

**STUDY OF HEAVY METAL INDUCED CHANGES IN SOME
SELECTED MEDICINAL PLANTS**

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**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN BOTANY**

BY

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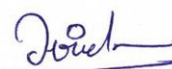
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DECLARATION

I hereby declare that the work presented in the thesis entitled "*STUDY OF HEAVY METAL INDUCED CHANGES IN SOME SELECTED MEDICINAL PLANTS*" is based on the original work done by me under the guidance of Dr. C. T. Anitha and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.



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CERTIFICATE

This is to certify that the thesis entitled “*STUDY OF HEAVY METAL INDUCED CHANGES IN SOME SELECTED MEDICINAL PLANTS*” Submitted by **Hridhya M. J**, in partial fulfilment of the requirements for the award of the **Degree of Doctor of Philosophy in BOTANY**, to University of Calicut, is a record of the original research work carried out by her under my supervision and guidance during the period 2018 to 2025 in the **Department of Botany**, Sree Narayana College, Nattika

This thesis has not previously formed the basis for the award of any degree, diploma, or fellowship in this or any other university and is, to the best of my knowledge, a genuine and independent piece of work.

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ABSTRACT

Heavy metal contamination is an environmental concern that directly threatens the safety and therapeutic quality of medicinal plants. The present study aimed to find the effects of copper (Cu), chromium (Cr), and zinc (Zn) on three commonly used medicinal species *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. The research aimed to examine the influence of heavy metals on seed germination, morphological growth, photosynthetic pigments, primary and secondary metabolites, antioxidant activity, and pharmacognostic properties, while also assessing the rate of metal bioaccumulation and the tolerant nature of each plant.

Experiments were conducted under controlled conditions using both germination assays and hydroponic culture systems. Seeds were exposed to Cu, Cr, and Zn at concentrations ranging from 25 ppm to 150 ppm. Germination percentage, axis length, vigour index, and phytotoxicity were recorded. Hydroponically grown plants were analyzed for morphological traits, chlorophyll content, biochemical composition, and phytochemicals, including key markers such as andrographolide, kaempferol, and quercetin using validated HPLC methods. Antioxidant potential was assessed through DPPH and SOD assays, and bioaccumulation of metals was quantified by atomic absorption spectroscopy.

The results revealed that Cu and Zn at low concentrations (25–50 ppm) stimulated germination, seedling vigor, and metabolite accumulation, whereas higher concentrations were inhibitory. Cr exerted consistent toxicity across all concentrations, suppressing germination, growth, and phytochemical content, with *Emilia sonchifolia* showing the highest sensitivity. *Tridax procumbens* demonstrated significant tolerance and retained higher growth and quercetin levels under stress, while *Andrographis paniculata* showed intermediate responses but a marked decline in andrographolide under higher doses. Antioxidant activity increased under moderate Cu and Zn exposure but decreased under severe stress. Metal uptake was dose-dependent, with Zn being absorbed more readily than Cr.

In conclusion, heavy metal stress profoundly affects both the growth and medicinal quality of plants, with Cu and Zn showing hormetic effects at low levels and Cr exerting strong toxicity. The study highlights the relative tolerance of *T. procumbens* and the vulnerability of *E. sonchifolia*, emphasizing the need for stringent quality control in the cultivation and use of medicinal plants. Beyond scientific relevance, the findings also serve to create awareness about the health risks associated with heavy metal pollution in herbal medicines and underscore the importance of cultivating medicinal plants in uncontaminated environments.

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CHAPTER 1
GENERAL INTRODUCTION

Heavy metals are metallic elements with high atomic weights that often exceed 5 g/cm³. They induce persistent toxicity in the environment. Copper (Cu) and zinc (Zn) are the heavy metals that are nutritious for plants and animals as they are required as micronutrients. Heavy metals like chromium (Cr) are nonessential elements and poisonous to plant and animal lives. Natural processes such as volcanic eruptions and rock weathering cause the release of these metals into the ecosystem, but the major cause is by industrial development and anthropogenic activities, including mining, smelting, electronic waste disposal, and the excessive use of agrochemicals. Sewage discharge and fossil fuel combustion of fossil fuel cause their dispersion in soil and the aquatic ecosystem. Once they enter in ecosystem it will cause depletion of soil fertility and reduction of microbial flora, and when they enter to food chain it will affect both plants and animals health

Excessive concentrations of Cu and Zn, despite their essential roles, can be detrimental, inhibiting root development, reducing photosynthetic efficiency, and inducing oxidative stress. Chromium, especially in its hexavalent form (Cr VI), is highly cytotoxic, causing cellular damage, stunted growth, and, in severe cases, plant mortality. Industrial effluents from electroplating, operation of tanneries and textile production are the main origins of Cr pollution, while Cu and Zn contamination comes from mining activities, agricultural run-off (e.g., fungicides and fertilisers) and urban waste water. These metals enter the ecosystem by means of unregulated waste disposal, groundwater leaching, and atmospheric dispersion, then taken up by the plants. Medicinal plants are, sensitive to heavy metal accumulation and pose a risk to human consumption.

Medicinal plants have been used in traditional and modern medicine for the treatment of various diseases, but most of the medicinal plants have the potential to absorb and accumulate heavy metals, especially when they are growing in polluted areas. This makes them unsafe to use, even though they have bioactive compounds with high therapeutic potential

In the present study, three medicinal plants *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumb* were studied for their uptake of Cu, Cr, and Zn. These species are widely used in traditional medicine for their anti-inflammatory, antimicrobial, and hepatoprotective properties. Studying their interaction with heavy metals is crucial to evaluate the safety of these plants used as herbal medicine since they are growing in urban and potentially polluted areas. evaluating their metal tolerance behaviour and the impact of heavy

metals on bioactive components can contribute valuable information for safer herbal medicine production and sustainable environmental practices.

1.2 AIM AND OBJECTIVES

Aim

The aim of the present research is to evaluate the impact of heavy metals copper (Cu), chromium (Cr), and zinc (Zn) on selected medicinal plants (*Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*) with reference to their germination, growth, phytochemistry, pharmacognostic properties, antioxidant activity, and bioaccumulation potential, in order to assess their tolerance and implications for medicinal use.

Objectives

To study the effect of heavy metals (Cu, Cr, and Zn) on the selected medicinal plants at different developmental stages, including seed germination, morphological growth, and physiological performance under hydroponic culture.

To evaluate the rate of bioaccumulation of Cu, Cr, and Zn in root, stem, and leaf tissues of the plants using atomic absorption spectroscopy.

To assess the pharmacognostic and phytochemical properties of the plants under heavy metal stress, including changes in primary metabolites (carbohydrates, proteins, lipids), secondary metabolites (alkaloids, flavonoids, tannins, saponins, terpenoids), and marker compounds (andrographolide, kaempferol, quercetin).

To determine the tolerant nature of each species by comparing their survival, morphological adaptations, and biochemical responses under increasing concentrations of Cu, Cr, and Zn.

To create awareness about the impact of heavy metal pollution on medicinal plants, particularly with respect to their safety, therapeutic quality, and potential role in phytoremediation.

1.3 REVIEW OF LITERATURE

Contamination of the ecosystem by heavy metals is becoming a serious threat to all life forms. There are many research works have been done to analyse the effect of heavy metals on biochemical constituents and the therapeutic properties of medicinal plants.

Copper (Cu), a transition metal with distinctive reddish-brown colouration (atomic number 29, molar mass 63.5 g/mol), ranks as the 25th most prevalent element in Earth's crust. With a density of 8.96 g/cm³, it stands as the world's third most utilised metallic element (Karlin and Tyeklár, 2012). Copper plays a peculiar dual role in plant systems as an essential micronutrient as well as exhibiting phytotoxicity at high concentrations, thus being of great research interest (Ameh and Sayes, 2019a). Copper is considered one of the eight essential micronutrients, necessary for normal growth and development of plants (Nazir et al., 2019).

Chromium (Cr) is an abundant element which is widely distributed in the earth crust and it can exist in a variety of oxidation states ranging from -2 to +6. Of these, the trivalent form [Cr(III)] and the hexavalent form [Cr(VI)] are the most stable and important in the environment. While small amounts of Cr(III) are beneficial as a micronutrient, Cr(VI) is highly toxic, easily moves through the environment, and is recognised as carcinogenic (Iyaka, 2009).

Zinc (Zn) is an essential micronutrient that plays a fundamental role in plant growth and development, and it acts as a structural or catalytic cofactor for more than 300 enzymes involved in diverse physiological and biochemical processes, including protein metabolism, nucleic acid synthesis, and hormone regulation (Rouached et al., 2012; Castillo-González et al., 2018).

There are many research works have been done to analyse the effect of heavy metals on biochemical constituents and therapeutic property of medicinal plants. For instance, research by Pandey et al. (2023) studied the impact of heavy metals Cd and As on the therapeutic value of *Andrographis paniculata*. Chen et al. (2021) studied the impact of heavy metals in medicinal plants commonly used in Chinese traditional medicine. Du et al. (2005) studied the effect of mercury (Hg) by seedlings of rice (*Oryza sativa* L.) grown in a hydroponic system. Antony and Nagella (2020) reported that heavy metal stress influenced andrographolide content and antioxidant activity in *Andrographis paniculata*. Chitolina et al. (2024) found that excess copper increased metabolite concentrations in *Tridax procumbens*.

A study conducted by Saud et al. (2022), in *Frontiers in Plant Science* explored the effects of the heavy metal stress for chromium (Cr) ions and its impact on the development of the plant

and several physiological traits. In conclusion heavy metals can potentially effect the phytochemistry and therapeutic potential of selected medicinal plants.

CHAPTER 2

**EVALUATING CONTAMINATION FROM FIVE SMALL-
SCALE INDUSTRIES**

2.1 INTRODUCTION

Heavy metal pollution from industrial and commercial activities causes serious damage to the ecosystem and all forms of life. Heavy metals are non-biodegradable and persistent. When they enter into food chain, they will accumulate in plants and animals. Bioaccumulation of heavy metals can potentially cause liver damage, neurological disorders and even cancer in human beings. Small scale industrial sectors like car wash stations, automobile repair services are very common in rural and urban areas they are producing effluent water which contains heavy metals like Cu, Zn, Ni etc. other key contributors of heavy metal pollution are electroplating and metal finishing workshops, textile dyeing units, and leather tanning units which often release toxic metals such as lead (Pb), cadmium (Cd), chromium (Cr), and zinc (Zn) into soil and water systems. Understanding the extent of heavy metal pollution from these sectors is crucial for developing effective mitigation strategies.

Car wash stations and automobile repair services discharge contaminated wastewater containing oil, grease, and metal particles from vehicle components, which may contain heavy metals like Zinc, nickel, and copper. And most of the carwash stations don't have efficient wastewater treatment in India. Similarly, electroplating and metal finishing workshops release high concentrations of chromium, nickel, and copper. Meanwhile, textile dyeing and leather tanning industries generate effluents with heavy metals used in dyes, mordants, and tanning agents, which can exacerbate environmental contamination. There must be a comprehensive assessment to identify pollution hotspots and evaluate compliance with environmental regulations.

This study aims to investigate heavy metal pollution from five small-scale industries located in Thrissur district, Kerala, India. By employing advanced analytical techniques, the research will quantify metal concentrations, and it can help to evaluate the ecological risks they can cause to the ecosystem and it will help to generate stricter regulatory measures to improve wastewater management practices for safeguarding environmental as well as public health.

2.2 LITERATURE REVIEW

Recent studies have highlighted the persistent issue of heavy metal contamination from industrial activities, with sector-specific pollution patterns emerging as a key concern and the excessive and unsustainable use of mineral resources worldwide has led to the generation of large-scale heavy metal pollution ([Gautam et al., 2016](#)). The rapid expansion of industrialisation and modernization in recent years has driven extensive utilization of heavy metals (HMs) became the prime cause of heavy metal pollution of natural resources (Cao, Zhao, Ma, Song, Zuo, Li, & Deng, 2021). Nogueira et al. (2013) reported that anthropogenic activities are the primary contributors to environmental pollution, with industrial discharges, automobiles, and roadways acting as major sources of heavy metal contamination due to the presence of elements such as cadmium, lead, and arsenic in particulate emissions. Onat et al. (2013) highlighted that large quantities of heavy metals enter the soil annually, from various human activities which contribute around one million tonnes of nickel and five million tonnes of lead into the ecosystem.

2.3 MATERIALS AND METHODS

This study investigated heavy metal contamination in wastewater from five small-scale industrial operations in Thrissur District, Kerala: a car wash facility in Chazhur, an automobile repair garage in Nattika, an electroplating unit in Ollur Industrial Estate, a textile dyeing centre in Kottappuram, and a leather tanning unit in Edathirinji. From each site, triplicate wastewater samples (1L each) were collected directly from discharge points during peak operational hours on three separate days to account for temporal variations in effluent composition. Samples were immediately filtered through 0.45µm cellulose nitrate membranes to remove suspended solids. After filtration acidified with ultrapure HNO₃ to pH <2 for metal stabilization. Samples were kept at 4°C until analysis in pre-cleaned polyethylene.

Metal concentrations (Pb, Cd, Cr, Ni, Zn, and Cu) were determined using Flame Atomic Absorption Spectroscopy (AAS, PerkinElmer PinAAcle 900T) with appropriate hollow cathode lamps and air-acetylene flame which was calibrated daily using matrix-matched standard solutions prepared from certified stock solutions (1000 mg/L). Method validation included analysis of certified reference materials (NIST 1643e), reagent blanks, and duplicate samples, demonstrating recoveries of 92-107% for all target metals and the detection limits ranged from 0.01 mg/L for Cd to 0.05 mg/L for Cr.

Statistical analysis included calculation of mean concentrations, standard deviations, and one-way ANOVA ($p < 0.05$) to evaluate significant differences between sampling days. While specific locations are provided for scientific reproducibility, individual business identities remain confidential in accordance with research ethics. This methodology ensured reliable quantification of heavy metal pollution while accounting for operational variability in these small-scale industrial units.

2.4 RESULT

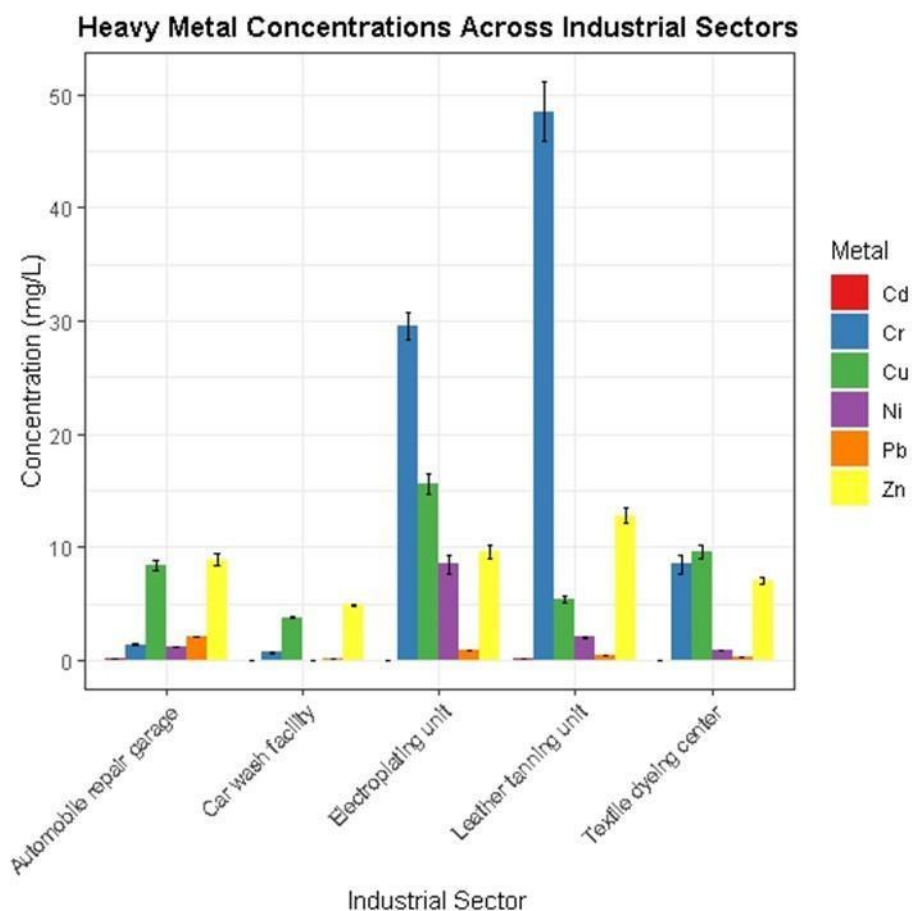


Fig.1. Heavy metal content in industrial effluent

Analysis of effluent samples from different industrial sectors revealed distinct contamination patterns. The wastewater from the leather tanning unit exhibited the highest chromium concentration (48.5 ± 2.6 mg/L), likely attributable to the use of chromium-based tanning agents. The electroplating unit effluent contained significant levels of copper (15.6 ± 0.9 mg/L) and chromium (29.5 ± 1.2 mg/L), reflecting the metal plating processes. Automobile-related facilities, including repair garages, produced effluents with measurable lead (2.1 ± 0.042 mg/L) and zinc contamination (ranging from 4.9 to 12.8 mg/L). The textile dyeing center effluent contained chromium (8.5 ± 0.8 mg/L) and copper (9.6 ± 0.6 mg/L), consistent with the use of metal-containing dyes and mordants. Cadmium concentrations were low across all samples, ranging from 0.02 to 0.15 mg/L. These results indicate that contamination patterns vary among industrial sectors; however, copper, chromium, and zinc were consistently detected in all effluent samples.

2.5 DISCUSSION

In the present study, chromium (Cr) was detected in effluent samples from all industrial sectors, with the highest concentration observed in the leather tanning effluent. This finding aligns with the study by Morão, Dilarri, and Corso (2017), who reported that leather tanning wastewater contains elevated Cr levels due to the use of chromium in stabilizing collagen fibers, thereby enhancing the strength and heat resistance of leather. Effluent from the electroplating industry contained Cr, Cu, Zn, and Ni, consistent with the observations of Mizushima, Tang, and Hansen (2005), who noted that electroplating processes generate wastewater rich in heavy metals through the discharge of plating bath solutions and rinse waters. Similarly, Masum, Islam, Anowar, Arsh, and Alam (2025) reported that textile industry effluents contain significant amounts of copper (Cu) and chromium (Cr), corroborating the present study's finding that wastewater from the textile dyeing unit contained substantial concentrations of Cu, Cr, and Zn. These results collectively indicate that industrial processes contribute specific heavy metal signatures to effluents, reflecting the materials and operations employed in each sector.

CHAPTER 3

INTRODUCTION TO SELECTED MEDICINAL PLANTS

3.1 *Andrographis paniculata* (Burm.f.) Nees

Andrographis paniculata (Burm.f.) Nees is an annual herbaceous plant belonging to the family **Acanthaceae**, and it is being used in Ayurveda and other traditional medicines like Siddha, Unani, and Chinese medicine. *Andrographis paniculata* (Burm.f.) Nees is native to India and Sri Lanka and grows erect and reaches up to 70 Cm. It is an annual herb. The leaves are slightly elongated with a length of up to 8.5 cm and a width of up to 3 cm. The flowers of these plants are white with a purple spot. Fruits are narrowly elliptical capsules, 1–2 cm long and 2–5 mm wide, compressed with longitudinal grooves on their broader surfaces, tapering sharply at both ends, and sparsely covered with glandular hairs. The seeds are small and numerous. It is known as the king of Bitters because of the extremely bitter taste of the leaves. *Andrographis paniculata*, commonly known as “Kalmegh,” or “Chuan Xin Lian. In Kerala, its common name is Kiriyaath.

Andrographis paniculata (Burm.f.) Nees is widely used in traditional medicine systems for the treatment of diarrhoea, flu, respiratory infections, sinusitis, syphilis, tuberculosis, and even HIV/AIDS (Kumar, Singh, & Bajpai, 2021). Roots and leaves of *Andrographis paniculata* are being used to prepare different ayurvedic and Unani medicines. Extensive research, particularly across Asia, has been undertaken following traditional Ayurvedic reports on the therapeutic potential of *Andrographis paniculata*. Phytochemical investigations indicate that the plant contains a variety of bioactive constituents with antioxidant, immunomodulatory and anti-inflammatory properties (Mishra, Sangwan, & Sangwan, 2007).



Fig. 2 *Andrographis paniculata*.

***Emilia sonchifolia* (L.) DC**

Emilia sonchifolia (L.) DC., commonly known as Lilac tassel flower or Cupid's shaving brush, is an annual herbaceous plant of the family Asteraceae, tribe Senecioneae and it is widely distributed in tropical and subtropical regions of Asia, Africa, and the Americas. It is a weed plant that commonly grows in farmlands and roadsides. The plant grows up to 50 cm in height, and the leaves are alternate with lyrate pinnatifid basal leaves and small sessile upper leaves. The inflorescence of *Emilia sonchifolia* is a capitulum with tubular florets, white or lilac. The fruits are small and with pappus hairs that will help in wind dispersal (Flora of North America, 2006; World Flora Online, 2025).

Emilia sonchifolia (L.) DC., is extensively utilised in Ayurveda, Siddha, Chinese, and folk systems for the treatment of fever, sore throat, sinus infections, and asthma. Wound healing properties of leaves are being utilised by many rural communities. Phytochemical analysis carried out in *Emilia sonchifolia* proves that this plant contains a wide variety of bioactive compounds with antioxidant, anti-inflammatory, antimicrobial, analgesic, gastroprotective, and cytotoxic properties, supporting many of its traditional uses (Shylesh et al., 1999; Sophia et al., 2012; Hussain, Komal, & Guruvayoorappan, 2023). However, due to the occurrence of hepatotoxic pyrrolizidine alkaloids, safety concerns remain regarding its prolonged use (Hsieh et al., 2015).



Fig. 3 *Emilia sonchifolia*

3.3 *Tridax procumbens* (L.) L

Tridax procumbens (L.) L. is a creeping perennial herb belonging to the family Asteraceae, and it is a prostrate, mat-forming weed, often found on roadsides and farmlands. The plant has slender, branched stems with roots emerging from nodal regions and grows up to 45 cm long, but in some regions, it can be found as a small, erect plant with a height of up to 25 cm. Leaves of *Tridax procumbens* are opposite and ovate with toothed margins. leaves and stems contain small hairs, and the inflorescence is solitary heads on long peduncles. Inflorescence contains centre yellow and outer white coloured florets. Pappus hairs for wind dispersal are present in the Seeds.

The common name of *Tridax procumbens* is Coat buttons or Tridax daisy. Even though the native place of this plant is tropical America, now it is being grown widely in Asia, Africa and other tropical regions. It is considered a weed plant, but the medicinal value of this plant is being utilised by many rural communities in Asia and Africa for the treatment of cataracts and respiratory infections. Hemostatic and antimicrobial properties have been identified in this plant by many researchers. Phytochemical studies indicate the presence of flavonoids, carotenoids, alkaloids, tannins, and sterols that contribute to its therapeutic potential (Bairwa, Gupta, & Gupta, 2010).



Fig. 4 *Tridax procumbens*

CHAPTER 4

EFFECT OF HEAVY METALS ON SEED GERMINATION

4.1 INTRODUCTION

Seed germination is the primary and pivotal stage of a plant's life cycle. It is the stage plant enters to active state from dormancy inside a seed. Seed germination is influenced by many external and internal factors. Abiotic stress, like high temperature presence of toxins and heavy metals, can alter the process of seed germination. So studying the impact of heavy metals can give valuable information about the toxicology of heavy metals.

Heavy metal pollution is a growing environmental concern that can affect all life forms. Among the most common heavy metals, copper (Cu), chromium (Cr), and zinc (Zn) are widely dispersed in ecosystems due to industrial activities, agricultural practices, and natural processes, and these can accumulate in plant tissues, potentially leading to detrimental effects on plant development and productivity. Plants are critical bioindicators of environmental pollution; they respond to pollution by many morphological changes. Germination percentage, axis length, vigour index, and germination index are common metrics used to estimate plant health and the response of plants to various stresses.

Germination percentage provides insights into the seed's ability to initiate growth under stress conditions, and it will decline as a result of toxicity in Germination can be a direct indication of toxicity, affecting the establishment of plant populations. "While the seed coat may serve as a primary barrier against the toxic effects of heavy metals, germination and seedling vigour generally decrease when exposed to heavy metal stress(Adrees et al., 2015). This poses a significant challenge to agricultural and forestry practices, making the impact of heavy metals on seed germination and seedling growth a critical area that warrants extensive investigation. Recent research indicates that heavy metals hinder seed germination and seedling growth by interfering with food reserve mobilisation, suppressing radicle emergence, disturbing cellular osmoregulation, and impairing proteolytic enzyme activities(Adrees et al., 2015).

4.2 REVIEW OF LITERATURE

The adverse effects of heavy metals on the germination of seeds and the growth and development of seedlings have been widely documented across various plant species. A study done by Liu, Zhang, Shan, and Zhu (2005) demonstrated that arsenate and arsenite significantly inhibited wheat seed germination and seedling growth, primarily by reducing amylolytic activity necessary for reserve mobilisation. Similarly, Ozdener and Kutbay (2009) found through their research that concentration-dependent inhibitory effects of copper, cadmium, nickel, lead, and zinc on seed germination and early growth in *Eruca sativa*, with copper exerting the strongest negative impact.

Copper toxicity has been further investigated in several studies. For example, Muccifora et al. (2013) showed that at low concentration, Cu does not affect the germination process, but the growth of the seedling was inhibited by its negative impact on mitosis. Infante-Izquierdo et al. (2020) found that *Spartina* species show a marked reduction in germination rate under Cu, Zn and Ni stress.

There are a few studies done to study the effect of Cr on germination and seedling growth. The study of Rath et al. (2021) found that chromium exposure in *Vigna mungo* not only inhibited seed germination but also triggered oxidative stress responses

Unlike chromium, copper and zinc demonstrate a dual role, which functions as an essential micronutrient and a toxicant depending on concentration. Imran et al. (2021) reported that zinc can enhance the seed germination in spinach under low temperature stress, and this study proves that Cu could reduce seed dormancy under low temperature. Studies done by Moreira, Martins, and Mourato (2020) proves that high concentration of zinc, along with cadmium, copper, and nickel can significantly inhibited germination and early seedling growth in lettuce cultivars and research done by Tao et al. (2007) further supported these findings and shows that cadmium exposure delayed germination and inhibited coleoptile growth in crop species.

