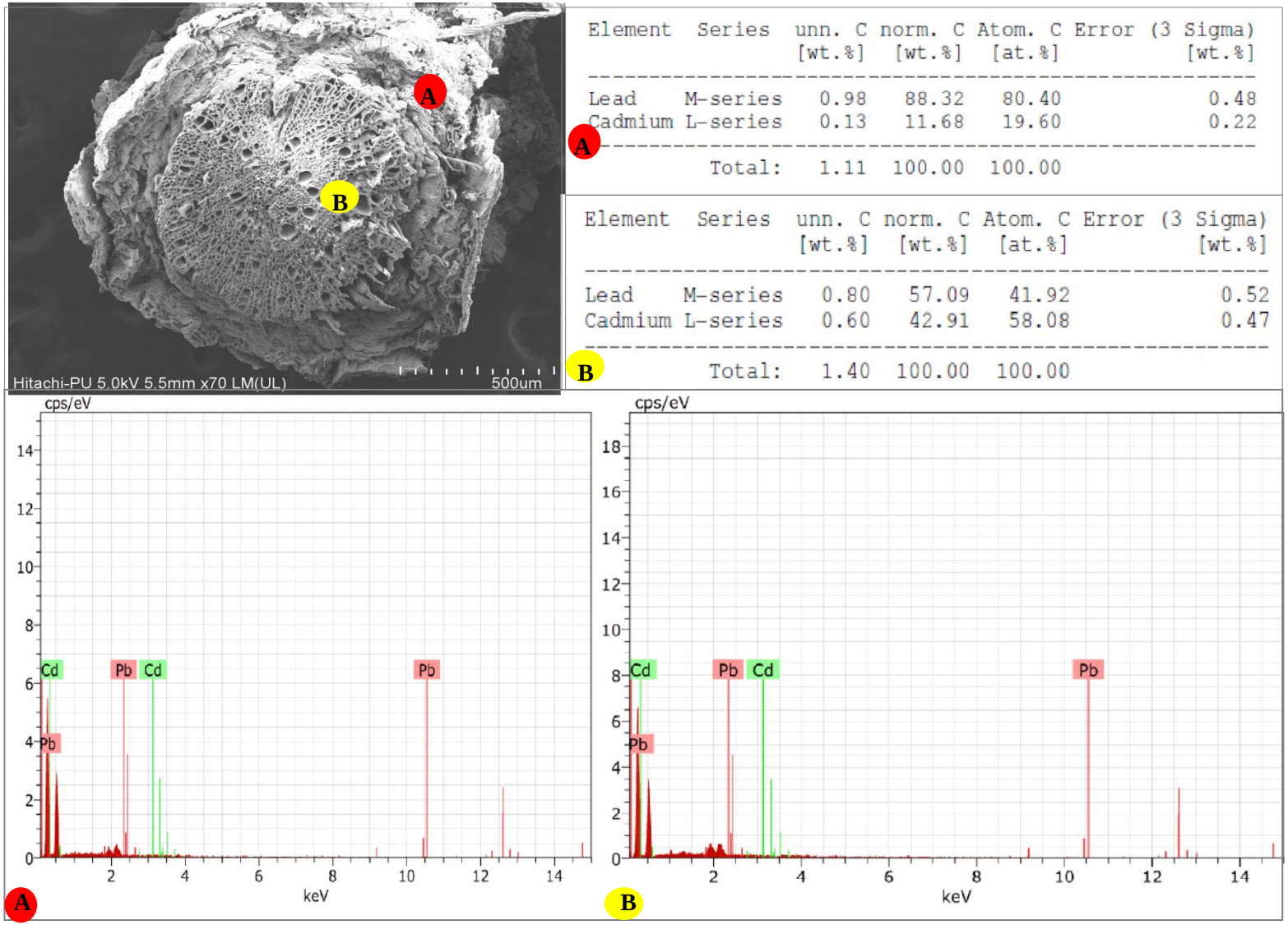

Fig. 6: Field emission scanning electron micrograph and energy dispersive x-ray spectroscopy (FESEM-EDS) of root cross-section of *Toona ciliata* ($Pb_{300}Cd_{25}$) in different tissues
(A. Epidermal and cortical region, B. Vascular region)