These studies indicate that the effects of heavy metals are diverse and concentration dependent. Heavy metals like Zn and Cu can stimulate seed germination at low concentrations since they are essential micronutrients, and they become toxic when exceeds the threshold concentration by inducing oxidative damage. The intensity of Cr impact on seed germination depends upon the plant species.

4.3 MATERIALS AND METHODS

Seed Collection

Seeds utilised in this study were harvested from robust and phenotypically healthy plants cultivated within the botanical garden and campus of Sree Narayana College, Nattika, Thrissur, Kerala. Morphologically uniform seeds were selected for seed germination experiment and before experimentation, all seeds were meticulously washed with tap water to remove surface contaminants.

Preparation of Heavy Metal Solutions

Stock solutions of each heavy metal (1000 ppm) were prepared by dissolving the required amount of metal salt in distilled water. Desired working concentrations (25, 50, 75, 100, 125, and 150 ppm) were obtained through serial dilution of the stock solutions. All solutions were stored in dark-colored glass bottles to prevent photodegradation.

Germination Studies

Germination experiments were conducted under controlled laboratory conditions using sterilized Petri dishes. Each Petri dish was lined with Whatman No. 1 filter paper, and ten seeds were placed per dish. Triplicates were maintained for each control and treatment. Seeds in the control group were germinated in distilled water, whereas treated seeds were exposed to the corresponding heavy metal solutions. The Petri dishes were maintained at room temperature, and observations were recorded after ten days. Germination percentage, seedling axis length, vigour index, and phytotoxicity were evaluated.

Germination Percentage

Germination percentage was calculated following the methodology described by Bewley and Black (1982).

Axis Length

Seedling axis length, encompassing both root and shoot, was measured according to the standard procedure outlined by Hartmann et al. (1997).

Vigour Index

The vigour index was calculated using the approach described by Abdul-Baki and Anderson (1973), incorporating both germination percentage and mean seedling length.

Phytotoxicity

Phytotoxicity was assessed to evaluate the inhibitory effect of heavy metals on seed germination and seedling growth. It was calculated using the formula (Ellis et al., 1981):

$$\text{Phytotoxicity (\%)} = (C - T) / C \times 100$$

C = Value from control (e.g., germination percentage or root/shoot length in the control group)

T = Value from treated group (e.g., germination percentage or root/shoot length under treatment)

This methodology allowed for a systematic assessment of heavy metal toxicity on seed germination and early seedling development.

4.5 RESULTS

Germination Percentage

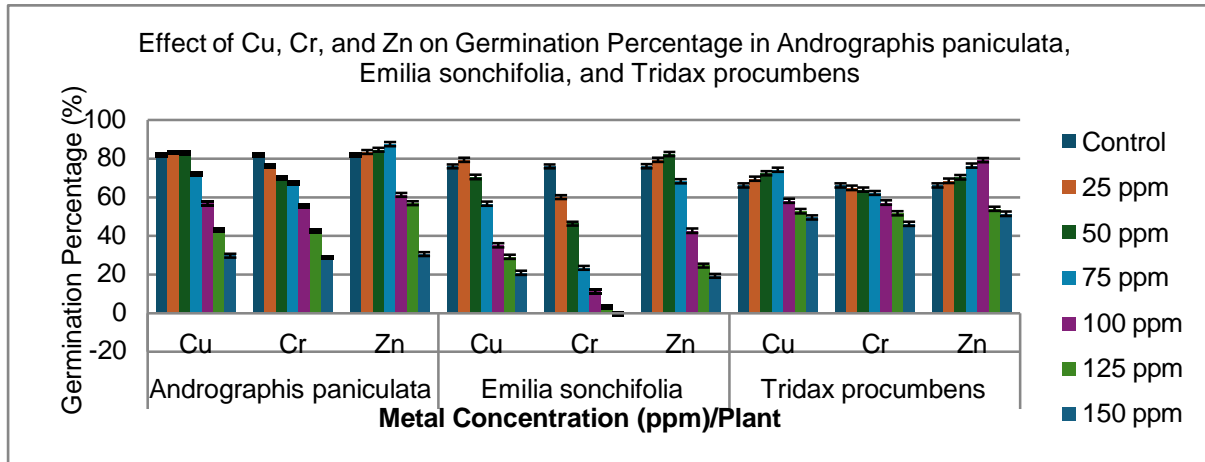


Fig.5 Effects of Metal Treatments on Germination Percentage in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

The germination response of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* to varying concentrations of copper (Cu), chromium (Cr), and zinc (Zn) exhibited distinct patterns. Low concentrations of Cu and Zn appeared to enhance seed germination, whereas concentrations exceeding 75 ppm resulted in a noticeable decline. In contrast, Cr exposure consistently reduced germination percentages in all three species, with the most pronounced inhibitory effect observed in *Emilia sonchifolia*. *Tridax procumbens* exhibited comparatively greater tolerance to Cr, showing only moderate reductions in germination under increasing Cr concentrations.

Axis Length

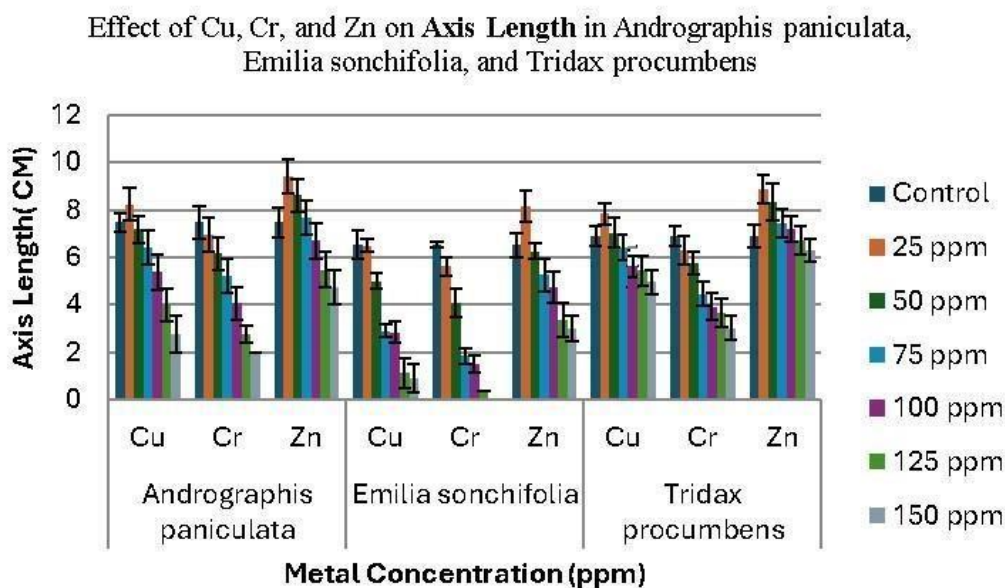


Fig.6 Effects of Metal Treatments on Axis Length in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

This study examined the effects of three heavy metals, copper (Cu), chromium (Cr), and zinc (Zn) on the axis length of three medicinal plant species: In all three plants, Cu cause a slight increase in axis length at 25 ppm and concentrations higher than 25 ppm cause a decline in axis length. An was able to cause a rise in axis length till the concentration of 75 ppm in *Andrographis paniculata* and *Tridax procumbens*. This positive impact of Zn on axis length was only at 25 ppm I the case of *Emilia sonchifolia*. Cr caused a sharp decline in axis, and this was most evident in the case of *Emilia sonchifolia*.

This study evaluated the effects of copper (Cu), chromium (Cr), and zinc (Zn) on the seedling axis length of three medicinal plant species. Exposure to Cu induced a slight increase in axis length at 25 ppm across all three species, whereas concentrations exceeding 25 ppm resulted in a progressive decline. Zinc treatment led to an increase in axis length up to 75 ppm in *Andrographis paniculata* and *Tridax procumbens*, while in *Emilia sonchifolia*, a positive effect was observed only at 25 ppm. Chromium exposure caused a pronounced reduction in axis length in all species, with the most severe impact observed in *Emilia sonchifolia*. These results highlight species-specific differences in seedling growth responses to heavy metal stress, as well as the dual role of Cu and Zn as essential micronutrients at low concentrations and as toxicants at higher concentrations.

Vigour Index

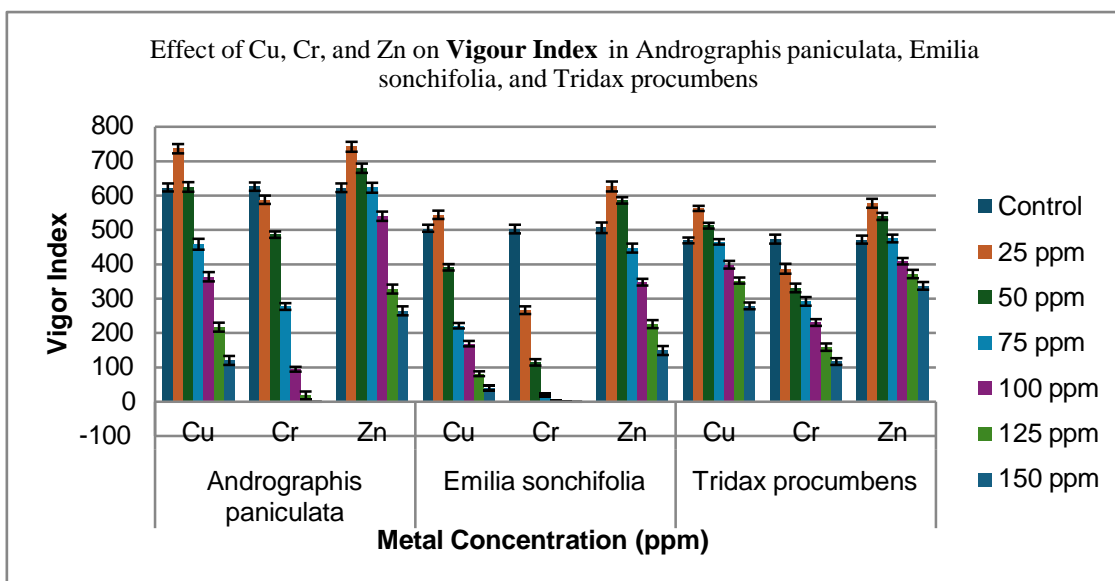


Fig. 7 Effects of Metal Treatments on Vigour Index in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

This study assessed the effects of copper (Cu), chromium (Cr), and zinc (Zn) on the vigour index of three medicinal plant species. Exposure to Cu resulted in an increase in vigour index at 25 ppm across all species. Zinc treatment enhanced vigour index at both 25 ppm and 50 ppm, with the greatest effect observed at 25 ppm. In contrast, Cr caused a pronounced reduction in vigour index in all three species, with the inhibitory effect intensifying as Cr concentration increased. These findings indicate that while Cu and Zn can stimulate early seedling vigour at low concentrations, Cr consistently exerts a toxic effect, underscoring the species- and concentration-dependent responses to heavy metal exposure.

Phytotoxicity

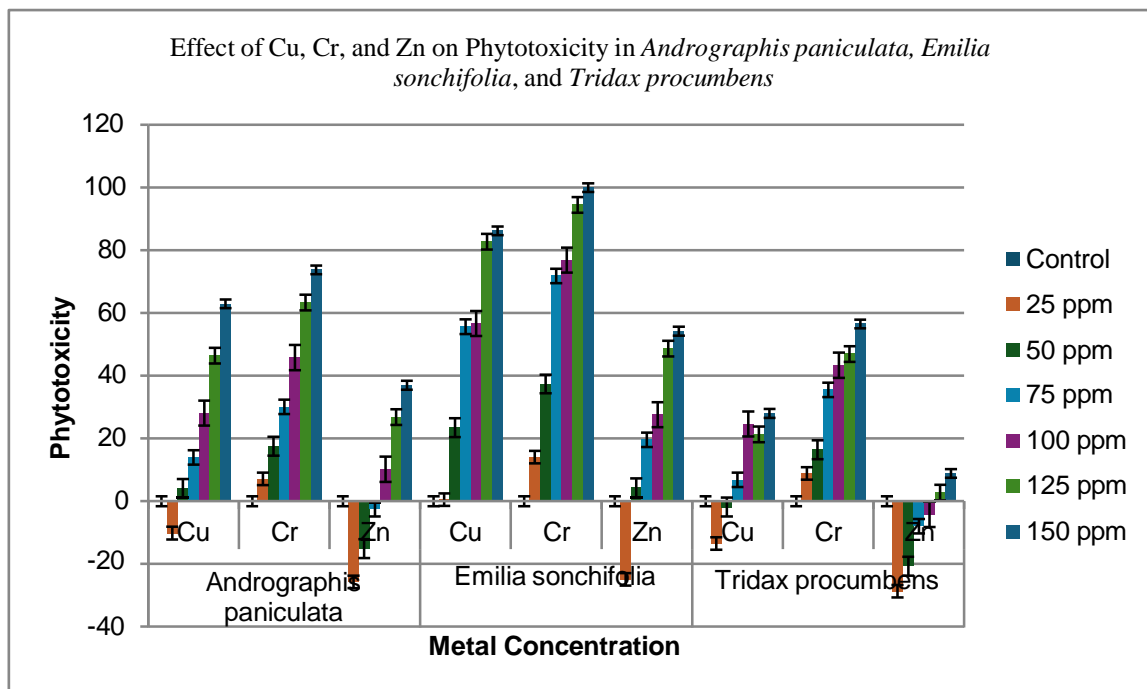


Fig.8 Effect of Cu, Cr, and Zn on Phytotoxicity in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

Phytotoxicity Assessment

Phytotoxicity analysis provides insights into the toxic potential of substances on seed germination and seedling growth. In the present study, Cu at 25 ppm and Zn at 25 and 50 ppm exhibited negative phytotoxicity values, indicating a stimulatory effect on seedling growth at these concentrations. In contrast, Cr induced the highest phytotoxicity, particularly in *Emilia sonchifolia*, where 150 ppm Cr was found to be the most toxic, demonstrating the species' heightened sensitivity to chromium exposure.

4.6 DISCUSSION

The present study examined the effects of copper (Cu), chromium (Cr), and zinc (Zn) on seed germination and early seedling growth in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. Exposure to 25 ppm Cu positively influenced seed germination across all three species, consistent with the findings of Hafeez et al. (2024), who reported that low concentrations of Cu promoted wheat germination. Conversely, higher Cu concentrations negatively affected seedling growth, aligning with the results of Mariano-da-Silva et al. (2022).

Chromium exhibited a phytotoxic effect even at 25 ppm in all three species, with *Emilia sonchifolia* showing the greatest sensitivity. These findings corroborate the study by Escudero-Villa et al. (2024), which demonstrated that Cr exposure inhibited lettuce germination and altered tissue morphology. Zinc displayed a positive effect on germination and early seedling growth at 25 and 50 ppm across all species. This stimulatory role of Zn is in agreement with the observations reported by Tolay et al. (2024).

Overall, these results indicate that Cu and Zn function as essential micronutrients at low concentrations, enhancing germination and seedling vigour, while Cr consistently exerts toxic effects in a concentration-dependent and species-specific manner.

CHAPTER 5

HYDROPONIC SYSTEM AND EXPERIMENTAL PROCEDURE

5.1 INTRODUCTION

Hydroponic Systems for Studying Heavy Metal Stress

Hydroponics provides a highly controlled and efficient system to investigate plant responses under abiotic stress such as heavy metals. In contrast of soil-based systems, in which metal availability is affected by complex interactions with organic matter, pH and microorganisms, in hydroponics, the plant is in direct contact with the treatments in uniform conditions. This means variation due to soil heterogeneity is removed and a set-up is gained that can be reproduced over time which is very useful in experimental work. Moreover, hydroponics offers the advantage of precise control of nutrient composition, aeration, and pH of the nutrient solutions, thus appreciably separating between the effects of nutrients and toxic metal stress.

In the present study, a hydroponic system was used to study the impact of copper, chromium and zinc at different doses on the growth and metabolism of plants. The system allowed for uniform nutrient supply, adequate aeration of the roots and a stable plant growth environment, and therefore no confounding effect due to non-metal specific treatment was responsible for the differences recorded. Controlled conditions have not only ensured reproducible results, but have also increased the comparability among treatments and species, rendering hydroponics a much better alternative to soil-based experiments for studying the effects of heavy metal stress in plants.

5.2 REVIEW OF LITERATURE

Hydroponic crop plants are known to be a powerful tool for studying plant responses to a range of environmental stresses including heavy metal treatment. In contrast to soil where there is variation in metal binding, nutrient buffering, and microbially interactions, hydroponics provide consistent levels of nutrients and metals which enhance the reproducibility and comparability of the results. As noted by Nguyen et al. (2016), hydroponic systems allow to precisely control nutrient composition and metal availability, and at the same time provide direct access to roots for physiological and biochemical investigations. In line, Tesi (2012) noticed that hydroponics has provided a standard technique of specific experimentation to plant biology, because of its contribution to external sources of variation reduction and support for controlled stress mimics.

A number of reports have made it clear the nondisputed significance of hydroponic setups in investigating heavy metal uptake, accumulation and tolerance from various plant species. For example, Barros et al. (2009) took the forage species in hydroponic culture to investigate phytoextraction and observed a significant difference in tracing metal+ uptake by roots and shoots. Utmazian et al. (2007) also demonstrated interclonal differences in tolerance and accumulation of metals in *Salix* and *Populus* clones, which presented marked differences in Cd and Zn partitioning between tissues. More recently, focusing on *S. perfoliatum* research showed that hydroponic treatment with copper, cadmium, lead and zinc gave rise to the significant changes in physiological parameters such as pigment content and stress markers, indicating the enhanced bioavailability of metals under solution culture (Şumālan et al., 2023). Taken together, these studies confirm that hydroponics is a powerful tool for mapping plant responses to heavy metals in a methodologically robust manner, and that it allows direct and cosmetic plant responses to heavy metals to be distinguished in a species-specific and dose-dependent manner.

5.3 MATERIALS AND METHODS

Plant Growth System

Plants were cultivated using the CityGreens Deep Water Culture (DWC) Hydroponic System (Model: DWC5PIKIT). Casa De Amor clay balls (hydrotons) served as the inert growing medium, providing root support without affecting nutrient or metal availability. A modified Hoagland's solution (pH 5.5–6.5) supplied essential nutrients, while continuous aeration via air pumps ensured adequate oxygenation of the roots. A 40 W full-spectrum LED light source (SanSi B22D LED Grow Light Bulb) maintained a stable photosynthetically active radiation (PAR) environment throughout the experiment.

Heavy Metal Treatments

Three heavy metals were evaluated: copper (Cu) as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, chromium (Cr) as $\text{K}_2\text{Cr}_2\text{O}_7$, and zinc (Zn) as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. Metals were added to the nutrient solution at concentrations of 0 (control), 25, 50, 75, 100, 125, and 150 ppm. The nutrient solution was renewed every seven days to maintain constant concentrations of nutrients and heavy metals. The total duration of treatment was 30 days.

Experimental Design

The experiment followed a Completely Randomised Design (CRD). Each hydroponic chamber contained five plants per treatment concentration, with three replicates per species. This design ensured statistical robustness while minimizing positional effects within the hydroponic system.

Data Collection

Morphological and phytochemical parameters were monitored to evaluate the effects of heavy metal exposure on plant performance. After 30 days, measurements included shoot and root length, leaf number, leaf area, biomass, and vigour index. Additionally, primary and secondary metabolite contents were quantified to assess metabolic responses to metal stress. Antioxidant activity assays and bioaccumulation studies were conducted to examine stress-induced physiological changes and the extent of metal uptake. Detailed methodologies for these analyses are described in the following sections.

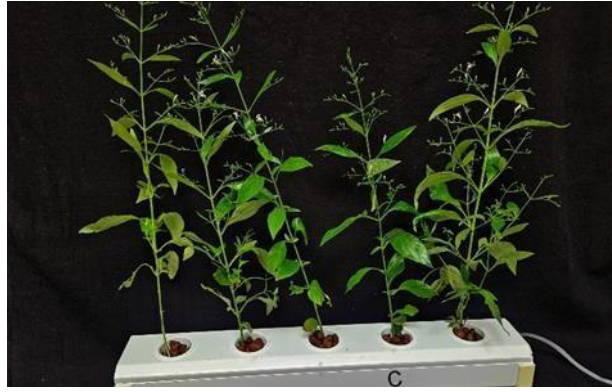


Fig. 9. *Andrographis paniculata* in a hydroponic system



Fig. 10 *Emilia sonchifolia* in a hydroponic system



Fig. 11 *Tridax procumbens* in a hydroponic system

CHAPTER 6

**STUDY OF THE EFFECT OF HEAVY METALS ON
MORPHOLOGICAL CHARACTERS**

6.1 INTRODUCTION

Impact of Heavy Metals on Plant Morphology and Productivity

Heavy metal treatment can induce various morphological, physiological, and biochemical responses in plants. Within them, macroscopic parameters, such as root and shoot lengths, leaf number, flowering and the total phytomass and productivity, are sensitive indexes of the response of plants to environmental stress. These parameters do not only indicate the adaption and tolerance of plants to metal toxicities, but also offer a preliminary perspective of ecological risks to terrestrial plant communities associated with soil contamination.

This knowledge gap is to be addressed in the present study by systematically examining the influence of Cu, Cr, and Zn at various concentration levels on key macroscopic parameters of the three aforementioned species. Parameters are finally root and shoot length, leaves number, flowers number, total Dry Weight and productivity. The aim of this research is to investigate the differences in response between these plants in order to determine tolerance thresholds, estimate potential lead phytotoxicity and propose potential uses in phytoremediation. In addition, the work provides insight into plant-metal interactions, which are important for environmental assessment, sustainable land use and protection of plant genetic resources in metal contaminated habitats.

6.2 REVIEW OF LITERATURE

Although a wide range of plants are affected by heavy metal exposure, amongst them the plant's root and shoot growth, morphology and physiology as well as stomatal properties and cell organization are entirely compromised. Estimations of Pb^{+2} , Zn^{+2} , Cr^{+6} and Cd^{+2} were done against rice (*Oryza sativa*) cultivars in an attempt to show that these heavy metals are responsible for reductions in the lengths of root and shoot, as well as lower in biomass which actually indicated that they had adversely affected a plant form (Alfaraas et al., 2016). Studies investigating Cd and Pb have indeed found leaf chlorosis, a reduced root number, decreased shoot height and microscopic damage like parenchyma degenerescence, changes in the vascular cylinder, cell wall thickening and dark deposits in the endodermal cells (Sabella et al., 2022). Seedlings of durum wheat showed a tendency towards Cd stress adaptation such as increase in primary root length and the number of root tips on exposure to low and high Cd whereas higher Cd and Cr stresses triggered marked declines in root and shoot growth, biomass, stomatal attributes and cellular architecture in the root epidermis, cortex, and xylem vessels of wheat seedlings (Yemets et al., 2021). In *Arabidopsis thaliana*, Cd, Ni, and Cu induced dose-dependent inhibition of primary root growth and root deformation due to the actin microfilament organization breakdown, while Zn stimulated slightly root growth along without pronounced morphological changes (Yemets et al., 2021). The effects of heavy metal stress on stomatal morphology have also been reported: Cd decreased guard cell length and width and increased circumference in pea, Pb reduced guard cell diameter and altered plastids in soybean, Cu damaged guard cell membranes in tomato, and combined Pb/Cd stress induced distortion of rice guard cell ultrastructure, which led to a decline in stomatal function and gas exchange (Guo et al., 2023). Other plant species growing on mine soils like dandelion (*Taraxacum officinale*) showed root thickening, decreased leaf size and changes in leaf morphology as a consequence of both toxic impact and potential adaptation strategies (Maleci, Buffa, Wahsha, & Bini, 2014). The abuse of plants to rapidly respond to heavy metal exposure system aggravates many more morphological and physiological changes in the plants in a broad metal plant responses would then be subject of plant species and its growth stages the former would be a variety of metals and its concentrations. Some plants evolved adaptive mechanisms which impede metal damage up to some extent, whereas in others growth is heavily restricted and cellular damage is noticeable that rephrases our conception about the mechanism of tolerance and potential use in phytoremediation (Farooq, 2022).

6.3 MATERIALS AND METHODS

Plant Material and Growth Conditions

The study focused on three medicinal plant species: *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. Healthy, morphologically uniform seeds of each species were selected for germination and subsequently cultivated under controlled hydroponic conditions. Environmental parameters, including nutrient composition, aeration, and light, were maintained consistently to ensure uniform growth and to minimize variability due to external factors.

Measurement of Macroscopic Parameters Following the completion of the 30-day heavy metal treatment, the macroscopic growth characteristics of each plant were assessed as follows:

Root Length (cm): Measured from the root tip to the base of the stem using a standard ruler.

Shoot Length (cm): Determined from the root–shoot junction to the apical bud.

Number of Leaves: Total number of leaves per plant was manually counted.

Flower Count: Total number of flowers per plant was recorded.

Dry Weight (g): Harvested plants were oven-dried at 60°C until constant weight, and dry biomass was recorded.

Productivity (g/day): Calculated as the total dry weight of each plant divided by the growth period (30 days), providing an estimate of biomass accumulation over time.

These measurements allowed a quantitative assessment of plant growth responses to varying concentrations of heavy metals, providing insight into their tolerance, adaptability, and potential ecological implications.

6.4 RESULTS

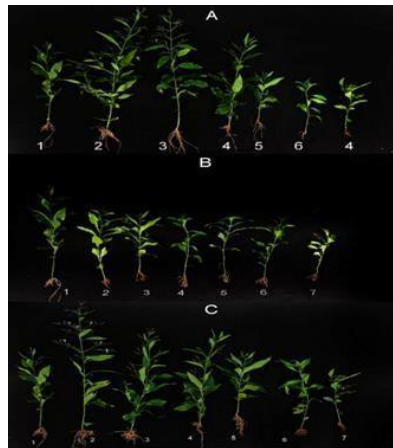


Fig. 12 *Andrographis paniculata* exposed to Cu (A), Cr (B), Zn (C). Concentrations control (1) , 25 (2), 50 (3), 75 (4), 100 (5), 125 (6), and 150 ppm (7).

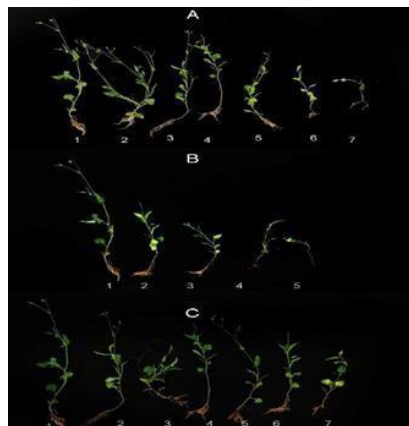


Fig. 13 *Emilia sonchifolia* exposed to Cu (A), Cr (B), and Zn (C) concentrations control (1) , 25 (2), 50 (3), 75 (4), 100 (5), 125 (6), and 150 ppm (7).

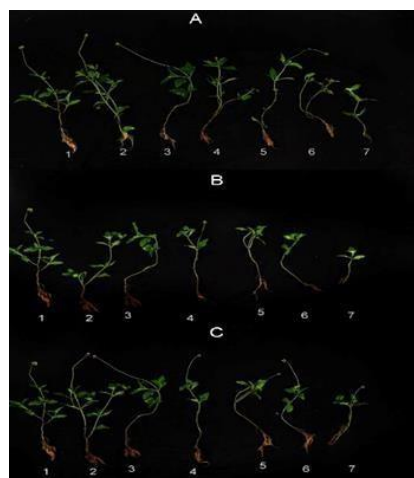


Fig. 14 *Tridax procumbens* exposed to Cu (A), Cr (B), and Zn (C) concentrations control (1) , 25 (2), 50 (3), 75 (4), 100 (5), 125 (6), and 150 ppm (7).

When Emilia sonchifolia plants were treated with 125 and 150 ppm of Cr, the plants did not survive for 30 days. Every Emilia sonchifolia plant sample treated with these concentrations Cr wilted within a few days

Effect of Heavy Metals on Root Length

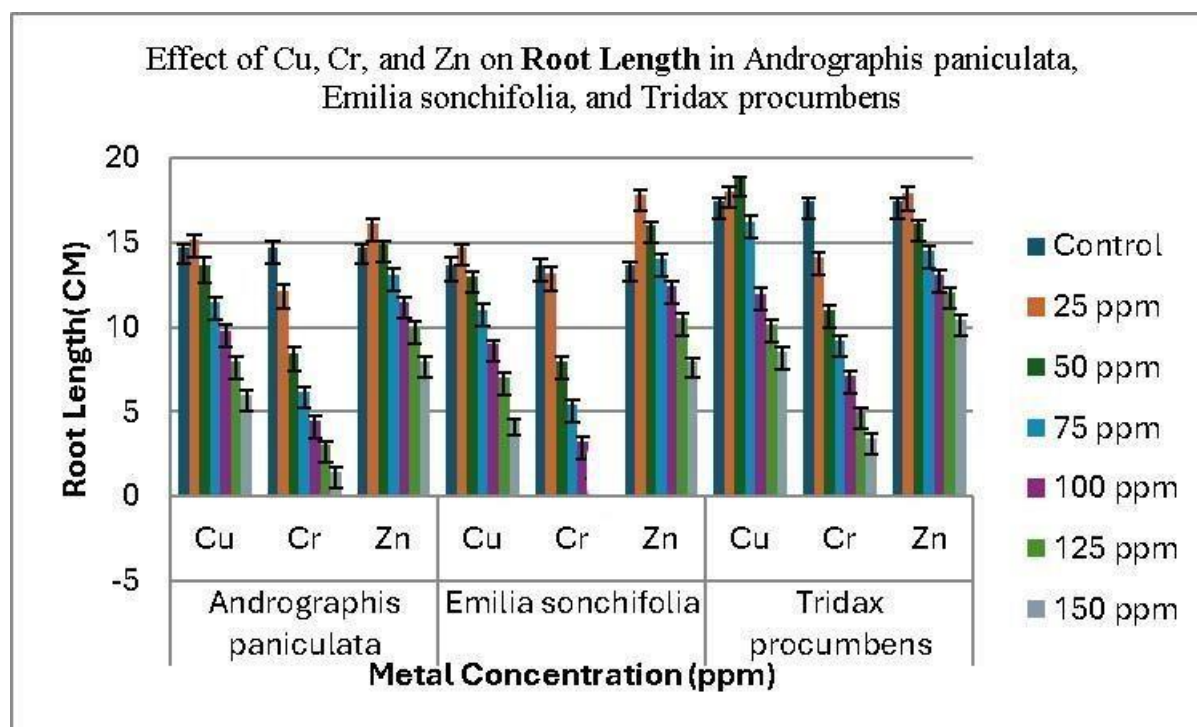


Fig. 15 Effect of Cu, Cr, and Zn on **Root Length** in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

The present study revealed that heavy metal treatments significantly influenced root length across the three medicinal plant species. Copper (Cu) at low concentrations (25–50 ppm) induced a slight increase in root length in all three species, while zinc (Zn) promoted root elongation up to 75 ppm. However, concentrations exceeding these thresholds resulted in a noticeable reduction in root length for both Cu and Zn treatments.

Chromium (Cr) consistently reduced root length in all species, with the inhibitory effect intensifying as Cr concentration increased. Among the species studied, *Emilia sonchifolia* was the most sensitive; seedlings exposed to Cr concentrations above 100 ppm failed to survive. In contrast, *Tridax procumbens* exhibited the highest tolerance, showing only minor reductions in root length across all metal treatments.

Andrographis paniculata displayed increased root length at 25 ppm Cu and at 25–50 ppm Zn compared to the control. Conversely, Cr exposure led to a decline in root growth even at 25 ppm, with the effect becoming increasingly pronounced at higher concentrations.

For *Emilia sonchifolia*, low concentrations of Cu (25 ppm) and Zn (25–50 ppm) resulted in a slight enhancement of root length relative to the control. However, higher Cr concentrations (125–150 ppm) were lethal, clearly indicating the species' sensitivity to chromium stress.

Tridax procumbens demonstrated increased root length under 25–50 ppm Cu and 25 ppm Zn treatments. At higher concentrations, root growth decreased, but the reduction was less severe compared to the other two species. Cr treatments led to a decline in root length, though the effect was comparatively moderate.

Overall, these findings suggest that *Emilia sonchifolia* is the most sensitive species to heavy metal stress, particularly Cr, whereas *Tridax procumbens* is comparatively more tolerant. *Andrographis paniculata* exhibited intermediate sensitivity, showing positive responses at low Cu and Zn concentrations but pronounced inhibition under elevated Cr exposure.

Effect of Heavy Metals on Shoot Length

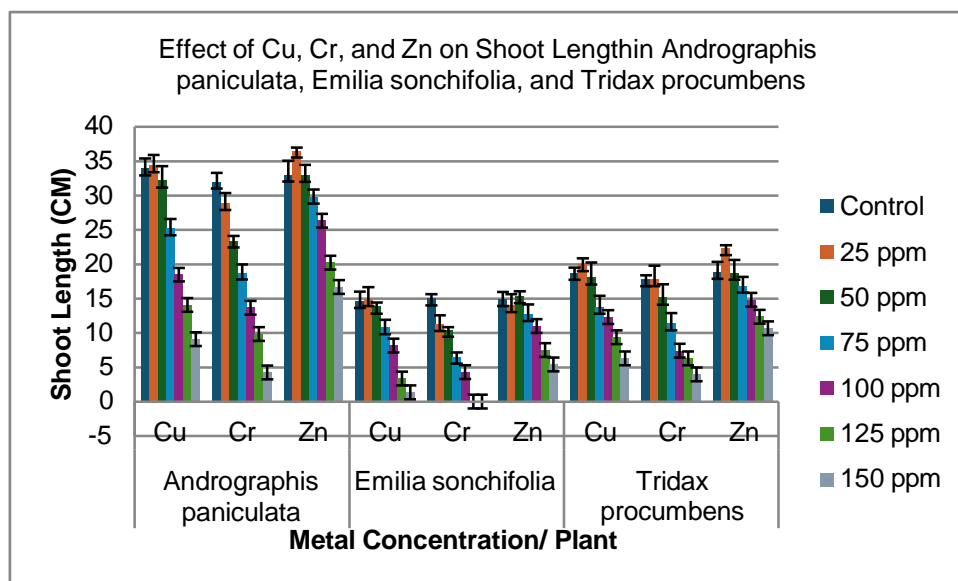


Fig . 16 Effect of Cu, Cr, and Zn on Shoot Length in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

The present study evaluated the impact of copper (Cu), chromium (Cr), and zinc (Zn) at concentrations of 25, 50, 75, 100, 125, and 150 ppm on the shoot length of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. Overall, shoot length declined with increasing metal concentrations across all three species.

At low concentrations (25–50 ppm), Cu and Zn exhibited moderate stimulatory effects on shoot growth in some cases, whereas higher concentrations (≥ 75 ppm) caused a pronounced reduction in elongation, occasionally resulting in negative growth, indicative of tissue damage or necrosis. Chromium emerged as the most inhibitory metal, with shoot elongation nearly arrested at 100 ppm and above, particularly in *Emilia sonchifolia*.

Tridax procumbens demonstrated the highest tolerance among the three species. Although shoot growth was reduced under elevated metal concentrations, significant elongation was still observed, especially under Zn stress, even at 150 ppm, albeit shorter than the control. Copper displayed intermediate toxicity: *Emilia sonchifolia* maintained modest growth at 25–50 ppm before sharply declining at higher levels. Zinc exhibited comparatively low toxicity, allowing moderate shoot growth in all species at low-to-intermediate concentrations.

Species-specific responses were evident. *Emilia sonchifolia* was the most sensitive, showing marked reductions in shoot length at concentrations ≥ 50 ppm across all metals. *Andrographis*

paniculata showed moderate tolerance to Cr and Zn at low concentrations but low tolerance to Cu. In contrast, *Tridax procumbens* consistently exhibited the highest tolerance, particularly to Zn, suggesting adaptive mechanisms that mitigate metal-induced stress.

Overall, these results indicate that heavy metal toxicity on shoot length is both concentration- and species-dependent, with Cr exerting the strongest inhibitory effect and Zn the least.

Effect of Heavy Metals on Leaf Yield

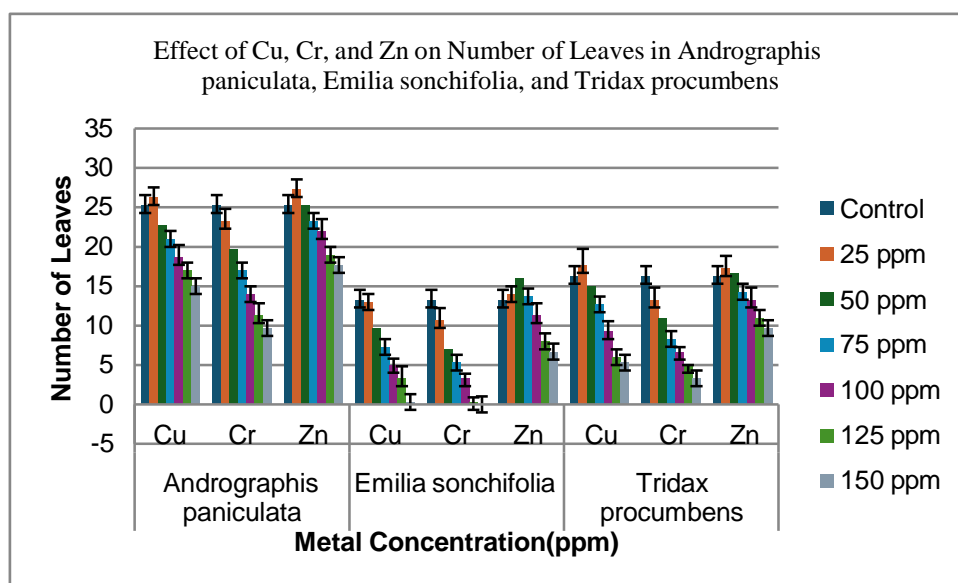


Fig. 17 Effect of Cu, Cr, and Zn on Number of Leaves in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

The influence of copper (Cu), chromium (Cr), and zinc (Zn) on leaf production in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* was assessed across concentrations ranging from 25 to 150 ppm. Overall, total leaf number decreased with increasing metal concentrations in all three species. At lower concentrations (25–50 ppm), the reduction in leaf number was minimal, whereas at concentrations exceeding 75 ppm, significant leaf shedding and reduced generation of new leaves were observed. In several replicates, negative values were recorded, indicative of necrosis or abscission.

Among the metals tested, Cu exhibited the highest toxicity. *A. paniculata* experienced near-complete defoliation at 150 ppm, whereas *T. procumbens* retained a few leaves, demonstrating relative resilience. Chromium (Cr) caused moderate decreases in leaf number between 25–100 ppm, with *E. sonchifolia* showing some tolerance at lower concentrations (25–50 ppm) but experiencing sharp declines beyond 100 ppm. Zinc (Zn) was comparatively less toxic; however, leaf production still declined with increasing concentrations. Notably, *T. procumbens* maintained substantial foliar mass even at the highest Zn concentrations, suggesting partial tolerance.

Species-specific responses were evident. *A. paniculata* was highly sensitive to all three metals, exhibiting marked reductions in leaf number at concentrations ≥ 50 ppm. *E.*

sonchifolia displayed moderate tolerance to Cr and Zn at low doses but was highly susceptible to Cu. *T. procumbens* consistently exhibited the greatest tolerance, particularly under Zn exposure, retaining a higher proportion of leaves at elevated metal levels.

These findings indicate that heavy metal stress significantly affects foliar development in a concentration- and species-dependent manner, with Cu being the most detrimental and Zn the least.

Effect of Heavy Metals on Flower Production

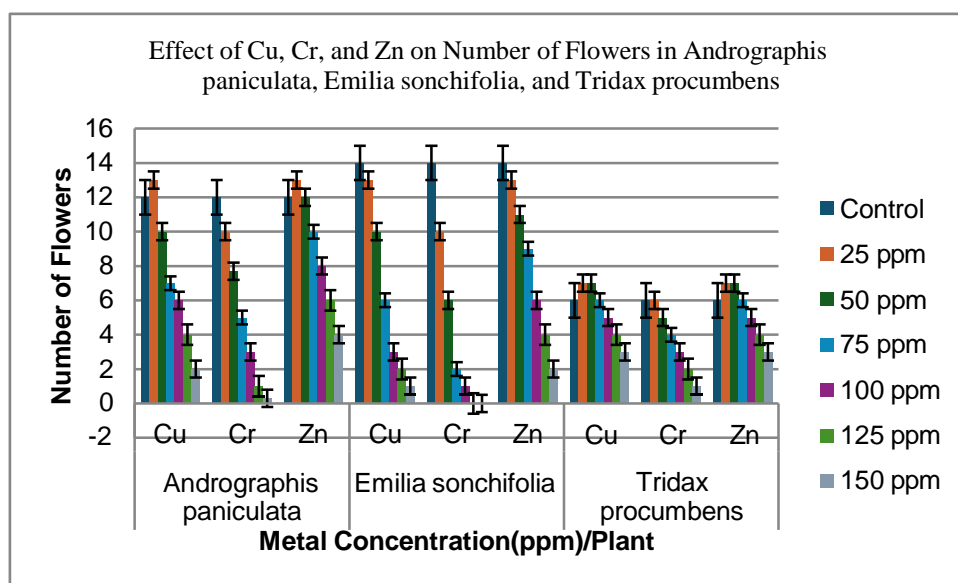


Fig. 18 Effect of Cu, Cr, and Zn on Number of Flowers in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

The impact of copper (Cu), chromium (Cr), and zinc (Zn) on flower production in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* was evaluated at concentrations of 25, 50, 100, and 150 ppm per plant. In general, increasing metal concentrations were associated with a significant decline in flower yield across all species. At lower concentrations (25–50 ppm), a moderate enhancement in flowering was observed with Cu and Zn treatments. However, at concentrations ≥ 75 ppm, flower production was strongly inhibited, often due to flower abortion or failure of flower initiation.

Chromium emerged as the most toxic metal, with *E. sonchifolia* exhibiting almost complete suppression of flowering at ≥ 100 ppm and failing to survive at higher concentrations. *Andrographis paniculata* showed slightly greater tolerance than *E. sonchifolia* but still experienced sharp reductions in flower numbers at elevated Cr levels. Cu exerted intermediate suppression, with *E. sonchifolia* showing slight resistance at lower concentrations, yet a significant decline was evident above 75 ppm. Zinc was comparatively less toxic; notably, *T. procumbens* maintained flower production even at the highest concentration, demonstrating strong tolerance.

Species-specific sensitivity was evident: *E. sonchifolia* was the most sensitive species overall, with flowering nearly abolished at ≥ 50 ppm for most metals. *A. paniculata* exhibited

moderate resistance to Cu and Zn at low concentrations but was highly sensitive to Cr. *T. procumbens* consistently displayed the greatest tolerance, particularly under Zn exposure, retaining reproductive capacity even at elevated metal levels.

These results indicate that flower production in medicinal plants is strongly influenced by metal type, concentration, and species, highlighting the importance of species selection in metal-contaminated environments.

Effect of Heavy Metals on Dry Weight

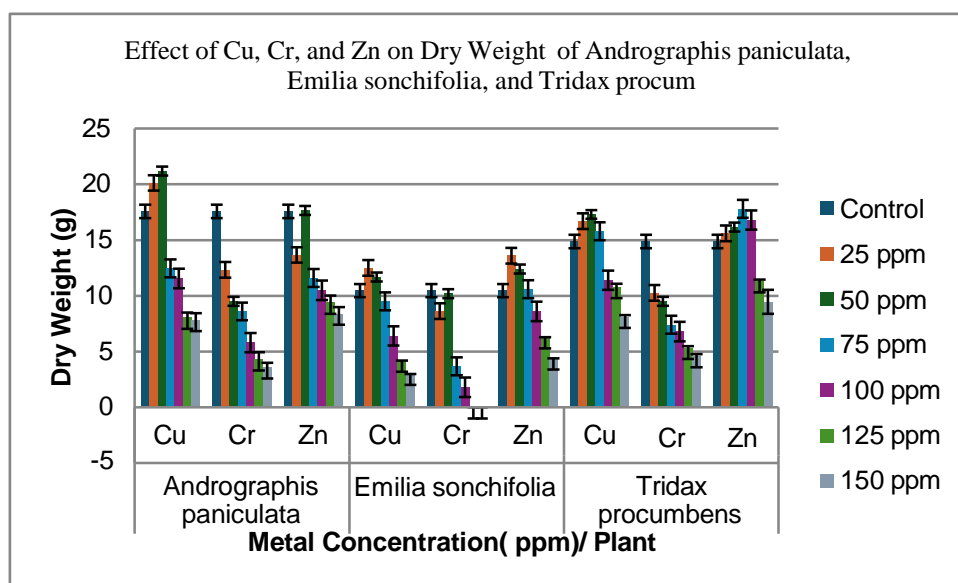


Fig. 19 Effect of Cu, Cr, and Zn on Dry Weight of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procum*

The influence of copper (Cu), chromium (Cr), and zinc (Zn) on the dry weight of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* was evaluated at concentrations ranging from 25 to 150 ppm. Overall, increasing heavy metal concentrations resulted in a consistent decline in biomass across all three species, although the differences among species were not statistically significant ($p > 0.05$).

At lower concentrations of Cu and Zn (25–50 ppm), a slight enhancement in dry weight was observed. In contrast, higher concentrations (≥ 75 ppm) caused marked reductions in biomass, indicating severe growth inhibition or plant mortality. Chromium proved to be the most toxic metal, with *E. sonchifolia* experiencing near-total loss of biomass at concentrations ≥ 100 ppm. *T. procumbens* showed moderate tolerance but still experienced substantial biomass reduction under high metal exposure. Zinc was the least detrimental; despite some suppression, *T. procumbens* maintained relatively higher dry weights even at 150 ppm, reflecting partial tolerance.

Species-specific responses were evident: *E. sonchifolia* was the most sensitive, exhibiting significant biomass reduction at concentrations ≥ 50 ppm for all metals. *A. paniculata* displayed moderate tolerance to Cu and Zn but was highly susceptible to Cr. *T. procumbens*

was the most tolerant species, particularly under Zn exposure, maintaining measurable dry weight across all concentrations.

These findings highlight the differential sensitivity of medicinal plants to heavy metals and underscore the potential of *T. procumbens* as a relatively tolerant species under metal stress conditions.

Effect of Cu, Cr, and Zn on Productivity

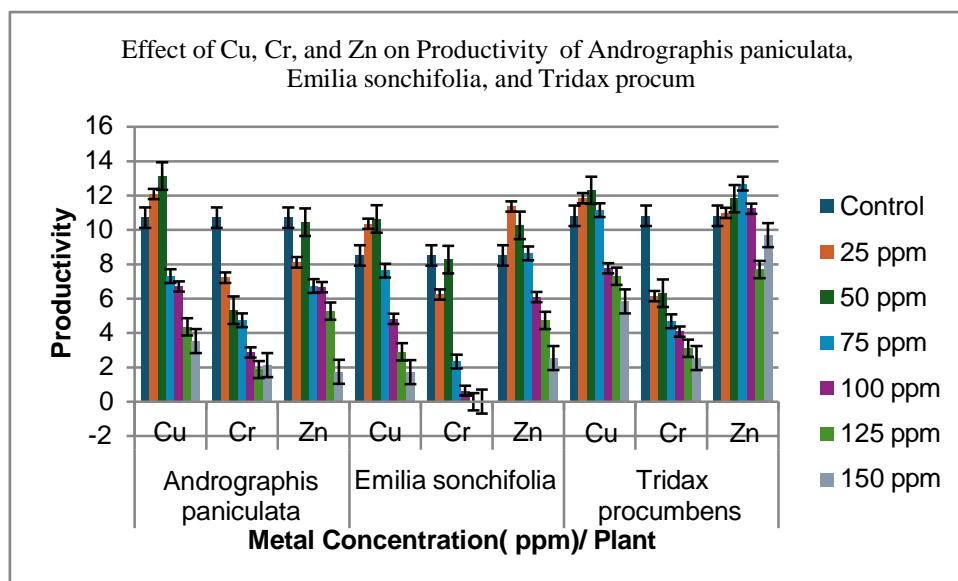


Fig. 20 Effect of Cu, Cr, and Zn on Productivity of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

The impact of copper (Cu), chromium (Cr), and zinc (Zn) on the bio-productivity of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* was evaluated at concentrations ranging from 25 to 150 ppm. Productivity of all three species declined in a concentration-dependent manner, with control plants showing the highest output. At higher metal concentrations, productivity decreased markedly, approaching zero at the highest doses, indicating severe growth inhibition or plant mortality.

Among the metals, chromium exhibited the highest toxicity, with productivity declining sharply above 50 ppm. *E. sonchifolia* was the most sensitive species, showing complete loss of productivity at 100 ppm Cr. Copper had intermediate effects, enhancing productivity at low concentrations but becoming inhibitory beyond threshold levels. Zinc was comparatively less detrimental; *T. procumbens* maintained measurable productivity even at 150 ppm, indicating partial tolerance.

Species-specific responses were evident. *E. sonchifolia* was the most susceptible, with productivity significantly reduced even at the lowest metal concentrations. *A. paniculata* displayed moderate tolerance to Cr and Zn at lower doses, while *T. procumbens* emerged as the most tolerant species, particularly under Zn exposure.

These results highlight the differential tolerance of medicinal plants to heavy metals and suggest that *T. procumbens* may be a suitable candidate for cultivation in metal-stressed environments.

6.5 DISCUSSION

Effect of Heavy Metals on Growth and Productivity of Three Medicinal Plants

The influence of varying concentrations of copper (Cu: 25–150 ppm), chromium (Cr: 25–150 ppm), and zinc (Zn: 25–150 ppm) on the growth and productivity of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* was investigated. Heavy metal treatments significantly affected root length across all three species. At lower concentrations (25–50 ppm), Cu and Zn marginally promoted root elongation; however, at 75 ppm and above, both metals caused a reduction in root length. In contrast, Cr consistently inhibited root growth in all species, with the inhibitory effect increasing with higher concentrations.

Species-specific responses were observed. *Emilia sonchifolia* was highly sensitive, showing severe growth reduction and no survival at Cr concentrations above 100 ppm. *Tridax procumbens* demonstrated the highest tolerance, showing minimal root length reduction even at elevated metal concentrations. *Andrographis paniculata* exhibited increased root length at 25 ppm Cu and 25–50 ppm Zn but experienced significant declines under Cr exposure, even at 25 ppm.

These observations align with previous studies documenting the toxic effects of heavy metals on plant growth. Espinola et al. (2025) reported reductions in root and shoot lengths in rice genotypes under heavy metal stress. Similarly, Rizvi (2020) observed concentration-dependent inhibition of root and shoot growth and biomass in wheat, with Cr showing greater toxicity than Cu. In *Arabidopsis thaliana*, Cd, Ni, and Cu caused dose-dependent inhibition of primary root growth, while Zn had relatively minor effects (Yemets et al., 2021).

Tolerance variations among species are also evident. *Tridax procumbens* has been recognized for its potential in phytoremediation due to its resilience to heavy metals, including mercury (Oliveira et al., 2015). *Emilia sonchifolia* can accumulate high levels of Ni, suggesting its feasibility for remediation of Ni-contaminated soils (Tjoa et al., 2021).

CHAPTER 7

EFFECT OF HEAVY METALS (Cu, Cr, Zn) ON PHYTOCHEMICAL COMPOSITION IN MEDICINAL PLANTS: *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

7.1 INTRODUCTION

Phytochemicals are heterogeneous compounds having biologically active molecules and synthesized in plants. They perform various metabolic, physiological and ecological roles. These substances are alkaloids, flavonoids, saponins, terpenoids. Phytochemicals also play a role in plant defense against biological and abiotic stresses. They have important role in plant growth and development too. (tibia spp.) Due to their strong pharmacological properties, such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects, phytochemical can be used to treat various diseases.

Qualitative phytochemical analysis disclosing the presence and range of these bioactive compounds in a plant and it provide information on how the synthesis of such secondary metabolites is likely to be affected by various stress including heavy metal toxicity throughout this analysis. Heavy metals like copper (Cu) and zinc (Zn) are essential elements for plant metabolism but their excess also results in the oxidative stress and the synthesis of phytochemicals. The majority of the plants are also highly sensitive to heavy metals like Chromium, Lead and Mercury These metals can affect plants negatively even in lower concentration this is because they do not have any significant role in the plant's growth and development.

The occurrence of different phytochemicals and the impact of Cu, Cr, and Zn on the phytochemical contents of three medicinal plants; *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbence* were studied. This will provide information related to to the well-known metabolic changes which occur in stress and further leads to pharmacological properties and the qualitative analysis of the plant will be crucial for further quantitative analysis to investigate the way adopted by plants to withstand the actions of metals.