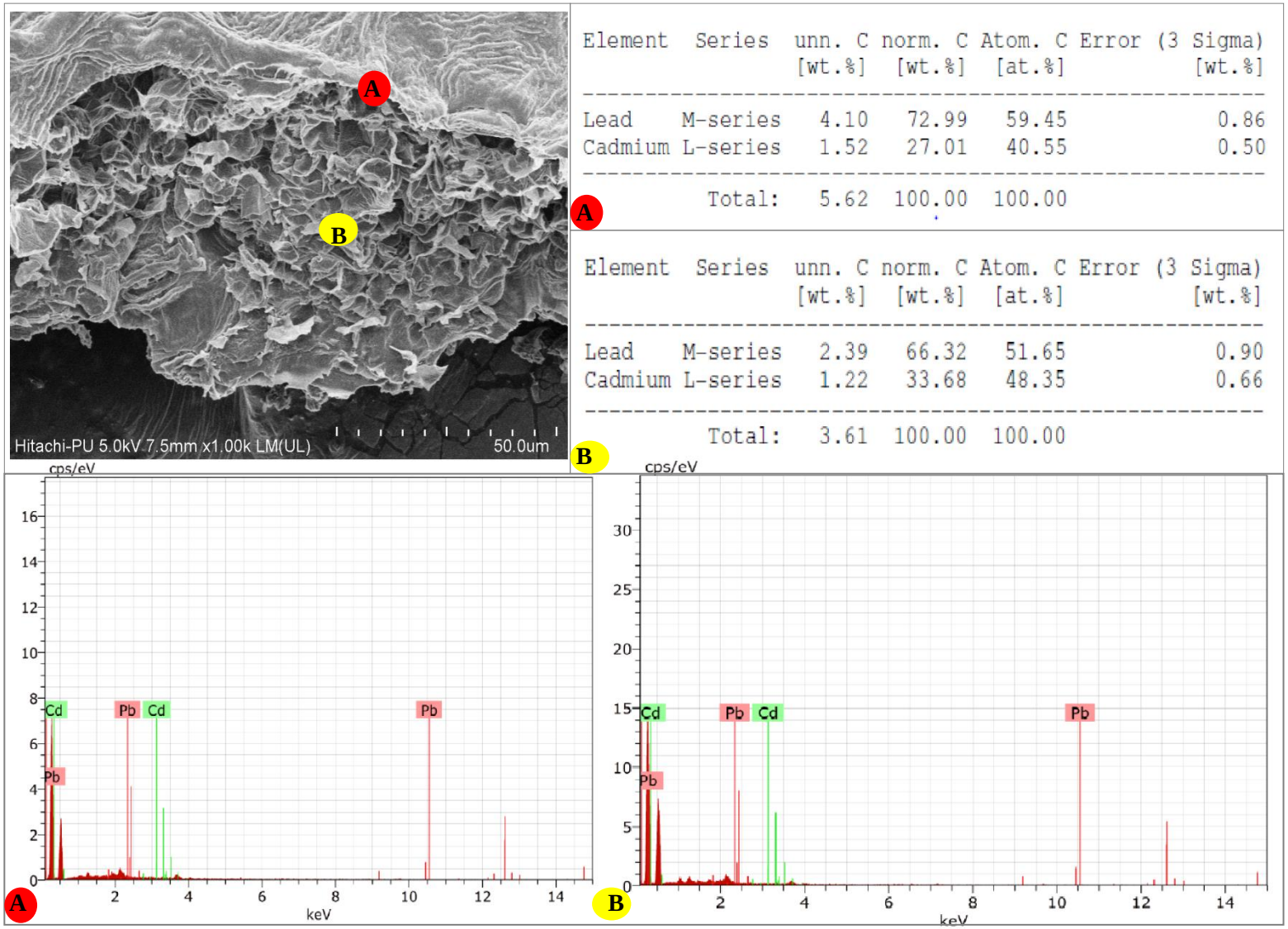

Fig. 7: Field emission scanning electron micrograph and energy dispersive x-ray spectroscopy (FESEM-EDS) of leaf cross-section of *Toona ciliata* ($Pb_{300}Cd_{25}$) in different tissues
(A. Epidermal and cortical region, B. Vascular region)

Appendix. Lead (Pb) and cadmium (Cd) concentration in soil with application of lead nitrate and cadmium nitrate

Lead concentration in soil (mg/kg)										
Total Pb (mg/kg soil)						Available Pb (mg/kg soil)				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	15.2	110.2	212.8	320.3	164.6	0.98	60.3	90.4	189.9	85.4
Cd_5	16.4	109.4	214.6	316.7	164.3	0.89	58.9	85.4	192.4	84.4
Cd_{15}	14.2	113.5	210.6	314.3	163.1	0.78	57.8	85.4	195.6	84.9
Cd_{25}	14.8	105.1	215.3	320.4	163.9	0.82	57.4	85	190.5	83.4
Mean	15.15	109.5	213.3	317.9		0.875	58.6	86.55	192.1	
Cadmium concentration in soil (mg/kg)										
Total Cd (mg/kg soil)						**Available Cd (mg/kg soil)**				
Treatments	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**	**Pb_0**	**Pb_{100}**	**Pb_{200}**	**Pb_{300}**	**Mean**
Cd_0	0.186	0.216	0.176	0.166	0.186	0.059	0.075	0.079	0.082	0.074
Cd_5	7.71	7.42	6.90	6.81	7.21	5.61	5.2	4.78	4.69	5.07
Cd_{15}	16.9	16.3	15.8	15.6	16.2	6.46	6.41	6.2	5.96	6.26
Cd_{25}	27.0	26.5	26.2	25.3	26.2	7.96	6.93	6.75	6.55	7.05
Mean	13.0	12.6	12.3	12.0		5.02	4.65	4.45	4.32	

VITA

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EDUCATIONAL QUALIFICATIONS

Bachelor degree	:	B.Sc. (Medical)
University	:	Panjab University, Chandigarh
Year of award	:	2016
%age	:	76.2%
Master‘s degree	:	M.Sc. (Botany)
University	:	Punjab Agricultural University, Ludhiana
Year of award	:	2018
OCPA	:	7.57/10.00
Title of Master‘s Thesis	:	Physiological, biochemical and anatomical basis of fruit cracking in lemon cv Punjab Baramasi
Ph.D.	:	Ph.D. (Botany)
University	:	Punjab Agricultural University, Ludhiana
Year of award	:	2022
OCPA	:	8.05/10.00
Title of Ph.D. Dissertation	:	Evaluation of morpho-physiological and metal accumulation potential of *Salix alba* L. and *Toona ciliata* M. Roemer grown on heavy metal contaminated soils
Awards/Distinctions/Scholarships	:	• Awarded with **PAU Merit fellowship** with highest OCPA in PhD degree (2019-2022) • Secured **Best poster presentation award (First prize)** at Golden jubilee national symposium (CIPME-2019) March 6-8, 2019. Guru Nanak Dev University, Amritsar • Got **Best poster presentation award (First prize)** at ISPP North Zonal Seminar-2022 (June 25, 2022) Indian Society for Plant Physiology and ICAR-Sugarcane Breeding Institute, Karnal, India