Alkaloids are nitrogenous organic compounds found in many plants. These compounds are known for their pharmacological activities including analgesic, anti-inflammatory, antimicrobial, and anticancer activities. On plants Alkaloids play the following functions:wound healing and protection from herbivory and infection. In addition to their significant bioactivities, the investigations of alkaloids are very significant for drug development and health care. Qualitative determination of alkaloids is one of the common methods of analysis for the presence of alkaloids in plant and these include several chemical assays, where such chemical assays can be used to determine the types of alkaloids based on their characteristic chemical behavior.

Flavonoids are a group of polyphenolic compounds widespread in plant kingdom. They are an important group of phytochemicals which act as important agents in plant defense mechanisms against biotic stress such as herbivory, pathogen infection, as well as most of the abiotic stresses including heavy metals and other environment pollutants, and flavonoids are supposed to be the major agents in these protective mechanisms. They are found in the vegetative as well as in reproductive plant parts and the biosynthesis of flavonoids is regulated by different intrinsic and extrinsic conditions including light intensity. Atmospheric temperature and nutrient availability.

Tannins are some classes of polyphenolic components found in plants that are known for their affinity towards proteins. These phytochemicals are of defense value in plants, protecting them from tapeworms, molecular worms, brinjal, etc. Tannins with antioxidative and antimicrobial activities have potential multifaceted pharmacological applications against several diseases.

Saponins exist extensively in plant kingdom, and possess amphipathic structure. This architecture will enable them to catalyze reactions, as they bind with different proteins, and lipids, on assembling different complexes, which will have particular biological roles. Saponins in plants reduce herbivores and pathogens loss through both their toxic as well as foaming activity. Saponins have also ability of growth control and plant regulation of environmental stress and pollution. These phytochemicals have been established to possess antimicrobial, antidiabetic, and anticancer activities

Terpenoids belong to the largest and wide array of plant secondary compounds which play crucial ecological role in plants' adaptation towards biotic and abiotic stresses and interactions. Pharmacological Terpenoids are of much interest due to their antimicrobial, antioxidant and anticancer activity.

Qualitative phytochemical screening is an interesting tool to ascertain the which bioactive compounds in a plant are present and how various stresses such as heavy metal toxicity will affect the synthesis of these secondary metabolites. Metals like copper (Cu) and zinc (Zn) also important as cofactors in plant metabolism but negate their excessive amount is a cause to oxidative stress, which leads to the increase the production of phytochemicals (Singh,2008). Most of these plants are really sensitive to heavy metals e.g. Chromium, Lead and Mercury As they don't have any functions in growth and development of plants in lower concentration these heavy metal effects very negatively the plants.

Here we have evaluated the presence of different phytochemicals and impact of copper (Cu), chromium (Cr) and zinc (Zn) on the phytochemical content of three medicinal plants such as

Andrographis paniculata, *Emilia sonchifolia*, and *Tridax procumbent* in this study. The results obtained would elucidate the metabolic rearrangement in plants during stress conditions and its possible influence on the pharmacological activity complex and QS will be the basis for their further quantitative analysis and also to investigate the adaptive strategies of plant which help to minimize the toxicity of metal stress.

7.2 REVIEW OF LITERATURE

Plants are important sources for biologically active substances and medicinal plants are widely used in traditional medicine and modern pharmacy. Their medicinal value is most commonly based on the phytochemical contents, which might be greatly affected by various environmental factors, including heavy metals. These findings highlight the necessity of assessing both the phytochemical composition and heavy metal burden in medicinal plants to protect the human use of safe, efficacious, and quality products.

A detailed investigation was accomplished on *Entandrophragma delevoiyi* from Zambia by Chibuye, Singh, Chimuka, and Maseka (2024), the study integrated metabolite profiling, phytochemical examination, heavy metals estimation, and risk evaluation. Their findings indicated that environmental metal pollution may change the chemical nature of folkloric medicinal plants, which could directly affect their ethnomedicinal efficacy and should be monitored periodically for their safety.

Medicinal plants have been examined using qualitative phytochemical screening, in addition to metal analysis, as a good method of assessment. Kawade, Ingole, Shinde, and Katade (2025) also support that the combination of these analyses gives a better perception of not only the efficacy but also the safety of herbal formulations, emphasizing the importance of standardization of the preparations and application for quality control.

Heavy metals may have carcinogenic and non-carcinogenic health risks in medicinal plants. Sulaiman et al. (47) evaluated several high-use-value plant species and concluded that exposure to heavy metals such as lead, cadmium, and arsenic was potentially hazardous to the health of consumers; this emphasized the requirement for quality control and quality assurance regulation.

Apart from its toxic effect, heavy metal stress may also impair the metabolite profile and secondary metabolite synthesis in the plant. Asiminicesei, Fertu and Gavrilescu (2024) demonstrated that: “ Some of the environmental heavy metals can reduce the antioxidant capacity and therapeutic efficacy of medicinal plants by modifying the important metabolic pathways This makes the environmental monitoring an important chapter in the field of medicinal plant cultivation”.

Adugna, Ezez, Guadie, and Tefera (2024) also studied some medicinal plants for their phytochemical profiling, heavy metal burden, antioxidant activity, and antibacterial activities. Their research validated that the accumulation of heavy metals is liable to affect the bioactive molecules and the quality of plants to a great extent, thereby warranting the necessity of thorough determination of plant materials, prior to their employment.

Together, these studies provide evidence that it is essential to study both phytochemical profiles and heavy metals for the safe and effective use of medicinal plants. Through the inclusion of these evaluations, researchers and practitioners can be assured that the therapeutic potential of medicinal plants is preserved and that health risks associated with environmental contamination are decreased.

7.3 MATERIALS AND METHODS

Qualitative Phytochemical Analysis

The presence of major phytochemical groups alkaloids, flavonoids, saponins, tannins, and terpenoids was evaluated using standard chemical assays as described below:

1. Alkaloids

Mayer's Test: 1 mL of plant extract was placed in a test tube, and 2 mL of freshly prepared Mayer's reagent was added. The formation of a dull white or creamy precipitate indicated the presence of alkaloids.

Dragendorff's Test: 1 mL of plant extract was mixed with 1–2 mL of Dragendorff's reagent. A yellow or orange-red precipitate confirmed the presence of alkaloids.

2. Flavonoids

Alkaline Reagent Test: Plant extract was dissolved in a test tube, and a few drops of sodium hydroxide solution were added. A strong yellow color appeared, which was decolorized upon addition of dilute HCl, indicating the presence of flavonoids.

Shinoda Test (Magnesium Hydrochloride Reduction Test): About 0.2 g of plant extract was dissolved in 2 mL of 50% methanol, and magnesium metal was added. The mixture was warmed gently, and a few drops of concentrated HCl were added. The development of an orange-red color indicated flavonoids.

3. Saponins

Foaming Test: 0.5 g of plant extract was dissolved in 5 mL distilled water and shaken vigorously. Persistent foam formation indicated saponins. Addition of 3 drops of olive oil, followed by vigorous shaking, forming an emulsion further confirmed saponins.

Sodium Bicarbonate Test: A small amount of extract was mixed with a few drops of sodium bicarbonate solution. Froth with a honeycomb appearance indicated the presence of saponins.

4. Tannins

Ferric Chloride Test: 0.5 g of plant extract was dissolved in 10 mL distilled water, boiled, and filtered. A few milliliters of 0.1% ferric chloride solution were added to the filtrate. A bluish-black color indicated gallic-type tannins.

Lead Acetate Test: A few drops of plant extract were treated with 1 mL lead acetate solution. Formation of a white precipitate indicated the presence of tannins and proteins.

5. Terpenoids

Salkowski Test: 0.5 g of plant extract was mixed with 2 mL chloroform, and 3 mL concentrated sulfuric acid was added carefully to form a separate layer. A reddish-brown color at the interface confirmed the presence of terpenoids.

Liebermann-Burchard Test: 4 mL of plant extract was mixed with 0.5 mL acetic anhydride and 0.5 mL chloroform. Concentrated sulfuric acid was added dropwise. The development of a red-violet color indicated terpenoids.

7.4 RESULT

Table 1 Qualitative Analysis of Alkaloid

Concentration (ppm)	<i>Andrographis paniculata</i>			<i>Emilia sonchifolia</i>			<i>Tridax procumbens</i>		
	Cu	Cr	Zn	Cu	Cr	Zn	Cu	Cr	Zn
Control	++	++	++	+	+	+	+	++	+
25 ppm	++	++	++	+	+	+	+	+	++
50 ppm	+++	++	+++	+	+	+	++	+	++
75 ppm	+++	+	+++	+	+	+	+	+	++
100 ppm	++	+	++	+	-	+	+	+	+
125 ppm	+	+	++	+	-	+	+	+	+
150 ppm	+	-	++	+	-	+	+	+	+

+++ : Strong positive result , ++ : Moderate positive result, + : Weak positive , - : Negative result

In *Andrographis paniculata*, qualitative assessment of alkaloids showed an increased presence in samples treated with 50 ppm and 75 ppm of copper (Cu) and zinc (Zn) compared to the control. However, at higher concentrations, a slight decrease in alkaloid content was observed. In *Emilia sonchifolia* and *Tridax procumbens*, the alkaloid content was generally lower than in *A. paniculata*. For *E. sonchifolia*, alkaloid levels remained largely unchanged under Cu and Zn treatments, but the plants could not survive at 125 and 150 ppm chromium (Cr), indicating higher sensitivity to Cr stress. In *T. procumbens*, alkaloid content slightly increased in plants treated with 50 ppm Cu and 25, 50, and 75 ppm Zn. Chromium exhibited a different pattern in *T. procumbens* compared to *A. paniculata* and *E. sonchifolia*: at 25 ppm Cr, a slight increase in alkaloid content was observed. These results suggest that heavy metals can induce changes in alkaloid levels, and the effects vary depending on the plant species and the specific metal involved.

Table 2 Qualitative Analysis of Flavonoids

Concentration (ppm)	<i>Andrographis paniculata</i>			<i>Emilia sonchifolia</i>			<i>Tridax procumbens</i>		
	Cu	Cr	Zn	Cu	Cr	Zn	Cu	Cr	Zn
Control	++	++	++	++	++	++	+++	+++	+++
25 ppm	+++	+++	++	++	+++	+++	+++	+++	+++
50 ppm	+++	+	+++	+++	++	+++	+++	++	+++
75 ppm	+++	+	+++	++	+	+++	+++	++	+++
100 ppm	++	+	+++	+	+	++	++	++	+++
125 ppm	+	+	++	+	-	+	++	+	++
150 ppm	+	-	++	-	-	+		+	++

+++ : Strong positive result , ++ : Moderate positive result, + : Weak positive , - : Negative result

The qualitative analysis of flavonoids in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* exposed to varying concentrations of copper (Cu), chromium (Cr), and zinc (Zn) revealed distinct changes in flavonoid presence. At lower metal concentrations (25 ppm), flavonoid intensity increased, indicating a potential protective or stress-adaptive response. In contrast, at higher concentrations (≥ 100 ppm), flavonoid intensity declined, likely due to the inhibitory effects of metal toxicity on flavonoid biosynthesis. Species-specific differences were evident, reflecting the complex interactions between metal stress and secondary metabolism in these medicinal plants.

Table 3. Qualitative Analysis of Saponins

Concentration (ppm)	<i>Andrographis paniculata</i>			<i>Emilia sonchifolia</i>			<i>Tridax procumbens</i>		
	Cu	Cr	Zn	Cu	Cr	Zn	Cu	Cr	Zn
Control	+++	+++	+++	+++	+++	+++	+++	+++	+++
25 ppm	+++	+++	+++	+++	+++	+++	+++	+++	+++
50 ppm	+++	+++	+++	+++	+++	+++	+++	+++	+++
75 ppm	++	++	+++	++	++	++	++	+++	+++
100 ppm	++	+	+++	++	+	++	++	+++	+++
125 ppm	++	+	+++	+	-	-	++	++	++
150 ppm	+	-	++	-	-	-	++	++	++

+++ : Strong positive result , ++ : Moderate positive result, + : Weak positive , - : Negative result

The qualitative analysis of saponins in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* under varying concentrations of copper (Cu), chromium (Cr), and zinc (Zn) revealed distinct trends across the three species. In the control group, all plants showed a strong positive reaction for saponins, indicated by the formation of a stable froth, demonstrating their natural abundance in these species. At lower metal concentrations (25 and 50 ppm), saponin levels remained largely unaffected, suggesting that the plants were able to sustain saponin biosynthesis despite the presence of heavy metals.

However, at higher metal concentrations (≥ 75 ppm), a decline in saponin intensity was observed, particularly in *Emilia sonchifolia*. For instance, at 100 ppm, the saponin reaction weakened, and at 125–150 ppm, it was minimal or absent, indicating inhibition of saponin synthesis likely due to metal-induced stress affecting metabolic pathways. In contrast, *Tridax procumbens* exhibited greater resilience, maintaining relatively stable saponin levels even at higher Cu and Zn concentrations, highlighting species-specific differences in tolerance to heavy metal stress.

Table .4 Qualitative Analysis of Tannins

Concentration (ppm)	<i>Andrographis paniculata</i>			<i>Emilia sonchifolia</i>			<i>Tridax procumbens</i>		
	Cu	Cr	Zn	Cu	Cr	Zn	Cu	Cr	Zn
Control	++	++	++	+	+	+	+++	+++	+++
25 ppm	+++	+++	+++	++	++	++	+++	+++	+++
50 ppm	++	++	+++	+	+	+	+++	+++	+++
75 ppm	++	+	+++	+	+	+	+++	+++	+++
100 ppm	++	+	+++	+	-	+	+++	++	+++
125 ppm	++	+	+++	+	-	+	++	++	+++
150 ppm	++	+	++	+	-	+	++	+	+++

+++ : Strong positive result , ++ : Moderate positive result, + : Weak positive , - : Negative result

The qualitative analysis of tannins in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* under different concentrations of copper (Cu), chromium (Cr), and zinc (Zn) revealed species-specific responses. At lower metal concentrations (25 ppm), tannin content in *Andrographis paniculata* and *Emilia sonchifolia* was higher than in the control, indicating a possible stimulatory effect of low-level metal exposure. However, as metal concentrations increased, tannin intensity declined, with chromium showing a more pronounced inhibitory effect compared to Cu and Zn.

In *Tridax procumbens*, a distinct pattern was observed: lower concentrations of metals did not significantly alter tannin levels. A marked reduction in tannin content occurred only at higher concentrations (125–150 ppm) of Cu and Cr, with chromium exerting the strongest negative effect. Zinc treatment had the least impact on tannin levels in *Tridax procumbens*, suggesting greater tolerance of this species to Zn stress.

Table 5. Qualitative Analysis of Terpenoids

Concentration (ppm)	<i>Andrographis paniculata</i>			<i>Emilia sonchifolia</i>			<i>Tridax procumbens</i>		
	Cu	Cr	Zn	Cu	Cr	Zn	Cu	Cr	Zn
Control	+++	+++	+++	++	++	++	+++	+++	+++
25 ppm	+++	+++	+++	++	++	++	+++	+++	+++
50 ppm	+++	++	+++	++	+	++	+++	++	+++
75 ppm	+++	++	+++	+	+	++	+++	++	+++
100 ppm	++	++	++	+	+	+	++	++	+++
125 ppm	++	+	++	+	-	+	++	+	+++
150 ppm	++	+	++	+	-	+	++	+	++

+++ : Strong positive result , ++ : Moderate positive result, + : Weak positive , - : Negative result

The qualitative assessment of terpenoids in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* under varying concentrations of copper (Cu), chromium (Cr), and zinc (Zn) demonstrated concentration- and species-dependent effects. At lower concentrations (25–50 ppm), all three metals had minimal impact on terpenoid content across the three plants. However, as metal concentrations increased, a progressive decline in terpenoid levels was observed, with chromium exerting the most pronounced inhibitory effect.

In *Andrographis paniculata*, Cu and Zn had little effect on terpenoid content up to 75 ppm, whereas concentrations above 75 ppm led to a noticeable decrease. Cr, however, reduced terpenoid levels even at 50 ppm, indicating higher sensitivity. In *Emilia sonchifolia*, Cu caused a decline in terpenoids at 75 ppm, Zn at 100 ppm, and Cr induced a significant reduction from 50 ppm onwards. *Tridax procumbens* displayed the greatest resilience, with Zn having minimal effect and Cr causing a decline starting at 50 ppm. These results suggest that terpenoid biosynthesis in these medicinal plants is susceptible to heavy metal stress in a species-specific manner, with Cr generally exerting the strongest negative influence.

7.5 DISCUSSION

Alkaloids: In *A. paniculata*, alkaloid content increased at moderate concentrations of Cu and Zn (50–75 ppm) compared to the control, suggesting an adaptive metabolic response to mild metal stress. At higher concentrations, a decline was observed, indicating that excessive metal exposure impairs alkaloid synthesis. *E. sonchifolia* showed minimal variation in alkaloid levels under Cu and Zn, but plants exposed to Cr at 125 and 150 ppm did not survive, highlighting its high sensitivity. *T. procumbens* exhibited a moderate increase in alkaloid content at 25 ppm Cu and 25–75 ppm Zn, while Cr induced a unique response, with a slight increase at 25 ppm, unlike the other species. These observations are consistent with previous reports indicating species-specific alkaloid responses to heavy metal stress (Pandey, Saini, & Raja, 2019).

Flavonoids: All three species exhibited increased flavonoid content at low metal concentrations (25 ppm), potentially reflecting an enhanced antioxidant defense mechanism. At higher metal concentrations (≥ 100 ppm), flavonoid levels declined, likely due to metal-induced inhibition of biosynthetic pathways.

Saponins: Control plants of all three species displayed high saponin content. Low metal concentrations (25–50 ppm) maintained this level, whereas higher concentrations, particularly in *E. sonchifolia*, caused a significant reduction, indicating disruption of saponin metabolism under severe metal stress.

Tannins: In *A. paniculata* and *E. sonchifolia*, tannin content increased at low metal concentrations but declined at higher Cr exposures. In *T. procumbens*, decreases were observed under Cu and Cr at 100–125 ppm, while Zn had minimal effect.

Terpenoids: Terpenoid levels were largely unaffected at low metal concentrations. However, Cr induced the most pronounced reduction, starting from 50 ppm in *T. procumbens* and lower in the other species. Cu and Zn also caused moderate decreases at 75–100 ppm in *E. sonchifolia*, whereas Zn had minimal impact on *T. procumbens*.

Overall, these results indicate that heavy metals induce complex, species-specific alterations in secondary metabolites, potentially affecting the pharmacological properties of medicinal plants. Elevated alkaloid and flavonoid levels at low metal concentrations likely reflect a protective response to oxidative stress. Conversely, high metal concentrations reduce

metabolite synthesis, reflecting toxicological effects due to metal accumulation. These findings align with reports suggesting that *T. procumbens* and *E. sonchifolia* can act as metal accumulators and bioindicators (Oliveira et al., 2025; Maulydia et al., 2024), while changes in *A. paniculata* confirm previous observations of secondary metabolite modulation under stress (Pandey et al., 2019; Saini et al., 2025). Collectively, this study emphasizes the importance of monitoring heavy metal contamination in medicinal plants, as it can significantly influence the content of bioactive compounds and, consequently, their pharmacological efficacy and safety for human use.

CHAPTER 8

QUANTITATIVE ASSESSMENT OF HEAVY METAL TREATMENTS ON PRIMARY AND SECONDARY METABOLITE DYNAMICS IN MEDICINAL PLANTS

8.1 INTRODUCTION

Primary and Secondary Metabolites and Heavy Metal Stress

Metabolites: primary and secondary Plant metabolites are any biochemical compounds that are produced by plants. Carbohydrates, protein, amino acids, and lipids are some primary metabolites that are required for growth and development of the plant. type secondary metabolites like phenolics, flavonoids, terpenoids, alkaloids etc provide effective defence to the plants against different biotic and abiotic stresses. Therapeutic effects of medicinal plants are attributed to the presence of numerous secondary metabolites. The biosynthesis and accumulation of these compounds is largely determined by intrinsic plant factors such as genotype and plant nutrient uptake as well as extrinsic environmental factors such as light. Water and nutrient availability. Extreme environmental conditions and contaminations - heavy metals in particular - can modify phytochemical profile of a plant.

Like other heavy metals, in addition to inhibiting metabolic pathways, copper (Cu), chromium (Cr), and zinc (Zn) are able to alter production of primary and secondary metabolism. Even Cu and Zn are cofactors for vital enzymes Nevertheless high levels of these metals can evoke oxidative stress and badly affect the biosynthesis of bioactive compounds. Knowledge of these metabolic variations is crucial, especially for medicinal plants as alterations in biochemical profiles may impair their nutritional and pharmacological potentials.

In the present work, the effect of Cu, Cr and Zn on the quantity and composition of primary and secondary metabolites of certain medicinal plants was studied. Based on the changes in some major biochemicals under treatment with various metals, this work can provide a theoretical reference to understand the regulation mechanism of heavy metals on metabolic pathways, and also the adaptive and medicated mechanism of the plants. The results will provide additional information to a comprehensive view of plant-metal interrelationship and its consequences in phytochemistry and quality of herbal drugs.

Primary metabolites are biochemical compounds that are essential for the living of plant and directly involve in growth and productivity of plant. Primary metabolites such as carbohydrates, proteins, amino acids, lipids and organic acids are the major classes of compounds in plants. They participate in respiration, photosynthesis, and the support of plant body. primary are present in all plant species and serve as essential metabolic intermediates.

have only limited influence on primary metabolites synthesis. recruitment: 1. The toxicity can affect the oxidative status to inhibit enzymatic activity and also damage the membrane permeability. Alteration in the membrane permeability may affect the ion uptake, which mediate the cancellation in the synthesis of sugars, proteins, and other primary metabolites. Study of these changes are very important to determine health status of plant and evaluation of nutritional quality especially in edible and medicinal plants. Knowledge on response of primary metabolites profile to the heavy metals will provide information for biochemical adaptations of plants to survive and retain their plant herbs metabolic pathway under the impact of stress.

Carbohydrates are key primary metabolites which serve as major energy sources and structural elements in plants. It is the major photosynthetic product and the key element in growth and development of the plant. Carbohydrates are necessary for energy accumulation, osmotic control of the cells and serve as carbon skeleton of secondary metabolites. Accumulation and distribution of carbohydrates is one of indicator of a plant's physiological status so it is also an indicator of environmental stress like heavy metals.

The effect of copper (Cu), chromium (Cr), and zinc (Zn) on their carbohydrate metabolism by quantifying carbohydrates in *A. paniculata*, *E. sonchifolia*, and *T. procumbens* was carried out in this study. Enzyme activities can be interfered by heavy metals and carbon allocation can be disturbed which in turn affect photosynthetic efficiency and that may vary the carbohydrate content of plants. In the present investigation carbohydrate was estimated in three medicinal plants to ascertain the effect of three heavy metals on medicinal quality and general health of the plant.

Metal ions, which include Cu, Cr, and Zn, are essential micronutrients that play crucial roles in a range of physiological and biochemical processes of plants, such as enzyme activation, photosynthesis, and protein synthesis (Freeman and Persans 1998). Their bioavailability in soil, however, encountered in the natural status and anthropogenic pollution, can greatly affect plant growth and metabolism functions. These metals are important in trace but their high levels may induce oxidative stress, inhibit nutrient utilization and disturb protein metabolism. The present investigation aims at identifying the effects of Cu, Cr, and Zn metals on the protein content of three medicinal plants *Andrographis paniculata* (green chiretta), *Emilia sonchifolia* (lilac tasselflower) and *Tridax procumbens* (coatbuttons). These plants are accepted in ethno-medicine to have a therapeutic value and require to know how heavy metals exposures impact on their biochemical composition, in particular protein level, as it is an important factor of their therapeutic interference.

Proteins are primary biomolecules responsible for plant defense response, structure and enzyme activities, thus quantification of these molecules is vital to evaluate the health and nutritional status of plants. Differential protein accumulation in response to heavy metals may suggest stress and adaptation processes in plants. It is the purpose of this paper to investigate if Cu, Cr, and Zn are beneficial or inhibitory to protein synthesis in these plants under aseptic culture techniques.

As important for the balance of a plant as are other necessary micronutrients (i.e., Cu, Cr, and Zn), these heavy metals can pose as potentially toxic at high levels, interfering with normal physiological and metabolic processes. Among these, lipid metabolism is more sensitive to metal stress, since lipids are critical to membrane structure, energy storage and signal transduction. Herbal plants, including *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* have been reported to possess medicinal value but with little information on their lipid responses in heavy metal-treated condition. It is important to investigate the effects of Cu, Cr and Zn on the lipid quantity in these species in considering the tolerance against metal (s) stress and medicinal potential .

Heavy metals and lipid metabolism may affect the nutritional pharmacology value of plants. Some metals are cofactors for enzymes responsible for lipid biosynthesis but over accumulation could cause oxidative stress and change on the lipid composition and content. This work seeks to understand how different Cu, Cr, Zn concentrations affect lipid accumulation in *A. paniculata*, *E. sonchifolia*, and *T. procumbens* thus shedding light on their adaptive strategies. The results may aid in the development of improved agricultural practices in metal-polluted soils, and the understanding of plant-derived lipid-based pharmaceuticals in response to environmental stress.

Secondary metabolites, complex organic compounds that are produced by plants and are comprised of wide spectrum of bioactive substances, perform indispensable functions beyond primary metabolism for the survival and reproduction of the plants (Hostettmann et al. These types of compounds, i.e., alkaloids, flavonoids, terpenoids, tannins and phenolic acids, are involved in plant defense, intercellular signaling and ecological interactions. Though they are not directly related to primary physiological processes; the secondary metabolites are also critical in facilitating plant defense to a wide range of biotic and abiotic stressors. Their importance in plant defense against herbivory, pathogen attack and environmental stresses such as heavy metal stress all indicate their ecological importance.

Heavy metal pollutants, such as Cu, Cr, and Zn are considered as common environmental stress factors that interfere with plant homeostasis and lead to oxidative stress and dysfunctional cellular process. In plant tissues, these metals on absorption can disrupt enzymatic processes and cell health, e.g. the biosynthesis of secondary metabolites. When plants are exposed to stress, secondary metabolites can be up-regulated, or converted into alternative forms as part of a defense responses aimed at helping them either to detoxify or sequester such metals.

Alkaloids are a class of nitrogen-containing secondary metabolites involved in plant defense against herbivores, pathogens and abiotic stresses, which also display pharmacological properties. For people, such bioactive molecules are supposed to have great pharmacological values because they form the core of a variety of drugs for their analgesic, antimicrobial, anticancer and/or anti-inflammatory properties. But, alkaloid content is very much susceptible to different growth conditions such as soil composition and metal availability. Micronutrients such as copper (Cu), chromium (Cr) and zinc (Zn) are essential trace elements and can affect enzyme activities and metabolic pathways which may change the alkaloid content. Studying the effects on alkaloid contents in medicinal plants will help to optimize the culture conditions for more leveraged therapeutics.

Trace metals interaction with alkaloid synthesis is still less known, especially in the plants having medicinal importance such as *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens*. When Cu, Cr and Zn are optimum for plant growth, they might not produce the required secondary metabolites, increase or decrease alkaloid content due to their excess or deficiency. This investigation evaluates the effects of these metals on alkaloid content, which is useful for agricultural applications to optimize bioactive compound production. These results also would be useful for learning metal-induced tolerance capacity and stress response in plants, thus could be utilized in pharmacological and environmental toxicology studies.

Flavonoids are a heterogeneous group of polyphenolic compounds that are widely distributed in plants and possessing various therapeutic effects such as anti oxidative, anti inflammatory, anticancer and anti microbial effects. These bioactive compounds are very important in plant resistance against environmental stresses like UV radiation, pathogen attack and heavy metal toxicity. In medicinal plants such as *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens*, the flavonoids are significantly involved in their pharmacological properties such as hepatoprotective, immunomodulatory and wound healing. The knowledge of flavonoid biosynthesis and regulation is crucial for the cure potential development in these plants, for example under external stimuli, such as heavy metal exposure.

Certain heavy metals (copper, Cu, chromium, Cr, and zinc, Zn) can have an effect on flavonoid production either as essential micronutrients or as stress factors in plants. Zn and Cu are essential for enzymatic activity and redox reactions, and high concentrations can induce oxidative stress, possibly activating the synthesis of flavonoids as a protective response. However, chromium is frequently toxic even at low concentration, and could inhibit secondary metabolites biosynthesis. That is why the present study is focused on the influence of Cu, Cr, and Zn on flavonoid content in AP, ES, TP, so as to control the metal exposure towards the production of flavonoids for medicinal applications. The results could help in the cultivation of the medicinal plants in a controlled environment and utilizing them more effectively for the health benefits.

Saponins are the major bioactive constituents in *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens*, which underlie the pharmacological activities of these plants including antiinflammatory, antimicrobial and immuno stimulant properties. These plants have been employed in the traditional medicine, in which saponins increase their medicinal properties. Nevertheless, environmental elements such as soil characteristics also may markedly affect saponin biosynthesis. Of these, trace metals such as Cu, Cr, and Zn have a critical but still less explored effect on secondary metabolite synthesis.

This work reports on the effects of Cu, Cr, and Zn on saponin content in *A. paniculata*, *E. sonchifolia*, and *T. procumbens*, and on how such metals can influence saponin accumulation. In this regard, environmental exposure of environmental metals from agricultural and industrial practices is on the increase; hence, it is imperative to assess their effect on medicinal plants. The results will indicate how growth conditions of 3kg saponins per meter will be most easily achieved, whilst maintaining appropriate levels of thein derivative and adjusting for possible metal-induced variation of bioactives for the use of the plant in herbal medicine.

Copper (Cu), chromium (Cr), and zinc (Zn) are heavy metals which are important for plant physiology regulating several biochemical processes including the synthesis of secondary metabolites. As polyphenolic compounds, tannins are reputed for their antioxidant and medicinal activities and are therefore important in pharmacological and ecological research. This study examines the effects of Cu, Cr and Zn on tannin content of three medicinal plants, *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* and which are known for several remedial properties. Insights into this modulation of tannin levels by these metals can

help comprehension of plant defence mechanism, and can help better understand adaptive response to environmental stress.

The accumulation of heavy metals in soil is able to induce the change of plant metabolic pathways that may promote or depress the tannin formation. Although some metals are catalytic cofactors of enzymes of the phenolic metabolism excessive amounts can result in oxidative damage either to increase in secondary metabolism. The objective of this study is to investigate the dose-dependent influence of Cu, Cr, and Zn on tannin contents of the studied plant species, in order to broaden the knowledge of the phytochemistry under metal exposure. The results could be considered for the preservation of ecology as well as for the propagation of medicinal plants with better balanced bioactive compounds profile.

Phytochemicals such as terpenoids are important secondary metabolites in medicinal plants that are responsible for the pharmacological activities including antioxidant, anti-inflammatory, and antimicrobial activities. *A. paniculata*, *E. sonchifolia* and *T. procumbens* are well known for their medical potential and terpenoids are the active constituent of these plants. But, its biosynthesis may be affected by environmental factors like trace metals like Cu, Cr, and Zn. Some metals are essential micronutrients that increase stress in secondary metabolite production and also enhance plant metabolic processes. With such information at hand, it should be possible to envisage how AM fungi can be used to improve plant growth conditions in order to stimulate a higher therapeutic potential.

Recent findings have underlined the double role of trace metals in plant physiology (they may either enhance or inhibit the synthesis of secondary metabolites as function of their concentration and bioavailability). Cu, Cr and Zn are also involved in enzymatic processes and oxidant response, and they could modulate terpenoid biosynthesis pathways, CCW included, directly or indirectly. The present study aims to report the influences of these metals on the accumulation of terpenoids contents in *A. paniculata*, *E. sonchifolia* and *T. procumbens*, to reveal their possibility as manipulators of bioactive compound synthesis. The results are applicable to the agricultural practices for growing the medicinal plants to have a better therapeutic potential with a consideration on the risk of metal induced phytotoxicity to the phytochemical profiles of the medicinal plants investigated.

8.2 REVIEW OF LITERATURE

A very high content of heavy metals [copper (Cu), chromium (Cr), zinc (Zn)] under visitation of ores can have a serious impact on synthesis and composition of secondary and some primary metabolites in plant tissues. At low levels, these metals are considered as essential micronutrients, however when present at high levels they may have toxic effects that block the growth and metabolic processes (Adamczyk-Szabela & Wolf, 2024; Fan et al., 2021). High levels of Cu and Zn have been found to interfere with carbohydrate metabolism via suppression of photosynthesis, and heavy metal stress can give rise to oxidative damage, which disturbs protein synthesis and stability via the active oxygen (ROS) accumulation (Salam et al., 2023). Lipid metabolism is also altered under metal stress which eventually may results in lipid peroxidation and loss of membrane integrity (Venkatasai et al., 25). Even exposure to Cu and Zn application has significantly increased the synthesis of secondary metabolites including alkaloids, flavonoids, tannins, terpenoids, and saponins which are responsible for plant defense and medicinal values (Son et al., 2024; Adamczyk-Szabela & Wolf, 2024; Syed et al., 2007). For example, research in *Artemisia annua* shows that dietary supplementation with Cu and Zn promotes the accumulation of flavonoids and phenolic acids, which are correlated with antioxidant and anti-inflammatory properties (Son et al., 2024). Tannins and terpenoids synthesis also regulated under metal exposure to species and metal type-dependent effects, while saponins levels (having antifungal and anticancer properties) also showed metal-imposed alteration (Venkatasai et al., 2025; Salam et al., 2023). These changes are due to the oxidative stress, enzyme activity modulation and gene expression of pretense biosynthesis (Salam et al., 2023). It is also critical to understand these effects in order to optimize the growing of medicinal plants for medicinal use and developing their medicinal value further emphasizing the need for further study of the complex effect of heavy metals on plant metabolic pathways (Adamczyk-Szabela & Wolf, 2024; Fan et al., 2021; Syed et al., 2007).

8.3 MATERIALS AND METHODS

Biochemical Estimations

Carbohydrate Content

Total carbohydrate content was measured using the phenol–sulfuric acid method, with D-glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

Protein Content

Protein concentration was determined following the Lowry method, using bovine serum albumin as the standard (Lowry, Rosebrough, Farr, & Randall, 1951).

Lipid Content

Total lipids were extracted and quantified according to the Bligh and Dyer method, employing chloroform–methanol extraction (Bligh & Dyer, 1959).

Alkaloid Content

Alkaloids were extracted and estimated following the procedure described by Harborne (1973).

Flavonoid Content

Flavonoid content was measured using the method outlined by Bohm and Kocipai-Abyazan (1974).

Saponin Content

Saponins were extracted by macerating 10 g of plant powder in 100 mL of 80% methanol for 24 hours. The filtrate was concentrated using a rotary evaporator at 40 °C, and total saponin content was determined using the vanillin–sulfuric acid assay (Hiai, Oura, & Nakajima, 1976).

Tannin Content

Tannin content was estimated according to the Folin–Ciocalteu method (Singleton & Rossi, 1965).

Terpenoid Content

Relative levels of terpenoids were determined using the Liebermann–Burchard reaction, with absorbance measured at 540 nm (Siddiqui, 2010).

Statistical Analysis

All biochemical assay data were subjected to statistical analysis. One-way and two-way ANOVA were employed to assess the effects of different heavy metal concentrations on biochemical parameters. Post hoc comparisons were performed using Holm's test to determine statistically significant differences among treatments.

8.4 RESULTS

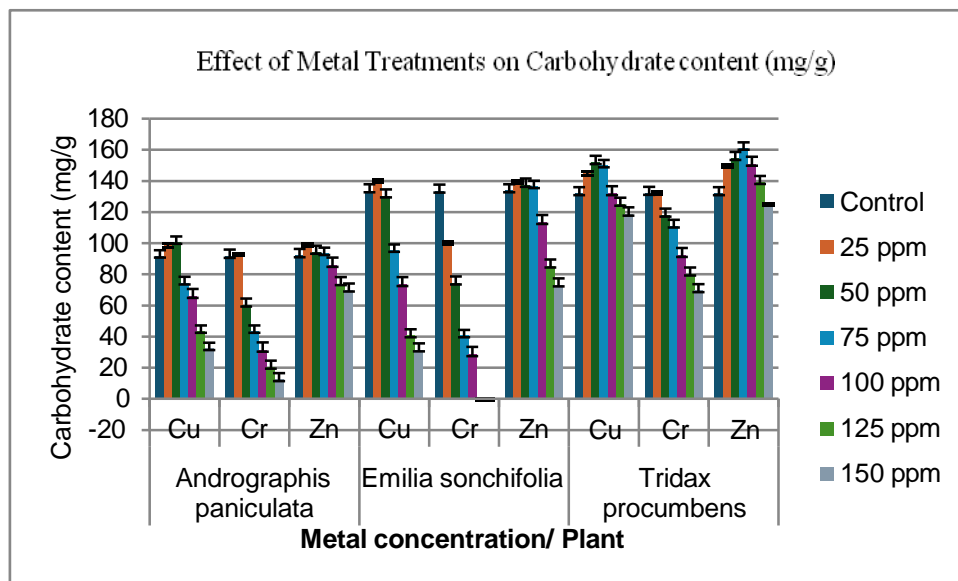


Fig. 21 Effect of Metal Treatments on Carbohydrate content

Carbohydrate Content

Quantitative analysis of carbohydrate content in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* revealed significant changes under different concentrations of copper (Cu), chromium (Cr), and zinc (Zn).

In *Andrographis paniculata*, carbohydrate levels were highest at lower concentrations of Cu (98.6 mg/g at 25 ppm) and Zn (99.1 mg/g at 25 ppm). However, at higher concentrations, a sharp decline was observed, with levels decreasing to 32.4 mg/g for Cu and 69.9 mg/g for Zn at 150 ppm. Chromium had the most pronounced effect, showing a dose-dependent decrease to 12.4 mg/g at 150 ppm, while a very slight increase was observed at 25 ppm.

For *Emilia sonchifolia*, treatment with low levels of Cu and Zn (25 ppm) caused a small increase in carbohydrate content (140.2 mg/g and 139.2 mg/g, respectively). Chromium induced a concentration-dependent decrease, with carbohydrate content dropping to 100.3 mg/g at 25 ppm, and the plants were unable to survive at 125 ppm and 150 ppm Cr, highlighting its high sensitivity.

Tridax procumbens demonstrated the greatest tolerance among the three species. Maximum carbohydrate accumulation was observed under Zn treatment at 75 ppm (161 mg/g), and even at the highest metal concentrations, significant carbohydrate levels were maintained (118.8

mg/g for Cu and 125.5 mg/g for Zn at 150 ppm). Chromium stress was less pronounced, with carbohydrate content remaining at 69.5 mg/g at 150 ppm.

Overall, chromium proved to be the most toxic metal, *Tridax procumbens* the most tolerant, and *Emilia sonchifolia* the most sensitive among the three medicinal plants studied.

Statistical Analysis

Table 6: Three-Way ANOVA

Source of Variation	Df	Sum of Squares	Mean Square	F-value	p-value
Species	2	1,13,305	56,653	3885.272	< 2e-16
Metal	2	69,877	34,939	2396.121	< 2e-16
Concentration	6	1,03,598	17,266	1184.139	< 2e-16
Species × Metal	4	4,855	1,214	83.231	< 2e-16
Species × Concentration	12	21,761	1,813	124.365	< 2e-16
Metal × Concentration	12	20,476	1,706	117.022	< 2e-16
Species × Metal × Concentration	24	3,044	127	8.699	< 2e-16
Residuals	126	1,837	15	—	—

Significance Codes: $p < 0.001$, $p < 0.01$, $p < 0.05$

The three-way ANOVA revealed that species, metal type, metal concentration, and their interactions significantly influenced carbohydrate content ($p < 0.001$). Among the main effects, species contributed the largest variance ($F = 3885.27$), followed by metal type ($F = 2396.12$) and concentration ($F = 1184.14$), demonstrating the strong independent impact of each factor on carbohydrate levels. Significant higher-order interactions including species × metal, species × concentration, metal × concentration, and species × metal × concentration indicate that carbohydrate responses are not only species-specific but also depend on the type and concentration of metal applied. These results highlight the complex and interdependent nature of plant responses to heavy metal exposure.

Carbohydrate Content under Metal and Concentration Treatments

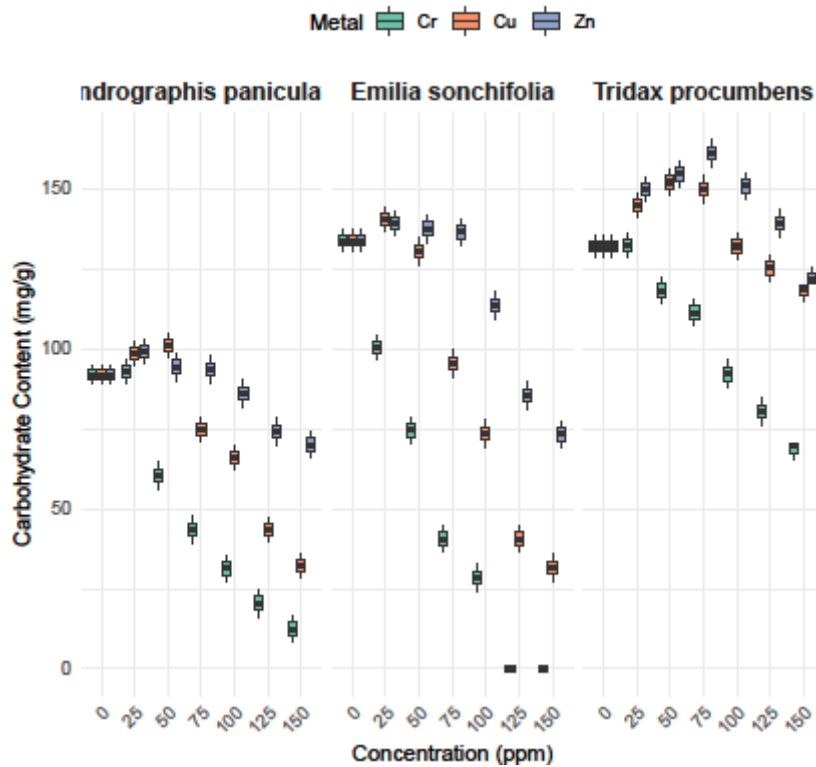


Fig. 22 Holm's Post Hoc Test

The GOM post hoc analysis indicates that both metal type and concentration strongly influence carbohydrate content in the three medicinal plants. *Andrographis paniculata* exhibited the highest carbohydrate accumulation, particularly at low concentrations of Cu and Zn. In contrast, *Emilia sonchifolia* and *Tridax procumbens* displayed intermediate to low carbohydrate levels, with pronounced reductions at higher metal concentrations and under Cr exposure. Among the metals tested, Cr had the strongest inhibitory effect, causing a concentration-dependent decline in carbohydrates, whereas Zn tended to elevate carbohydrate levels, especially in *Andrographis paniculata*. These patterns highlight species- and metal-specific responses, suggesting that differences in tolerance and metabolic adaptation to metal stress are closely associated with the type and concentration of metal exposure.

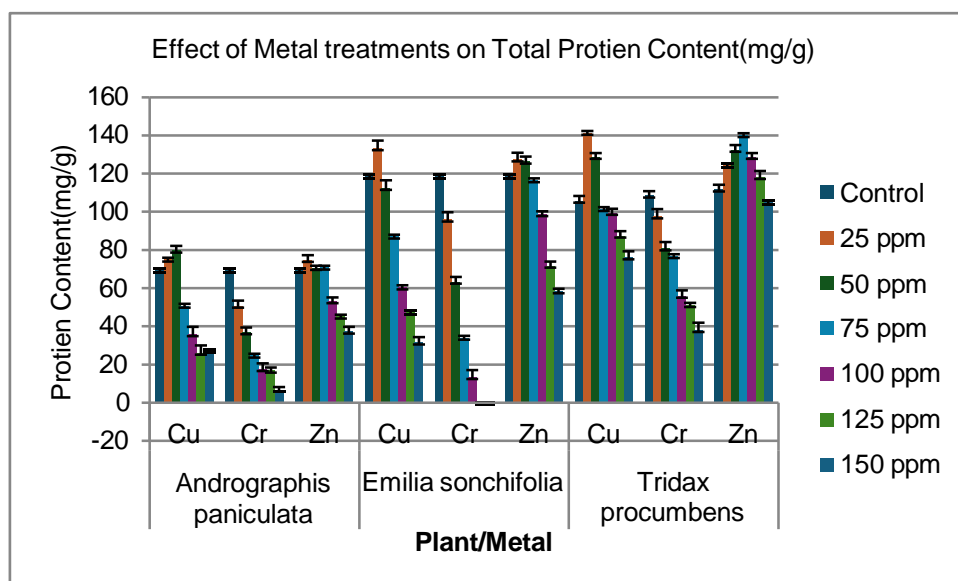


Fig. 23 Effect of Metal treatments on Total Protein Content

The present study revealed notable variations in protein content among the three selected plants *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* exposed to varying concentrations (0–150 ppm) of copper (Cu), chromium (Cr), and zinc (Zn). At the control (0 ppm), all three species exhibited similar basal protein levels. Low metal concentrations (25–50 ppm) initially enhanced protein content in some cases, particularly Cu in *A. paniculata* (highest 79.63 mg/g at 50 ppm) and Zn in *T. procumbens* (highest 140.14 mg/g at 75 ppm). However, higher concentrations (75–150 ppm) caused a significant decline in protein levels across all species, with Cr being the strongest inhibitor. Proteins in *E. sonchifolia* were completely depleted at concentrations above 125 ppm.

Although *E. sonchifolia* had the highest initial protein content (118.62 mg/g at control), it proved to be the most sensitive to heavy metal stress, whereas *T. procumbens* exhibited greater resilience, particularly under Zn exposure, maintaining high protein content even at elevated concentrations. These differential responses reflect species-specific tolerance to heavy metal stress, with the degree of protein suppression generally following the trend Cr > Cu > Zn. The findings suggest that low doses of these metals may enhance protein metabolism, while higher, toxic concentrations inhibit protein synthesis in a dose-dependent manner, with the plant species' physiological adaptations influencing the extent of the effect.

STATISTICAL ANALYSIS

Table. 7 Three-Way ANOVA

Source of Variation	Df	Sum of Squares	Mean Square	F value	p-value
Plant	2	87,570	43,785	6892.83	< 2e-16
Metal	2	65,821	32,910	5180.86	< 2e-16
Concentration	6	92,275	15,379	2421.03	< 2e-16
Plant × Metal	4	4,554	1,139	179.23	< 2e-16
Plant × Concentration	12	14,932	1,244	195.89	< 2e-16
Metal × Concentration	12	17,860	1,488	234.3	< 2e-16
Plant × Metal × Concentration	24	3,091	129	20.27	< 2e-16
Residuals	126	800	6.35		

Note: _ ‘ indicates significance at $p < 0.001$.

The ANOVA results revealed highly significant effects ($p < 0.001$) of all tested factors and their interactions on protein content, with an extremely low p-value ($< 2 \times 10^{-16}$). Among the main effects, plant species ($F = 6892.83$), metal type ($F = 5180.86$), and concentration ($F = 2421.03$) all had significant influences, with plant species accounting for the largest proportion of variance (sum of squares = 87,570).

Two-way interactions (Plant × Metal, Plant × Concentration, Metal × Concentration) and the three-way interaction (Plant × Metal × Concentration, $F = 20.27$) were also significant, indicating that the effects of metals on protein content are not simply additive but depend on the specific combination of factors. The small residual variance (Mean Square = 6.35) suggests that nearly all variability in protein content can be attributed to the experimental factors and their interactions.

These results statistically confirm that protein content responses to heavy metal stress are strongly dependent on plant species, metal type, and concentration, with each parameter significantly contributing to the observed variability.

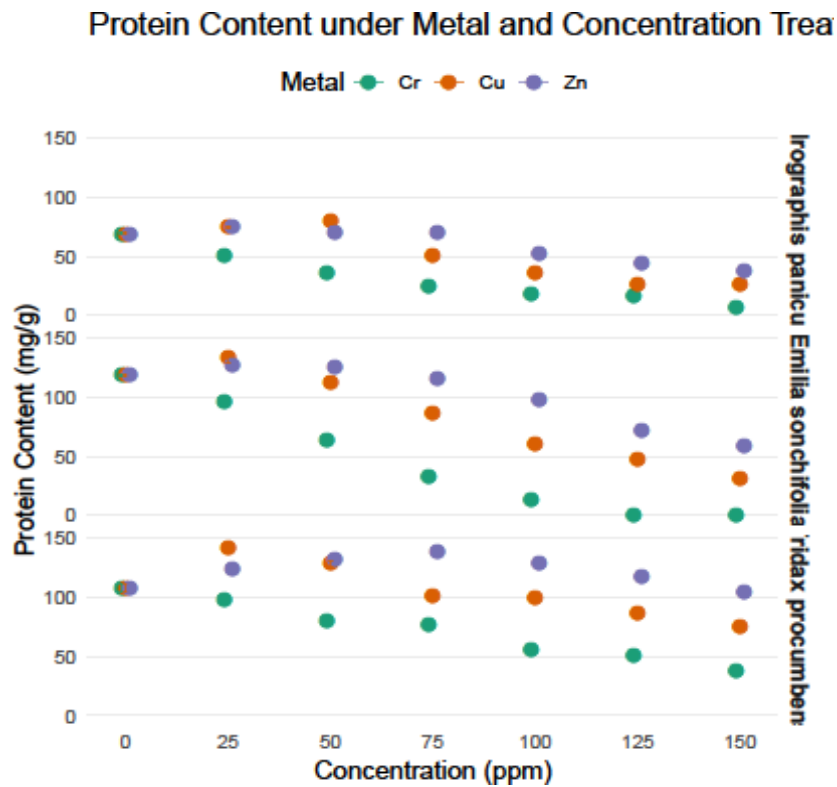


Fig. 24 Holm Post Hoc Test

The plotted data clearly demonstrate variations in protein content among plant species, metal type, and treatment concentration. Across all species, protein levels exhibited a downward trend with increasing metal doses, indicating a dose-dependent inhibitory effect. *Andrographis paniculata* showed the lowest protein content under heavy metal exposure, particularly with chromium and copper treatments. In contrast, *Emilia sonchifolia* maintained relatively higher protein levels, reflecting greater tolerance to metal stress. *Tridax procumbens* displayed intermediate responses. Among the metals, chromium caused the most pronounced reduction in protein content, followed by copper, while zinc had the least inhibitory effect. These patterns underscore species- and metal-specific differences in physiological tolerance, with *Emilia sonchifolia* emerging as the most resilient among the selected medicinal plants..

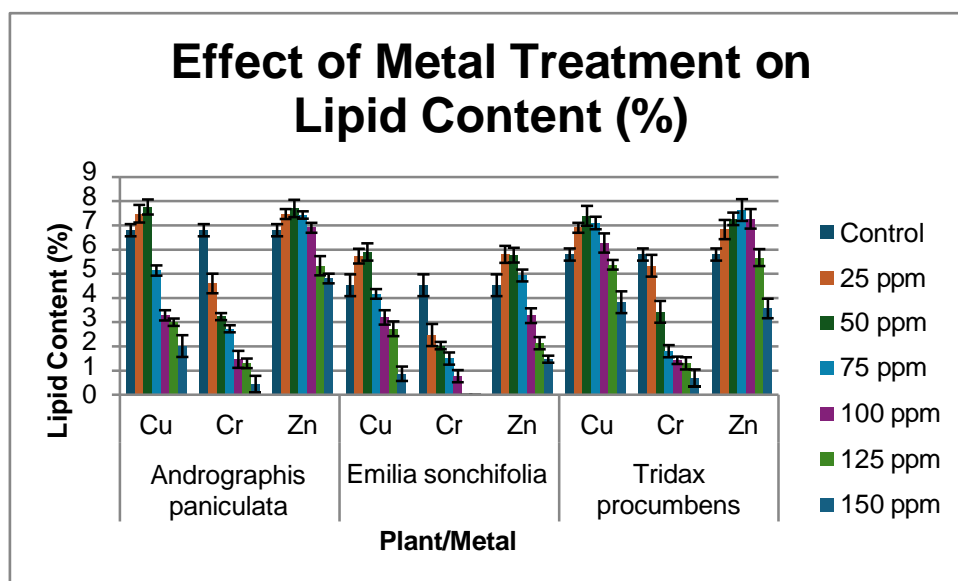


Fig. 25 Effect of Metal Treatment on Lipid Content

This study investigated the impact of different concentrations of copper (Cu), chromium (Cr), and zinc (Zn) on the lipid content of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. In *A. paniculata*, Cu induced an increase in lipid accumulation at lower concentrations (7.48 mg/g at 25 ppm and 7.76 mg/g at 50 ppm), but lipid content declined steadily at higher concentrations, reaching 2.01 mg/g at 150 ppm. Cr caused a consistent reduction in lipids (6.8 mg/g at 25 ppm to 0.43 mg/g at 150 ppm), whereas Zn initially promoted lipid accumulation up to 50 ppm (7.7 mg/g) before decreasing to 4.8 mg/g at 150 ppm.

E. sonchifolia displayed a similar pattern: Cu increased lipid content at low concentrations (5.73 mg/g at 25 ppm) and decreased at higher doses (0.87 mg/g at 150 ppm). Cr exposure resulted in complete lipid depletion at 125 and 150 ppm, while Zn had a moderate effect, peaking at 5.8 mg/g at 25 ppm and decreasing to 1.47 mg/g at 150 ppm.

In *T. procumbens*, Cu and Zn promoted lipid accumulation at lower concentrations (7.4 mg/g and 7.63 mg/g at 50–75 ppm, respectively), with a decline at higher doses. Cr exposure caused a marked reduction, from 5.8 mg/g to 0.7 mg/g.

Overall, low levels of heavy metals tended to enhance lipid content in all three species, whereas higher concentrations were inhibitory. Chromium emerged as the most potent inhibitor of lipid accumulation across all species, highlighting its toxicity relative to Cu and Zn.

Statistical Analysis

Table.8 Three-Way ANOVA

Source of Variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Species	2	139.8	69.88	700.62	< 2e-16	***
Metal	2	356.8	178.4	1788.76	< 2e-16	***
Concentration	6	370.5	61.76	619.19	< 2e-16	***
Species × Metal	4	21.8	5.45	54.62	< 2e-16	***
Species × Concentration	12	14.9	1.24	12.45	< 2e-16	***
Metal × Concentration	12	86.4	7.2	72.22	< 2e-16	***
Species × Metal × Concentration	24	32.7	1.36	13.65	< 2e-16	***
Species	2	139.8	69.88	700.62	< 2e-16	***
Residuals	126	12.6	0.1			

Significance codes: „ p < 0.001; „ p < 0.01; „ p < 0.05; „ p < 0.1

The three-way ANOVA demonstrated that plant species, metal type, and concentration all had significant effects on lipid accumulation in the medicinal plants analyzed ($p < 0.001$). Among the main effects, metal type contributed the most to variance in lipid levels ($F = 1788.76$), followed by species ($F = 700.62$) and concentration ($F = 619.19$), indicating that lipid content is strongly dependent on the type and level of heavy metal treatment as well as the plant species.

All interaction terms were also highly significant ($p < 0.001$), highlighting the complex interplay between species, metal type, and concentration. The Species × Metal interaction ($F = 54.62$) suggests differential responses of plant species to each metal, while the Metal × Concentration ($F = 72.22$) and Species × Concentration ($F = 12.45$) interactions indicate that lipid responses vary with both treatment level and species. The significant three-way interaction ($F = 13.65$) further underscores that the combined effects of species, metal type, and concentration can substantially influence lipid metabolism. These results emphasize the importance of considering species-specific and metal-specific strategies for optimizing phytoremediation and the cultivation of medicinal plants under heavy metal stress.

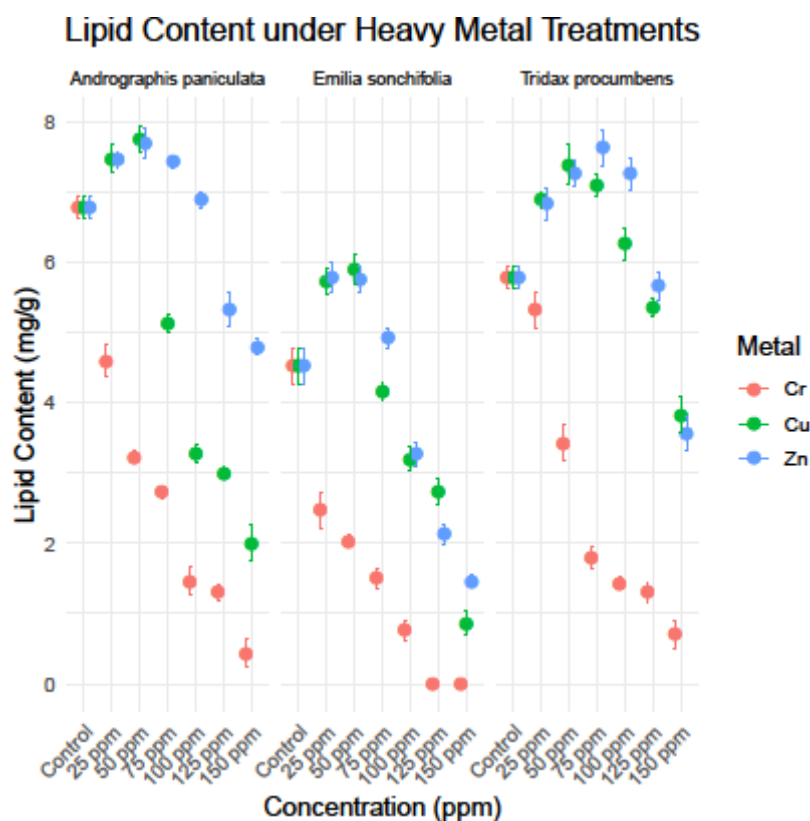


Fig. 26 Holm's post hoc test

The post hoc Holm's test for lipid content in the three medicinal plants *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* demonstrated significant differences among species under increasing concentrations (25–150 ppm) of heavy metals (Cu, Cr, and Zn). Overall, lipid content declined with higher metal levels across all species; however, the extent of this reduction varied depending on the plant species and type of metal. *Andrographis paniculata* exhibited the highest sensitivity, particularly under Cu and Cr exposure, showing the greatest decreases in lipid content. In contrast, *Emilia sonchifolia* and *Tridax procumbens* displayed relatively smaller reductions, with Zn generally exerting the least effect. These observations highlight a clear three-way interaction between metal type, concentration, and species, reflecting species-specific capacities to cope with heavy metal-induced stress in lipid metabolism.

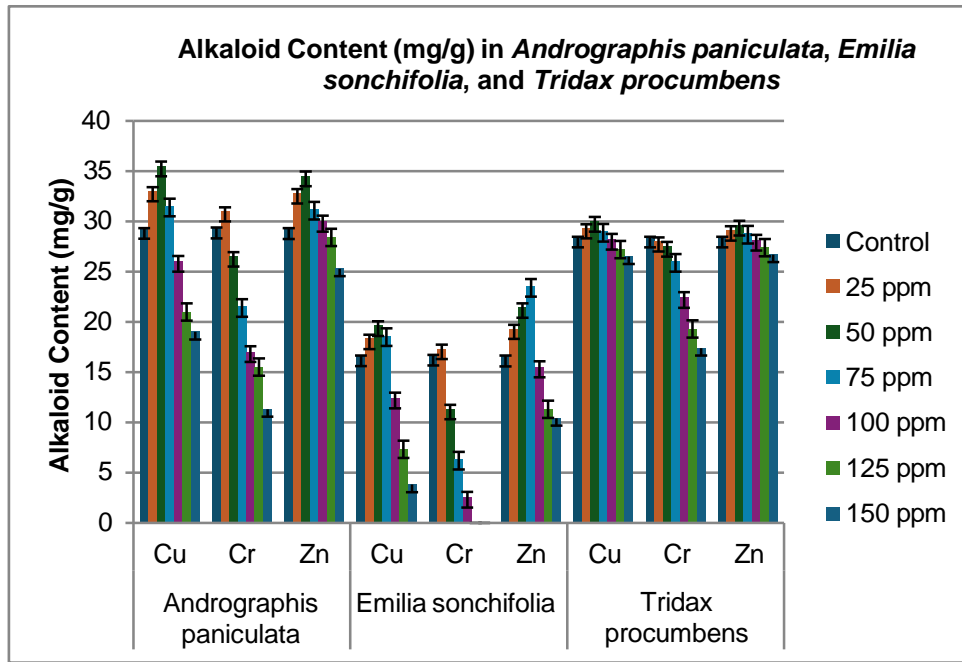


Fig. 27 Alkaloid Content (mg/g) in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

The results revealed significant differences in **alkaloid content** among the three medicinal plants, metal treatments, and concentrations. *Emilia sonchifolia* was the most sensitive to both the type and concentration of metals, with Zn treatment consistently producing the highest alkaloid levels (21.4 mg/g at 50 ppm), whereas Cr exposure led to almost complete depletion at higher concentrations (0 mg/g at 125–150 ppm). *Andrographis paniculata* showed moderate alkaloid accumulation, reaching a maximum of 35.5 mg/g under Cu at 50 ppm, but levels decreased at higher concentrations, particularly with Cr. In *Tridax procumbens*, alkaloid content remained relatively stable under most treatments, ranging from 26.5–30 mg/g, indicating greater resilience. Overall, Zn generally enhanced alkaloid production across all species, whereas Cr reduced alkaloid levels, with maximum yields typically observed at intermediate metal concentrations (50–75 ppm), followed by a decline at higher doses.

Table 9 Three-Way ANOVA

Source of Variation	Df	Sum of Squares	Mean Square	F Value	Pr(>F)	Significance
Species	2	8220	4110	651145	< 2e-16	***
Metal	2	1807	904	143161	< 2e-16	***
Concentration	6	3072	512	81121	< 2e-16	***
Species × Metal	4	157	39	6219	< 2e-16	***
Species × Concentration	12	427	36	5640	< 2e-16	***
Metal × Concentration	12	655	55	8649	< 2e-16	***
Species × Metal × Concentration	24	226	9	1491	< 2e-16	***
Residuals	126	1	~0			

The three-way ANOVA revealed that plant species, metal type, and concentration each had highly significant effects ($p < 0.001$) on alkaloid content. In addition, all interaction terms including the two-way interactions (Species × Metal, Species × Concentration, Metal × Concentration) and the three-way interaction (Species × Metal × Concentration) were also highly significant ($p < 0.0001$). These results indicate that the influence of metal exposure on alkaloid accumulation is complex, species-specific, and dependent on the type and concentration of metal. The very low residual variance further emphasizes that nearly all variation in alkaloid content can be attributed to the experimental factors and their interactions. Overall, this analysis demonstrates that metal-induced changes in alkaloid synthesis are not uniform across species, reflecting a multifaceted response to metal stress.

Estimated Alkaloid Content (mg/g) by Species × Metal × Con

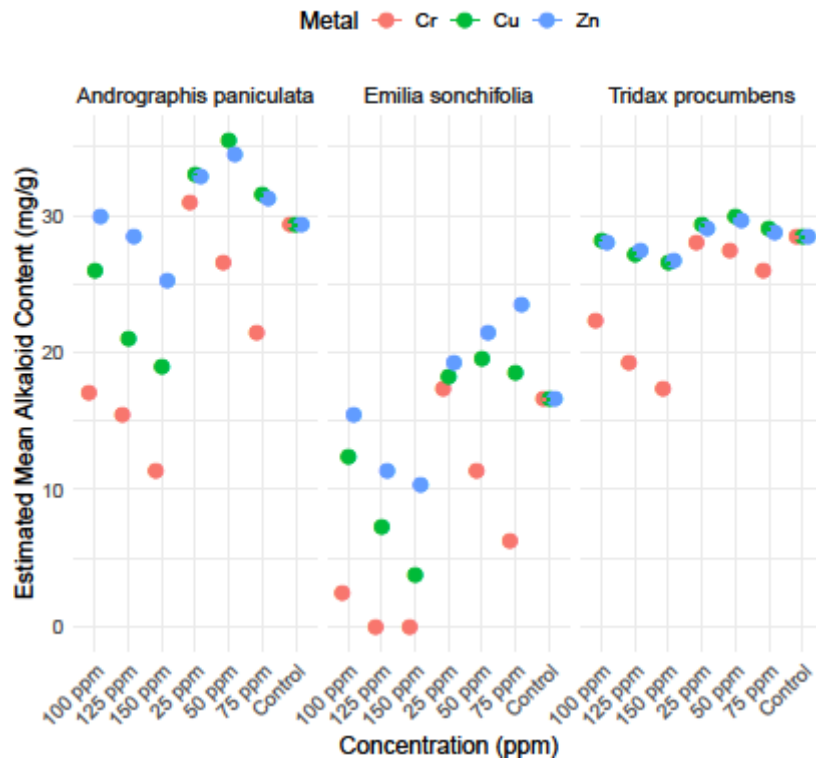


Fig. 28 Holm Post Hoc Test

The Holm post hoc multiple comparisons, as depicted in the graph, clearly demonstrate a significant interaction effect of metal type (Cr, Cu, Zn), concentration (0–75 ppm), and plant species (*Andrographis paniculata*, *Emilia sonchifolia*, *Tridax procumbens*) on alkaloid content. Overall, *A. paniculata* consistently exhibited the highest alkaloid levels across treatments, particularly at medium concentrations of Cu and Zn, suggesting a stimulatory effect at these levels. In contrast, *T. procumbens* and *E. sonchifolia* displayed more variable responses, with alkaloid content declining at higher metal concentrations, especially under Cr exposure, likely due to metal-induced stress. The Holm-adjusted multiple comparisons confirmed that the majority of these differences were highly significant, highlighting the species-specific and concentration-dependent regulation of alkaloid synthesis in response to metal treatments.

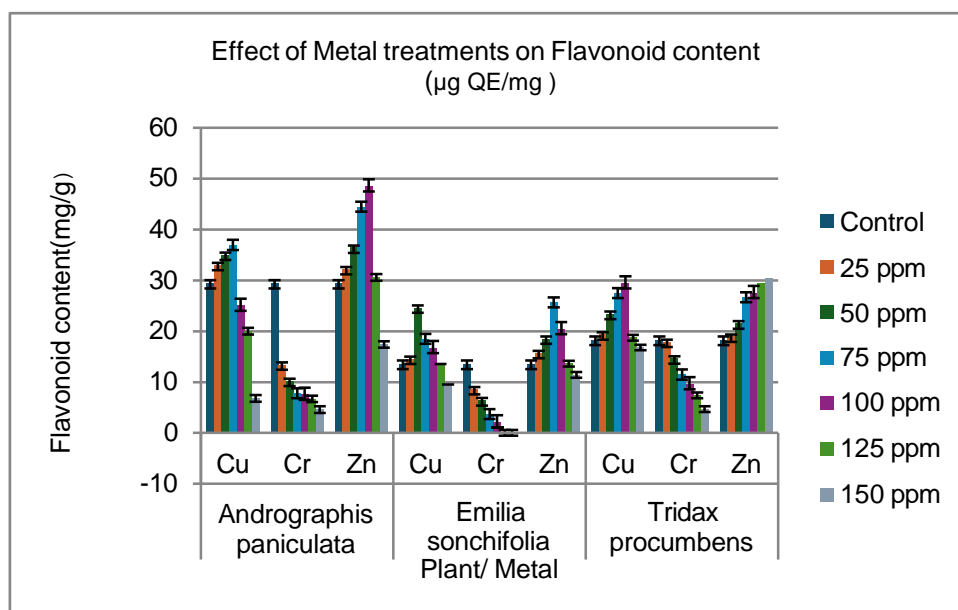


Fig. 29 Effect of Metal treatments on Flavonoid content

The results indicate that varying levels of heavy metals (Cu, Cr, Zn) significantly influenced flavonoid production in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. In *A. paniculata*, flavonoid content increased notably with Cu treatment (maximum 37.0 ± 0.4 ppm at 75 ppm) and reached its highest under Zn exposure at 100 ppm (48.5 ± 0.8 ppm). *Emilia sonchifolia* exhibited enhanced flavonoid levels in the presence of Cu (24.7 ± 0.3 ppm at 50 ppm), whereas Cr treatment caused a drastic reduction (0.0 ± 0.0 ppm at 125–150 ppm), indicating Cr-induced stress. In *T. procumbens*, flavonoid accumulation rose with Zn exposure (maximum 30.4 ± 0.2 ppm), while Cu and Cr also led to significant increases. Overall, these results reveal metal-specific and concentration-dependent effects, with Zn generally promoting flavonoid synthesis at higher concentrations, whereas Cr acted as a suppressor, reflecting species-specific adaptive strategies to heavy metal stress.

Table. 10 Three-Way ANOVA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Concentration	6	2520	420	14672	< 2e-16
Metal	2	8614	4307	150464	< 2e-16
Plant	2	4193	2096	73241	< 2e-16
Concentration × Metal	12	2710	226	7891	< 2e-16
Concentration × Plant	12	1179	98	3433	< 2e-16
Metal × Plant	4	773	193	6749	< 2e-16
Concentration × Metal × Plant	24	1454	61	2117	< 2e-16
Residuals	126	4	0		

A three-way ANOVA revealed that concentration, metal type, and plant species had significant main effects on flavonoid content ($p < 0.001$), and their three-way interaction (Concentration × Metal × Plant) was also highly significant. All two-way interactions (Concentration × Metal, Concentration × Plant, Metal × Plant) were likewise significant, indicating that the effect of one factor is dependent on the levels of the others. These results demonstrate a complex, species- and metal-specific response, where flavonoid production is influenced not only by the type and dose of heavy metal but also by the plant species. In general, Zn tended to enhance flavonoid accumulation, particularly at higher concentrations, while Cr strongly suppressed it, and Cu showed intermediate effects. This highlights the intricate regulation of flavonoid biosynthesis under heavy metal stress and the differential tolerance strategies of the three medicinal plants.

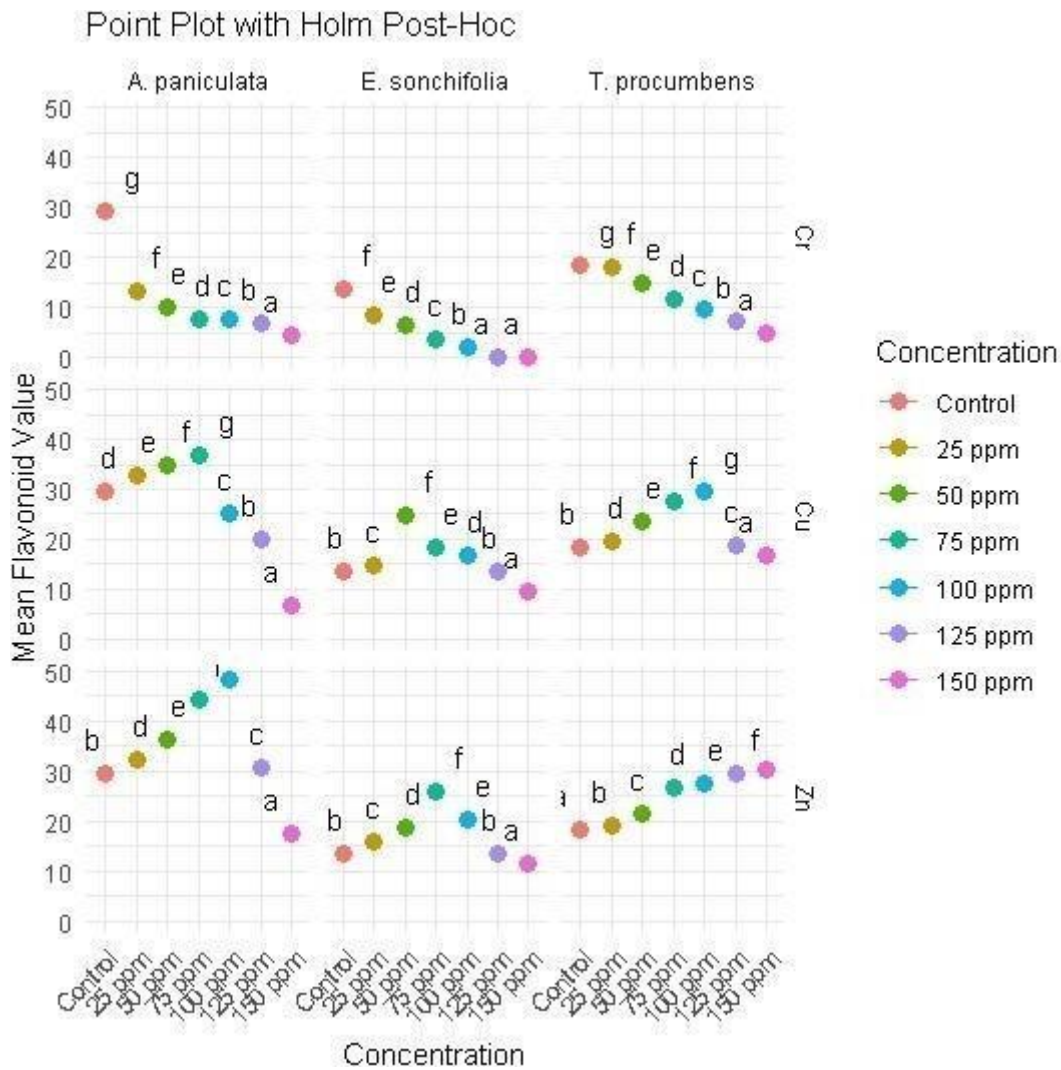


Fig. 30 Holm post hoc test

The Holm's post-hoc test results, illustrated in the point plot, indicate statistically significant differences in flavonoid content among the three medicinal plants (*Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*) across varying metal exposures. *A. paniculata* exhibited the highest flavonoid accumulation, particularly at moderate concentrations (~50–75 ppm), reflecting a strong stress-responsive enhancement under metal exposure. In contrast, *E. sonchifolia* showed the lowest flavonoid content, especially at higher metal concentrations, suggesting either species sensitivity or a pronounced inhibitory effect of metals on flavonoid biosynthesis. *T. procumbens* displayed moderate flavonoid levels, with an increase at higher concentrations, indicative of an adaptive tolerance mechanism. Holm-adjusted p-values, shown in the plot, confirm that these interspecies differences are statistically significant. Overall, the findings highlight species-specific metabolic responses and adaptive strategies under heavy metal stress.

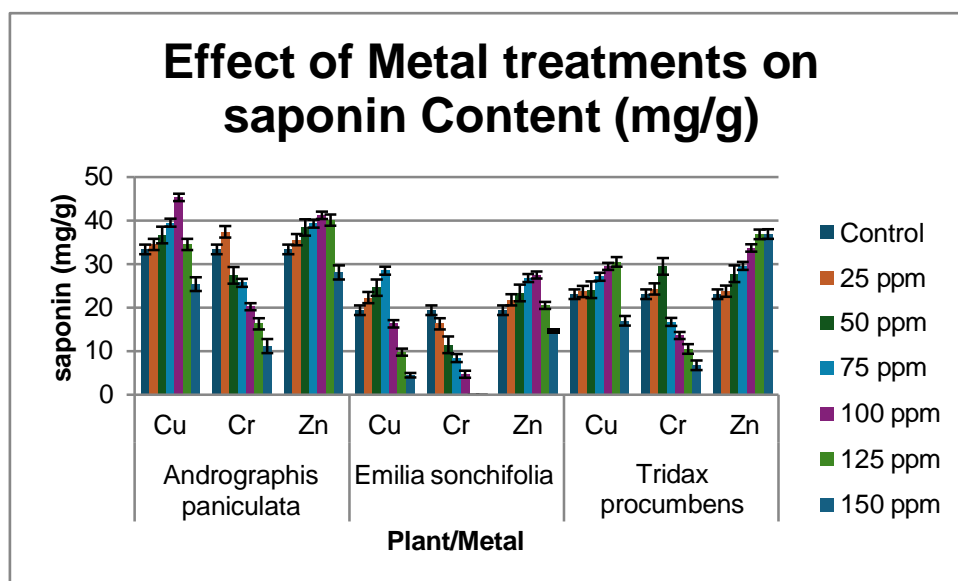


Fig. 31 Effect of Metal treatments on saponin Content

The saponin fractions of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* exhibited metal- and concentration-dependent responses. In *A. paniculata*, Zn treatment led to a maximum saponin accumulation of 41.2 mg/g at 100 ppm, while Cu enhanced saponin content to 45.3 mg/g at the same concentration. In contrast, Cr exposure caused a continuous decline beyond 25 ppm, reaching a minimum of 11.2 mg/g at 150 ppm. *E. sonchifolia* displayed a biphasic response to Cu and Zn: saponin content increased up to 75–100 ppm (28.5 and 27.5 mg/g, respectively), then sharply decreased. Cr exerted a strong inhibitory effect, with saponin levels dropping from 19.4 mg/g (control) to complete inhibition at 125–150 ppm. In *T. procumbens*, Zn consistently promoted saponin accumulation across all concentrations, achieving 36.9 mg/g at 150 ppm. Cu induced a gradual increase in saponin content up to 125 ppm (30.5 mg/g) before declining, whereas Cr caused a pronounced toxic effect, reducing levels from 23.1 mg/g to 6.8 mg/g at 150 ppm. Collectively, these results indicate that Zn generally stimulates saponin biosynthesis, Cr strongly inhibits it, and Cu has a stimulatory effect at low concentrations but becomes inhibitory at higher levels, with species-specific differences in tolerance and metabolic responses.

Statistical Analysis

Table 11 Three-Way ANOVA

Source of Variation	Df	Sum of Squares	Mean Square	F value	Pr(>F)	Significance
Species	2	8089	4045	5878.40	< 2e-16	***
Metal	2	5350	2675	3888.11	< 2e-16	***
Concentration	6	2583	430	625.64	< 2e-16	***
Species × Metal	4	131	33	47.44	< 2e-16	***
Species × Concentration	12	863	72	104.53	< 2e-16	***
Metal × Concentration	12	3020	252	365.76	< 2e-16	***
Species × Metal × Concentration	24	978	41	59.22	< 2e-16	***
Residuals	126	87	0.69			

The three-way ANOVA showed that species ($F(2,126)=5878.40$, p99% of the variance (residual SS=87 total SS=16021)).

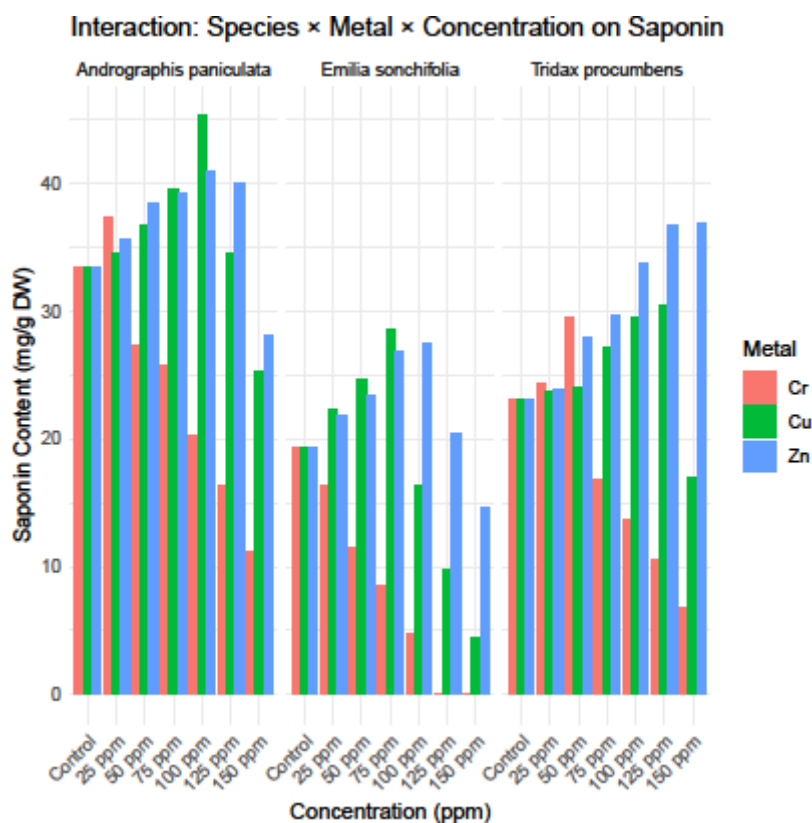


Fig. 32 Holm Post Hoc Test

The study demonstrates that saponin accumulation is significantly influenced by species identity, type of metal, and metal concentration ($p < 0.001$). Saponin content varied markedly among *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*, with Zn generally enhancing biosynthesis, Cr causing strong inhibition, and Cu showing a stimulatory effect at lower concentrations but a reduction at higher doses (>100 ppm), indicating potential metabolic perturbation. These findings highlight the species-specific nature of saponin regulation under metal exposure and underscore the potential for developing metal-based elicitation strategies tailored to individual Asteraceae species to optimize saponin yield while minimizing phytotoxic effects.

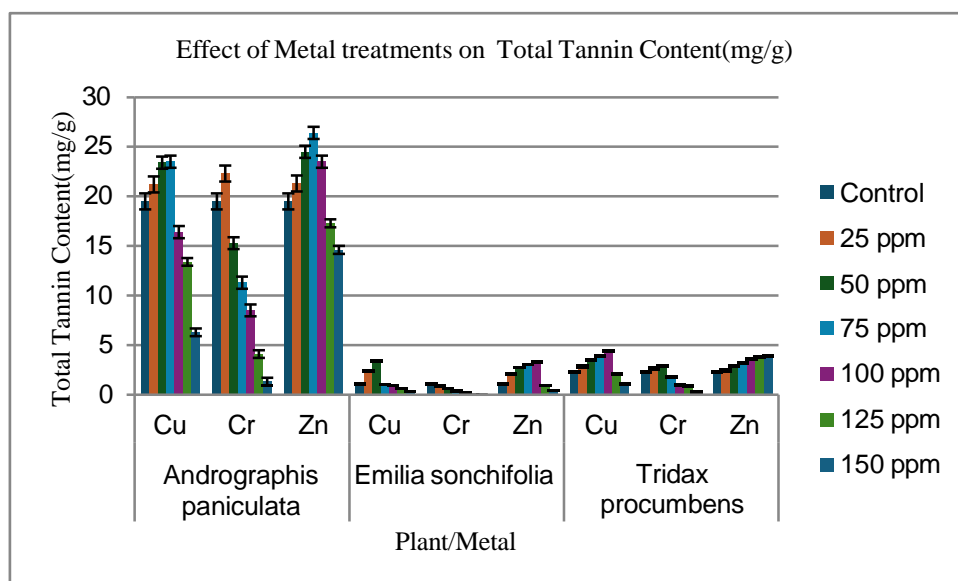


Fig. 33 Effect of Metal treatments on Total Tannin Content (mg/g)

The tannin content of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* was significantly influenced by copper (Cu), chromium (Cr), and zinc (Zn) across concentrations of 25–150 ppm. *A. paniculata* exhibited the highest baseline tannin level (19.5 mg/g) among the three species, which increased under low Cu and Zn treatments (23.5 and 26.4 mg/g at 75 ppm, respectively), suggesting a stimulatory effect at moderate concentrations. However, higher metal concentrations caused a marked decline, particularly under Cr stress, where tannin content dropped to 1.33 mg/g at 150 ppm, highlighting the inhibitory effect of chromium on tannin biosynthesis.

In contrast, *E. sonchifolia* displayed consistently lower tannin levels across all treatments and experienced complete depletion under high Cr and Cu stress, indicating a high sensitivity and limited capacity to adjust secondary metabolite accumulation under metal stress. *T. procumbens* maintained moderate tannin content, with slight enhancement under Zn treatment up to 3.9 mg/g at 150 ppm, demonstrating better tolerance, but also showed decreases under Cr and Cu stress, reaching 0.31 mg/g at 150 ppm Cr.

Overall, these results indicate species-specific responses to heavy metal exposure: *A. paniculata* shows dynamic regulation of tannin synthesis, particularly in response to Zn, *E. sonchifolia* is highly sensitive, and *T. procumbens* maintains relatively stable tannin levels. The interaction between species, metal type, and concentration underscores the complex regulation of tannin metabolism under heavy metal stress.

Statistical Analysis

Table 12 Three-Way ANOVA

Source of Variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	2	9364	4682	71871.6	< 2e-16 ***
Metal	2	539	270	4139.8	< 2e-16 ***
Concentration	6	753	126	1926.8	< 2e-16 ***
Species × Metal	4	430	108	1651.7	< 2e-16 ***
Species × Concentration	12	884	74	1130.3	< 2e-16 ***
Metal × Concentration	12	269	22	344.4	< 2e-16 ***
Species × Metal × Concentration	24	287	12	183.8	< 2e-16 ***
Residuals	126	8	0		

Three-way ANOVA revealed that Species, Metal type, and Metal Concentration all had highly significant effects on tannin content ($p < 0.001$). Additionally, all two-way interactions (Species × Metal, Species × Concentration, Metal × Concentration) and the three-way interaction (Species × Metal × Concentration) were highly significant ($p < 0.001$), indicating that tannin accumulation depends on the specific combination of plant species, metal type, and concentration. Among the main effects, species contributed the most variation ($F = 71,871.6$), highlighting the dominant role of plant genotype in regulating tannin biosynthesis under heavy metal stress. The high F-values for all main and interaction terms reflect the complex interplay between environmental factors and intrinsic plant biology in modulating secondary metabolite production in medicinal plants.

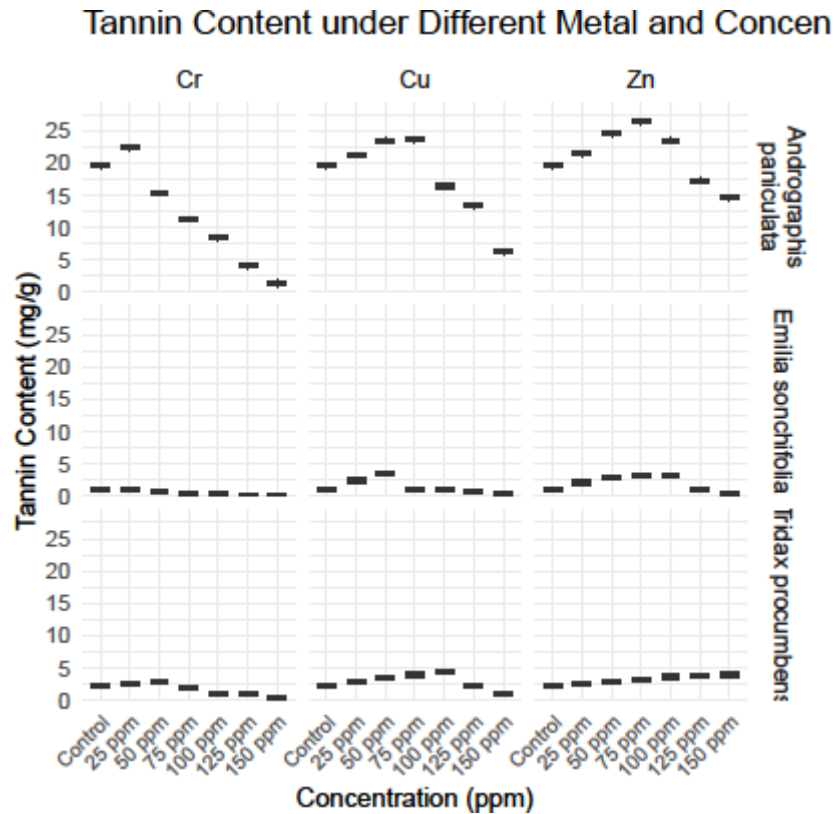


Fig. 34 Holm's Post Hoc Test

Post hoc comparisons using Holm's adjustment demonstrated that tannin content exhibits a **metal- and species-specific response**. The largest decreases were observed in *Andrographis paniculata*, where tannin levels dropped significantly under **Cu and Cr treatments**, with a smaller decrease under Zn exposure. In *Emilia sonchifolia*, tannin concentration was consistently low and declined steadily across all treatments, indicating a limited metabolic adjustment. *Tridax procumbens* maintained low tannin levels that showed minimal change even after metal treatments, suggesting a relatively stable but low tannin metabolic response. The marked reduction in *A. paniculata* under Cu and Cr stress likely reflects inhibition or enhanced degradation of tannins due to oxidative stress or metal toxicity. These findings corroborate the ANOVA results, highlighting significant interaction effects, and clearly identify *Andrographis paniculata* as the most sensitive and responsive species in terms of tannin modulation under heavy metal exposure.

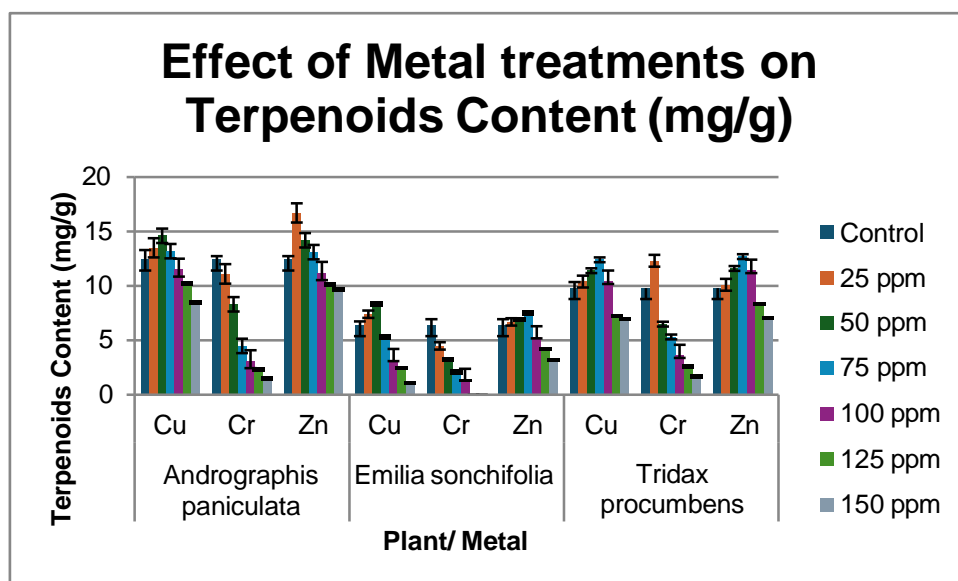


Fig . 35 Effect of Metal treatments on Terpenoids Content

Concentration-dependent effects of **Cu, Cr, and Zn** on terpenoid content were observed in all three plants. In *Andrographis paniculata*, Cu at 50 ppm increased terpenoid levels (14.6 mg/g versus 12.4 mg/g in control), whereas Cr caused a dose-dependent decrease, reaching 1.5 mg/g at 150 ppm. Zn treatment produced maximum stimulation at 25 ppm (16.7 mg/g). In *Emilia sonchifolia*, a similar trend was noted: Cu at 50 ppm enhanced terpenoids (8.3 mg/g compared with 6.4 mg/g in control), while Cr completely suppressed terpenoid synthesis at concentrations ≥ 125 ppm. *Tridax procumbens* showed the strongest response to Zn (12.7 mg/g at 75 ppm), whereas its response to Cu and Cr was biphasic, with initial increases (Cu: 12.4 mg/g at 75 ppm; Cr: 12.3 mg/g at 25 ppm) followed by decreases at higher concentrations. Overall, low-to-moderate levels of Cu and Zn (25–75 ppm) stimulated terpenoid synthesis, whereas Cr consistently inhibited it, establishing a toxicity rank order of **Cr > Cu > Zn**.

Statistical Analysis

Table 13 Three-Way ANOVA

Source of Variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	2	1147.5	573.8	14,61,444	<2e-16
Metal	2	784.3	392.2	9,98,902	<2e-16
Concentration	6	842.7	140.5	3,57,763	<2e-16
Species × Metal	4	77.8	19.5	49,548	<2e-16
Species × Concentration	12	46.9	3.9	9,961	<2e-16
Metal × Concentration	12	239.3	19.9	50,800	<2e-16
Species × Metal × Concentration	24	98.9	4.1	10,497	<2e-16
Residuals	126	0	0		

A three-way ANOVA revealed strong main effects of Species, Metal, and Concentration on terpenoid content (all $p < 0.001$). In addition, all interaction terms Species × Metal, Species × Concentration, Metal × Concentration, and Species × Metal × Concentration were highly significant ($p < 0.001$), indicating that terpenoid accumulation depends not only on individual factors but also on complex interactions among species, metal type, and concentration. The high F-values for both main effects and interactions (Table 3) highlight the substantial influence of these variables on terpenoid biosynthesis and accumulation in the medicinal plants studied.

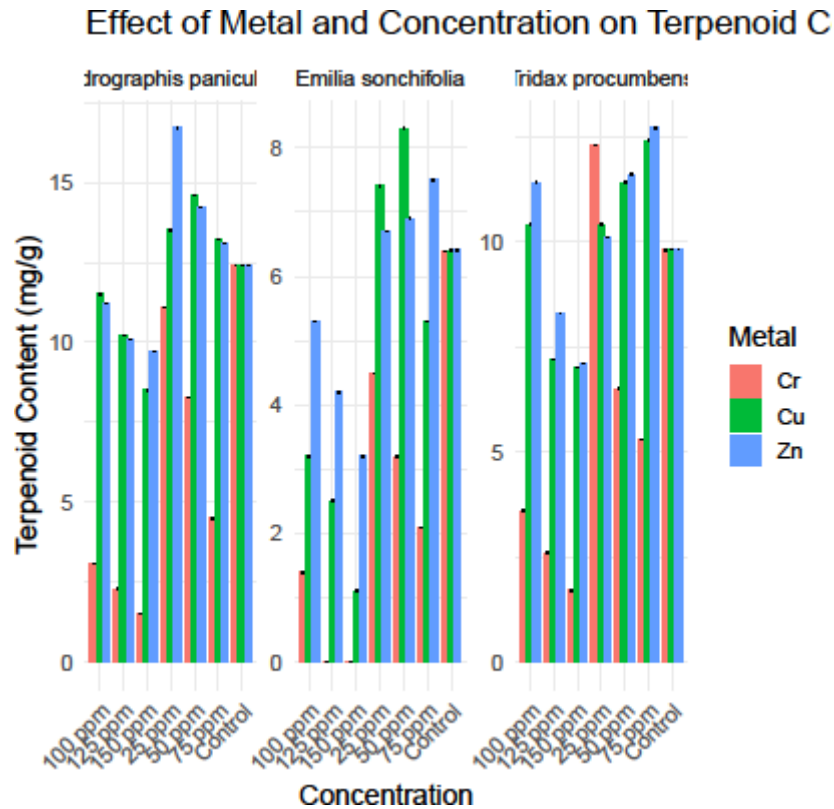


Fig. 36 Holm's Post Hoc Test

The Holm post hoc test revealed that terpenoid content in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* varied significantly across different concentrations of heavy metals (Cu, Cr, and Zn). Overall, **A. paniculata** exhibited the highest terpenoid accumulation, with a pronounced peak under Cu treatment at 50–75 ppm. In contrast, **E. sonchifolia** and **T. procumbens** showed lower total terpenoid contents, with steeper declines at higher metal concentrations, particularly under Cr stress. The observed differences between concentrations and metal types, visually represented by spacing and error bars in the plots, are supported by Holm-adjusted pairwise comparisons. These findings confirm that terpenoid biosynthesis is strongly influenced by species identity, metal type, and concentration, highlighting a species-specific metabolic response to heavy metal stress.

8.5 DISCUSSION

Species- and Metal-Specific Metabolic Responses in Asteraceae Medicinal Plants

The present study investigated the quantitative effects of copper (Cu), zinc (Zn), and chromium (Cr) on the metabolism of carbohydrates, proteins, and lipids in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. Across all species, low levels of Cu and Zn initially stimulated the accumulation of these primary metabolites, whereas higher concentrations caused sharp declines. Cr consistently exhibited the strongest inhibitory effect. Such trends align with the dual role of Cu and Zn as essential micronutrients at low levels but toxicants in excess (Xu et al., 2024; Emamverdian et al., 2015). For instance, in *A. paniculata*, carbohydrate and lipid levels increased at 25–50 ppm Cu and Zn but decreased steeply at 150 ppm, indicating a threshold for metabolic disruption. Comparable observations in wheat seedlings exposed to Cu and Cr support this concentration-dependent stimulation and inhibition of protein and proline synthesis (Muslu & Ergün, 2013).

Among the species, *E. sonchifolia* was the most sensitive to heavy metal stress, particularly Cr, which completely depleted carbohydrates, proteins, and lipids at ≥ 125 ppm. These results corroborate findings that toxic metals, such as Cd and Cr, disrupt nutrient uptake, induce oxidative stress, and depress metabolic pathways (Sharma et al., 2020; Paul et al., 2025). Conversely, *T. procumbens* displayed remarkable tolerance, maintaining high carbohydrate (161 mg/g at 75 ppm) and protein (140.14 mg/g at 75 ppm) levels under Zn exposure, likely through efficient detoxification mechanisms including chelation, antioxidant defense, or compartmentalization (Ejaz et al., 2023; Emamverdian et al., 2015). Protein metabolism appeared particularly sensitive, with *E. sonchifolia* exhibiting complete depletion under high Cr concentrations, whereas *T. procumbens* retained higher protein levels, highlighting species-specific tolerance thresholds. Lipid metabolism followed a similar trend, showing slight stimulation under low Cu and Zn concentrations but severe inhibition under increasing metal stress, particularly Cr, consistent with metal-induced lipid peroxidation and membrane destabilization (Sharma et al., 2020; Paul et al., 2025). Overall, Cr emerged as the most toxic metal, while *T. procumbens* demonstrated the highest resilience, indicating its potential for phytoremediation and growth in metal-contaminated soils.

Secondary metabolite patterns exhibited a clear, metal- and dose-dependent hormetic response, where mild exposure often stimulated biosynthesis, whereas severe treatment

suppressed it sometimes with fatal consequences, particularly in *E. sonchifolia*. Low-level metal inputs may act as abiotic elicitors, transiently stimulating ROS and defense-related pathways such as phenylpropanoid and MEP/MVA pathways, whereas high concentrations disrupt redox homeostasis and metabolism (Morkunas et al., 2018; Vicidomini et al., 2024).

Zn supplementation generally enhanced secondary metabolite production, with moderate doses promoting maximum alkaloid accumulation, increased flavonoid and saponin levels, and terpenoid responsiveness. These findings are consistent with previous studies in *Artemisia annua* and *Dendrobium nobile*, where Zn improved phenolics, flavonoids, and other medicinal compounds (Balafrej et al., 2020; Fan et al., 2021). Similarly, nano-ZnO has been shown to upregulate biosynthetic genes and antioxidant enzymes, supporting the dose-dependent enhancements observed in our hydroponic experiments (Rashidi Asl et al., 2020). Cu exhibited a biphasic response: moderate levels (25–75 ppm) stimulated metabolite synthesis, whereas higher concentrations caused oxidative stress and metabolic inhibition, in agreement with prior reports (Asiminicesei et al., 2024). In contrast, Cr produced strong, dose-dependent suppression of metabolites, including total depletion of alkaloids and saponins in *E. sonchifolia*, reflecting the well-documented phytotoxicity of Cr(VI) (Morkunas et al., 2018; Asiminicesei et al., 2024).

Species-specific responses were evident: *T. procumbens* was overall the most resilient, maintaining flavonoid and alkaloid-saponin yields under Zn stress; *A. paniculata* showed high but plastic metabolite outputs peaking at intermediate exposures; *E. sonchifolia* was highly sensitive to Cr. These patterns reflect genotype-specific thresholds for —beneficial stress— versus toxicity and the amplitude of secondary metabolite responses (Balafrej et al., 2020; Vicidomini et al., 2024).

Mechanistically, elicited metabolite responses likely involve metal-induced ROS signaling and transcriptional activation of biosynthetic genes at low concentrations, whereas high doses induce oxidative stress, enzyme inhibition, and metabolic breakdown (Vicidomini et al., 2024; Fan et al., 2021). Collectively, our findings suggest that moderate Cu and Zn exposures can serve as elicitors to enrich phytochemicals, whereas Cr represents a major risk of metabolic suppression. These insights are especially relevant for hydroponic cultivation and elicitation strategies, where careful dosing within the hormetic range is essential.

CHAPTER 9

DIFFERENTIAL EFFECTS OF COPPER, CHROMIUM, AND ZINC ON CHLOROPHYLL A AND B IN THREE MEDICINAL PLANTS”

9.1 INTRODUCTION

Chlorophyll, the central pigment affording light absorption in photosynthesis, was more sensitive to the stress of heavy metals. Chlorophyll a (Chl a) and chlorophyll b (Chl b) play alternate roles in light-harvesting by channeling the energy toward photochemical reactions and the Chl a/b ratio represents an important indication of the activity and state of the photosynthetic apparatus. Chlorophyll biosynthesis is often impaired, pigment degradation is enhanced and the ultrastructure of chloroplasts is disturbed under metal stress, resulting in ecologically relevant decreased photosynthesis rates and, as a consequence, in the diminished productivity of a plant.

The objective of the present study, therefore, is to investigate the effects of different levels of Cu, Cr and Zn on three medicinally important plants such as *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens* on chlorophyll profiles viz., chlorophyll a, chlorophyll b, a/b ratio, total chlorophyll content. These plants have known pharmacological effects, and determining how their photosynthetic machinery is affected by stress from heavy metal contamination may shed light on their ability to respond and on any impacts to their medicinal value in polluted areas.

9.2 REVIEW OF LITERATURE

Copper is a micronutrient required for different enzymatic processes, although its high concentration in plant tissues is known to have a negative impact on chlorophyll synthesis and photosynthesis (Shakya et al., 2008). The reduction of chlorophyll-a, chlorophyll-b and total chlorophyll content of the mosses and leafy liverworts by copper was significantly severe (Shakya et al., 2008). Ultrastructural changes in chloroplasts that occur in response to excess copper, such as a decrease of thylakoid membranes, have been reported (Quartacci et al., 2000). An increased amount of copper may also interfere with the biosynthesis of the photosynthetic apparatus, and affect the photosynthetic membrane pigments and proteins (Elefteriou & Karataglis, 1989). Chromium increases the generation of reactive oxygen species in plants, leading to significant damage of macromolecules like membranes, lipids, and proteins due to impaired function (Cheng et al., 2024). Although zinc tends to accumulate in plant roots, its role in the development of stem, leaf, and subcellular structures is essential for normal plant growth. An overabundance of zinc may be toxic to plants in that it may interfere

with processes such as growth and development, including seed germination, root lengthening, stem elongation, chlorophyll production, and may even cause plant death. These detrimental zinc effects have been associated to changes in enzyme activities, cytostructural damage and nitrogen metabolism disturbances induced by zinc (Blasco et al., 2019).

9.3 MATERIALS AND METHODS

Collection of Plant Samples

Leaves were harvested from *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* after 30 days of exposure to different heavy metal treatments. For statistical reliability, at least three independent samples were collected from each treatment group and species.

Chlorophyll Analysis

Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll content were determined following the method of Arnon (1949). Excised leaves were homogenized in 10 mL of 80% acetone and incubated in darkness at room temperature for 24 hours. The chlorophyll concentrations were measured spectrophotometrically at 645 nm and 663 nm and calculated using the following formulas:

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

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

$$\text{Total chlorophyll (mg/g)} = \text{Chl a} + \text{Chl b}$$

The chlorophyll a/b ratio was also calculated by dividing Chl a by Chl b content.

Statistical Analysis

Data were analyzed using two-way ANOVA to assess the effects of different metal concentrations and plant species on chlorophyll content. Significant differences between treatment means were evaluated at $p < 0.05$.

9.4 RESULTS

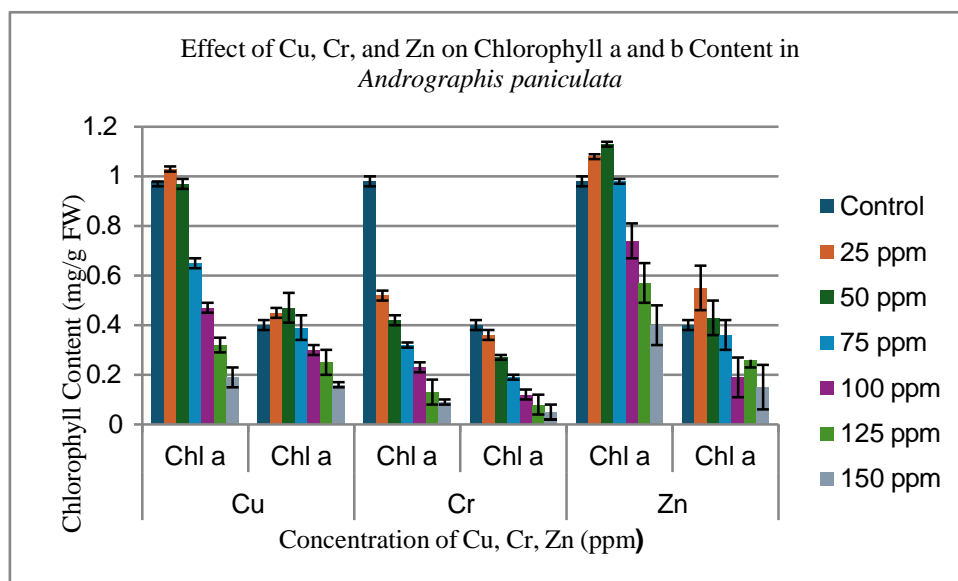


Fig. 37 Effect of Cu, Cr, and Zn on Chlorophyll a and b Content in *Andrographis paniculata*

Chlorophyll a

In the control group, chlorophyll a (Chl a) content was 0.98 ± 0.02 mg/g. With increasing concentrations of heavy metals, Chl a content exhibited a clear dose-dependent decline. At 150 ppm, the lowest values were observed: 0.19 ± 0.04 mg/g for chromium (Cr), 0.09 ± 0.01 mg/g for copper (Cu), and 0.32 ± 0.25 mg/g for zinc (Zn). These results indicate that Cr and Cu exerted the strongest inhibitory effects on Chl a accumulation.

Chlorophyll b

A similar trend was observed for chlorophyll b (Chl b), with reductions most pronounced at 150 ppm. The Chl b content decreased to 0.16 ± 0.01 mg/g (Cr), 0.05 ± 0.03 mg/g (Cu), and 0.15 ± 0.09 mg/g (Zn). Again, Cr and Cu demonstrated more substantial negative impacts on Chl b compared to Zn, highlighting their greater toxicity toward the photosynthetic pigment system.

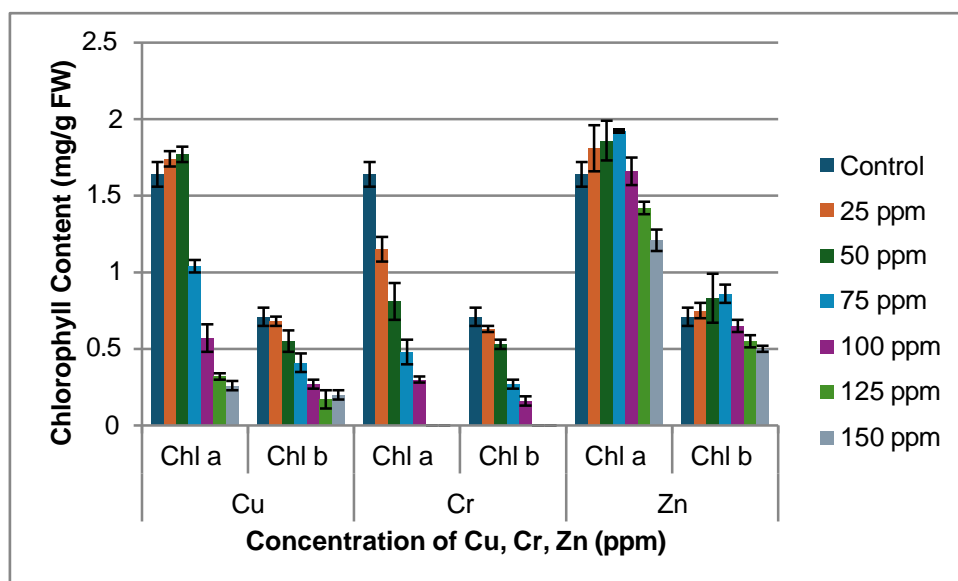


Fig . 38 Effect of Cu, Cr, and Zn on Chlorophyll a and b Content in *Emilia sonchifolia*

The chlorophyll a (Chl a) content of *Emilia sonchifolia* declined with increasing concentrations of heavy metals, with the most pronounced effect observed under chromium (Cr) treatment. The plants did not survive at 125 and 150 ppm Cr, and at 100 ppm, Chl a content was 0.30 ± 0.06 mg/g. In contrast, zinc (Zn) treatments up to 75 ppm caused an increase in Chl a, exceeding the control levels. Copper (Cu) at 25 and 50 ppm also stimulated Chl a significantly, but higher concentrations led to a gradual decline. A similar pattern was observed for chlorophyll b (Chl b), with reductions being more severe at higher metal concentrations. At 150 ppm Zn, Chl b reached 0.49 ± 0.07 mg/g, showing a less pronounced decrease compared to Cr and Cu treatments, indicating that *E. sonchifolia* is more sensitive to Cr and Cu than to Zn under the experimental conditions.

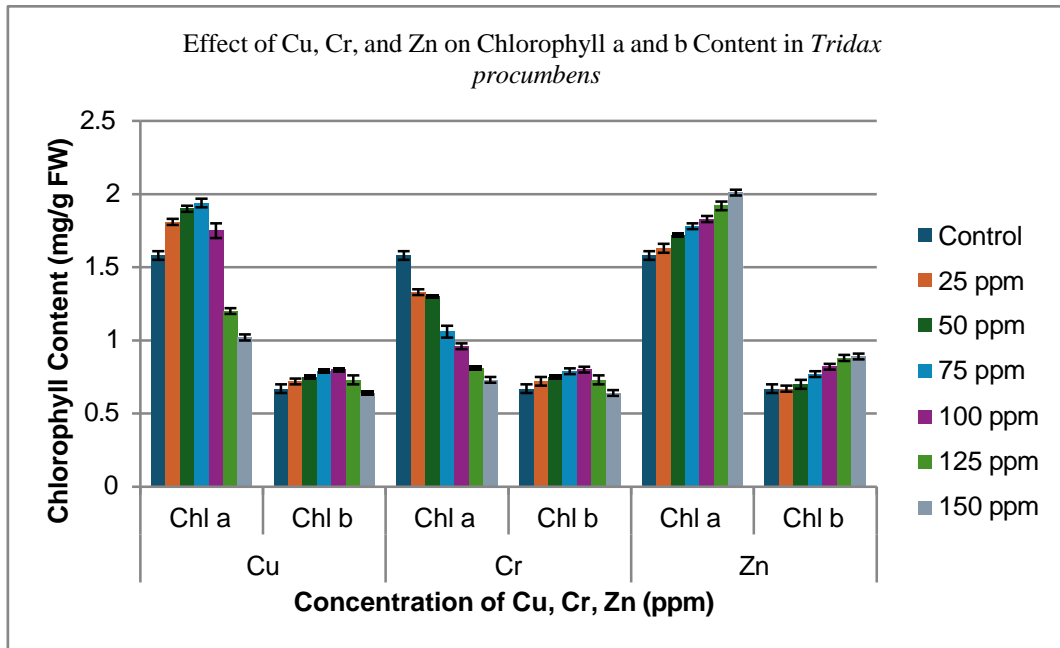


Fig. 39 Effect of Cu, Cr, and Zn on Chlorophyll a and b Content in *Tridax procumbens*

In *Tridax procumbens*, chlorophyll content exhibited a concentration-dependent response to Cu, Cr, and Zn treatments. At lower concentrations (25–100 ppm), both Chl a and Chl b increased, particularly under Cu and Zn, indicating a stimulatory effect at moderate metal levels. For example, at 75 ppm, Chl a reached 1.94 ± 0.02 mg/g under Cu, while Chl b was 0.80 ± 0.01 mg/g in control plants. However, at higher concentrations (125–150 ppm), chlorophyll levels decreased significantly, especially under Cr exposure. At 150 ppm, Chl a declined to 1.02 ± 0.02 mg/g (Cu) and 0.73 ± 0.02 mg/g (Cr), while Chl b decreased to 0.64 ± 0.01 mg/g (Cu) and 0.64 ± 0.02 mg/g (Cr). In contrast, Zn treatments consistently maintained or increased both Chl a and Chl b across all concentrations, suggesting that *T. procumbens* is relatively tolerant to Zn stress, whereas higher levels of Cu and Cr induce oxidative stress that impairs chlorophyll synthesis and stability.

Total Chlorophyll (Chl a + Chl b) Under Heavy Metal Stress

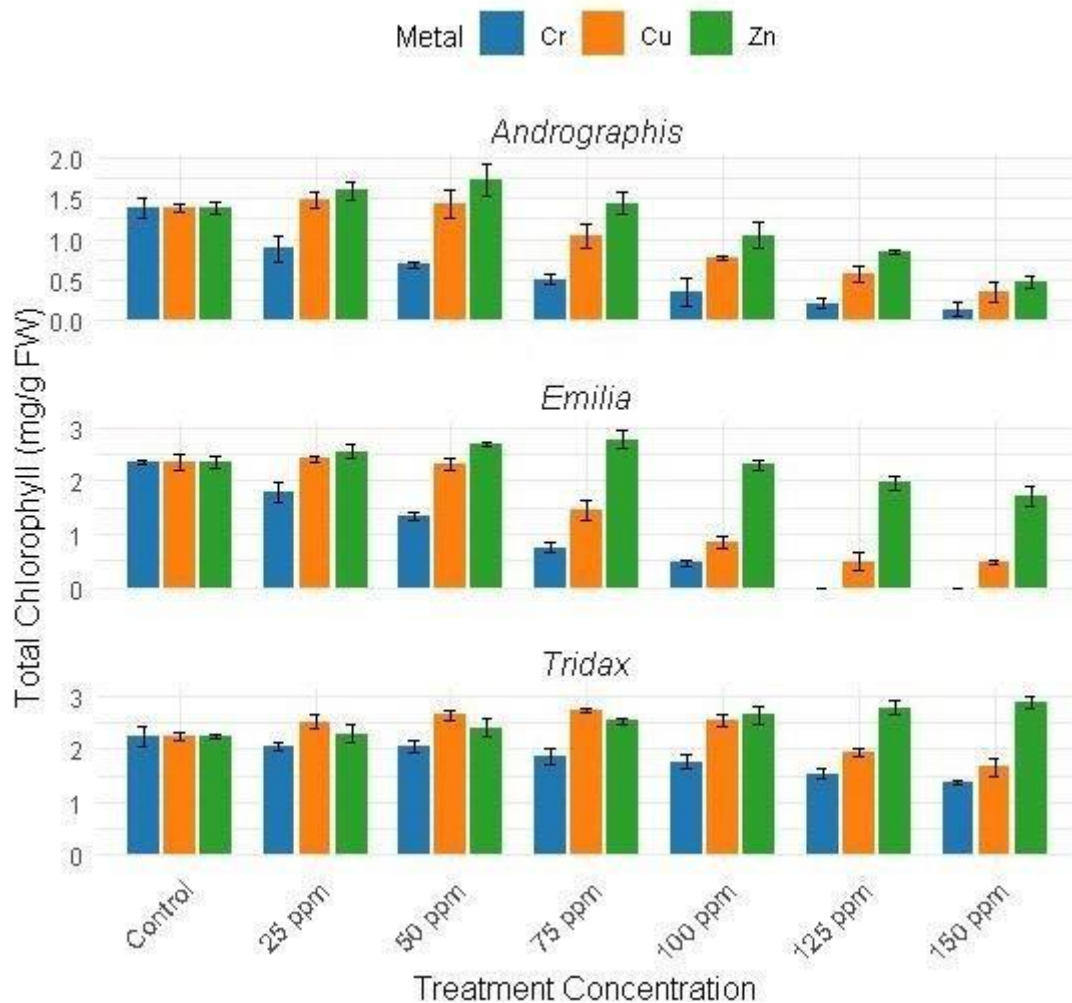


Fig. 40 Chlorophyll a+b under metal stress

Among the three medicinal plants, *Tridax procumbens* exhibited the highest tolerance to all three heavy metals, maintaining relatively high chlorophyll content across treatments, particularly under Zn and Cu exposure. In contrast, *Emilia sonchifolia* and *Andrographis paniculata* were more sensitive, showing marked decreases in chlorophyll even at lower concentrations of Cr and Cu, indicating strong adverse effects of these metals. Chromium was the most deleterious across all species, causing substantial chlorophyll loss even at moderate levels and nearly complete depletion at 150 ppm. Interestingly, low concentrations (25 ppm) of Cu and Zn transiently stimulated chlorophyll synthesis in *A. paniculata* and *E. sonchifolia*, consistent with reports that trace metals can act as essential micronutrients. However, this stimulatory effect was short-lived, as toxicity manifested at higher doses. The observed variations in chlorophyll content likely reflect multiple mechanisms, including

inhibition of chlorophyll biosynthesis, disruption of the photosynthetic apparatus, and direct damage to chloroplast structures, all potentially mediated by oxidative stress. The steep declines at elevated metal concentrations suggest impaired photosynthetic efficiency and reduced plant growth and productivity.

Chlorophyll a/b Ratio Under Heavy Metal Stress

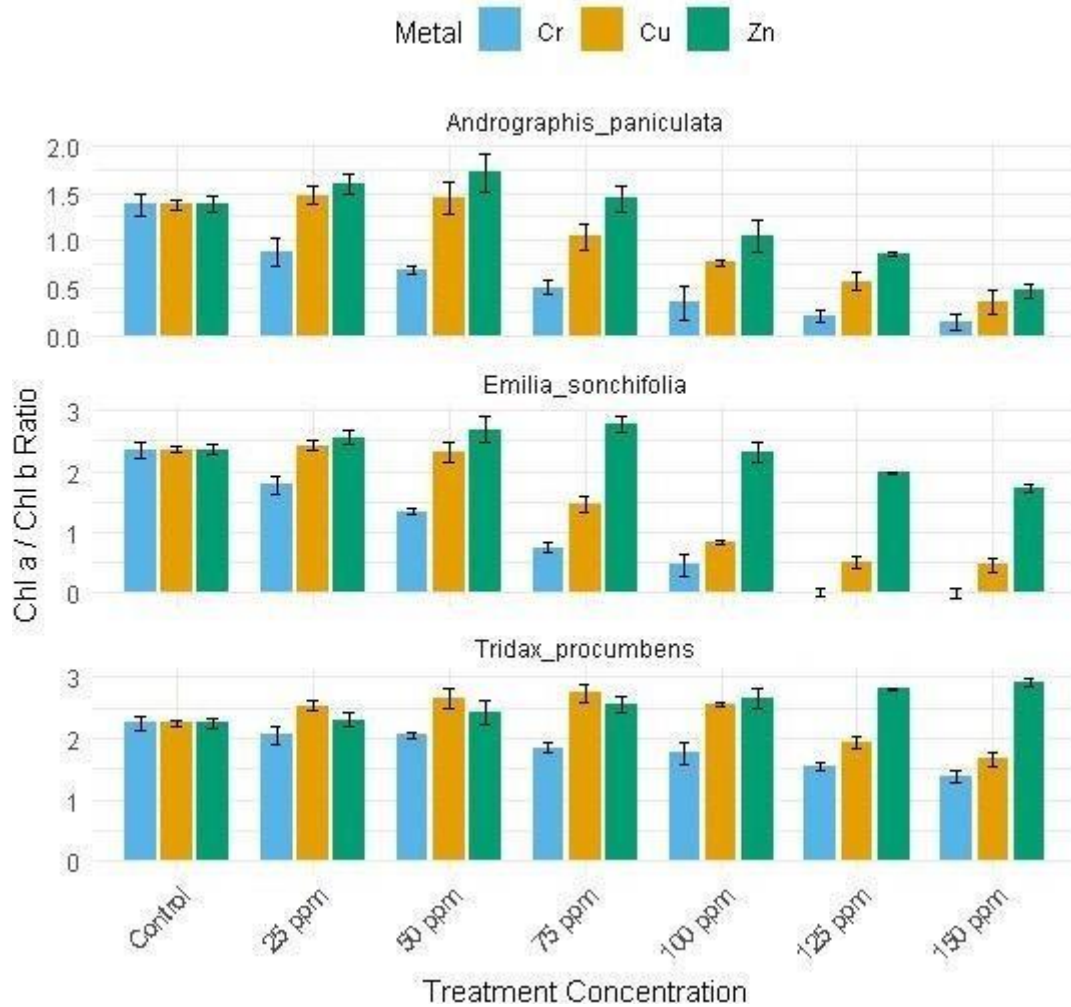


Fig. 41 Chlorophyll a/b

This study investigated the influence of Cu, Cr, and Zn on the chlorophyll a/b ratio in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. Overall, increasing concentrations of heavy metals caused a reduction in the chlorophyll a/b ratio, indicating disturbances in chlorophyll synthesis, although the responses were species- and metal-specific. In *A. paniculata*, both Cu and Cr treatments significantly decreased the chlorophyll a/b ratio, with the reduction being more pronounced under Cu, particularly at higher concentrations. Zn, however, exhibited a moderate effect, slightly increasing the ratio at 100 ppm before decreasing at higher levels, suggesting a temporary protective influence of Zn on chlorophyll balance. A similar trend was observed in *E. sonchifolia*, where Cr caused a marked inhibition of the chlorophyll a/b ratio even at lower concentrations, with higher concentrations leading to extreme toxicity, as evidenced by missing values at 150 ppm. In

contrast, *T. procumbens* showed relatively minor changes in the chlorophyll a/b ratio, with Cu and Zn treatments maintaining or slightly increasing the ratio at certain concentrations. While Cr still exerted a negative effect, it was less severe than in the other two species, highlighting the higher stress tolerance of *T. procumbens*. Collectively, these findings indicate that Cr poses the greatest risk for disrupting chlorophyll balance, whereas Zn is the least destructive. The metal stress tolerance index (MSTI) was highest in *T. procumbens*, identifying it as the most tolerant species, and lowest in *A. paniculata* and *E. sonchifolia*, which were most sensitive, especially to Cu and Cr. These results underscore the distinctive impacts of heavy metals on photosynthetic processes, leaf growth, and potentially the medicinal quality of these plants.

Statistical Analysis

Table: 14 ANOVA Results for Chl a and Chl b in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

Source	df	F	p-value
Species	2	2961.53	<0.001
Metal concentration	6	547.71	<0.001
Chlorophyll type	1	8567.16	<0.001
Metal type (Cu/Cr/Zn)	2	1862.06	<0.001
Interactions (all)	–	Significant	<0.001

The two-way ANOVA revealed that chlorophyll a (Chl a) and chlorophyll b (Chl b) content in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* were significantly affected by plant species, metal type (Cu, Cr, Zn), and metal concentration ($p < 0.001$). Species identity played a major role, with *T. procumbens* maintaining higher chlorophyll levels across treatments, while *E. sonchifolia* and *A. paniculata* were more sensitive, particularly to Cr and Cu. Metal type also had a pronounced effect: Cr caused the most severe reductions in Chl a and Chl b, Cu showed intermediate inhibition, and Zn had the least negative impact, occasionally stimulating chlorophyll synthesis at low concentrations. Increasing metal concentration generally led to a dose-dependent decrease in chlorophyll, although low levels of Cu and Zn sometimes promoted Chl accumulation. Significant interactions between species, metal type, and concentration indicate that chlorophyll responses are highly species-specific and metal-specific, with certain combinations exacerbating toxicity or allowing partial tolerance. Overall, these results highlight the differential sensitivity of photosynthetic pigments to heavy metal stress and the superior tolerance of *T. procumbens* relative to the other species.

9.5 DISCUSSION

The present study demonstrates that heavy metals (Cu, Cr, and Zn) significantly alter leaf chlorophyll content in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*, with clear species-specific and metal-dependent effects. A four-way ANOVA revealed highly significant differences ($p < 0.001$) across species, metal type, concentration, and exposure time, highlighting both main effects and interactions. *Tridax procumbens* consistently maintained higher chlorophyll levels across treatments, suggesting strong tolerance, whereas *Andrographis paniculata* and *Emilia sonchifolia* were more sensitive, particularly under Cr and Cu stress. These results indicate that *T. procumbens* could serve as a potential phytostabilizer in metal-contaminated soils, while *A. paniculata* may require protective measures in polluted environments. To further elucidate the underlying mechanisms of these responses, measurements of non-photochemical quenching and antioxidant enzyme activities are recommended.

CHAPTER 10

ANTIOXIDANT RESPONSES OF MEDICINAL PLANTS TO HEAVY METAL STRESS: INSIGHTS FROM SOD AND DPPH ASSAYS

10.1 INTRODUCTION

Environmental contamination, especially soil and water contamination, with heavy metals (Cu, Cr, Zn) is a serious global problem that is particularly prevalent in places being influenced by industrial and agricultural cycle. These metals have a reputation for the tendency to accumulate in the ecosystems with related toxic effects on plant growth, development, and physiological processes. Abiotic stress mediated heavy metal stress triggers excess generation of reactive oxygen species (ROS) that oxidizes cellular components including lipids, proteins and nucleic acids leading to decline in plant health and productivity.

Plants have developed elaborate antioxidant defense systems to neutralize the harmful impacts of ROS when plants are exposed to stress. These defence systems include the enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as well as non-enzymatic antioxidants such as flavonoids, phenolics, and ascorbic acid. These antioxidants are crucial for cellular homeostasis and for protecting cells against oxidative damage resulting from a variety of environmental stress, including exposure to heavy metals.

Andrographis paniculata, *Emilia sonchifolia* and *Tridax procumbens* are widely studied medicinal plants where they are introduced due to the presence of pharmacological properties such as anti-inflammatory, antioxidant and antimicrobial properties. These plants are a source of structure diversity of bioactive “secondary” metabolites with a broad pharmacological value. Nevertheless, the effect of heavy metal exposure on antioxidant potential of these plants is not widely studied.

In this context, the present investigation was undertaken to study the effect of Cu, Cr and Zn on the antioxidant functionality of *A. paniculata*, *E. sonchifolia* and *T. procumbens*. This study will quantify the degree of alteration in enzymatic (and non-enzymatic) antioxidant response in these species under different concentrations of these metals. The results are anticipated to clarify the mechanisms by which these plants regulate their antioxidant defense systems under heavy metal exposure induced oxidative stress, and provide new findings about their possible role in phytoremediation technologies and their persistence in medical service under contaminated conditions.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely used for the analysis of the free radical scavenging and antioxidative effects of plant extracts, compounds, and natural products. This test is based on the antioxidant activity of antioxidants to donate electrons or hydrogen to free radicals, scavenging and stabilizing them, and limiting oxidative damage.

Deep purple DPPH radical is a stable nitrogen-centred free radical. The colour streak on DPPH were decolorized as a result of the radical being reduced through scavenging activity of the antioxidant.

The DPPH assay has found wide applications in the field of botanical studies for measuring the antioxidant activity of plant extracts, especially extracts of medicinal plants such as *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens*. These species are well known for their pharmacological potency and their secondary metabolites including flavonoids, phenolics and other bioactive contents were believed to be responsible for their antioxidant activities.

DPPH assay is preferred because of its simplicity, speed of performance and ability to provide instant results, which are good characteristics for initial evaluating the antioxidant activity of plant extracts. Furthermore, it enables comparison of the antioxidative potential of different plants species, and how environmental abiotic pollutants, e.g., exposure to heavy metals (Cu, Cr, Zn), might affect antioxidant response of these plants. Heavy metal-induced stress triggers ROS production in plants that can result in oxidative destruction of plant cells. Therefore, it is important to know how plants themselves control their antioxidant systems under these stress conditions, both for the ecology and for the medicine.

This study used the DPPH assay to measure the antioxidant potentials of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* after treatment with different levels of copper (Cu), chromium (Cr), and zinc (Zn). The results of the present study will promote a precise assessment of the antioxidant properties of the plants and widen our knowledge on their covering potential of heavy metal-induced oxidative damages. In addition, the findings might provide important knowledge about the involvement of these plants in phytoremediation and their medicinal and ecological importance.

Superoxide Dismutase (SOD) belongs to the main antioxidant metalloenzyme playing a fundamental role in detoxifying oxidative stress in plants especially under environmental challenges like metal toxicity. The toxic heavy metals such as Copper (Cu), Chromium (Cr), Zinc (Zn) are reported to generate highly toxic reactive oxygen species (ROS) especially superoxide anions (O_2^-) that are relatively high reactive and can act in damage to the lipids, proteins and nucleic acids by initiating the release of free radicals. It catalyzes the dismutation of superoxide anions into the less toxic hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) and is, therefore, a key component of the defence mechanism to achieve cellular redox balance.

In the present study, the quantitative SOD assay will be employed to assess the enzymatic activity of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* against various concentrations of Cu, Cr, and Zn. In measuring SOD activity, we expect to show the level of oxidative stress generated by the heavy metal exposure and the plants' ability to overcome superoxide radicals. This study will provide insight into the biochemical detoxification pathways mediating plant tolerance to metal-triggered oxidative stress, which will shed light on the efficacy of phytoremediation and plant adaption to anthropogenic-metal depleted habitats.

10.2 REVIEW OF LITERATURE

Heavy metal exposure has been well documented to alter physiological responses in plants, stimulating mainly the synthesis of secondary metabolites and the activity of antioxidants. Heavy metal stress could modulate phytochemical levels including andrographolide as well as change the overall antioxidant activity of plant in *Andrographis paniculata* (Antony and Nagella, 2021). The antioxidant potential of *A. paniculata* has been explored in animal models and it showed to protect against liver, kidney, heart, lung and spleen damage due to oxidative stress induced by nicotine effects, thus corroborating systemic antioxidant property (Neogy et al., 2008). Furthermore, the natural bioactive molecule andrographolide has been reported to possess strong natural antioxidant activity that could scavenge ROS and support the cell's defense network (Mussard et al., 2019).

In the same way, *Emilia sonchifolia* has an important antioxidant potential. In vitro assays, the n-hexane extract was established to possess DPPH radical scavenging potential and phytochemical content as well (Sophia et al., 2011). Additional animal experiments have also shown that *E. sonchifolia* ameliorates high-protein diet-induced oxidative stress in the pancreas of Wistar rats, further supporting its effect on protection in biological system (Sophia et al., 2012).

Tridax procumbens also demonstrated improved antioxidant defense system under stress environment. *T. procumbens* extracts were found to increase the hepatic antioxidant enzyme activity in the lipopolysaccharide-induced hepatitis rat (Ravikumar, Shivashangari, and Devaki 2005). Further investigations showed that it possessed antioxidant, antimicrobial and antihyperuricemic activities, reflecting diverse bioactivity profiles associated with amelioration of oxidative stress (Andriana et al., 2019).

In addition to species-level studies, effect of heavy metals on antioxidant properties has been studied among plant lineages. *Betula pendula* was analyzed by Makuch-Pietraś, Grabek-Lejko, Górka, and Kasprzyk (2023), metal transport from soil to plant organs is promoting antioxidant reactions in metal-challenged plants, indicating a conserved mechanism of defense among the different plant species facing metal stress. These results all together underscore that the complex biochemical adaptations are induced upon exposure to heavy metal such as modulation of phytochemical profiling and enzymatic antioxidant systems like superoxide dismutase (SOD), catalase and glutathione peroxidase, to alleviate oxidative stress.

Taken together, these studies lay a foundation to dissect the antioxidative defence mechanisms in medicinal plants like *A. paniculata*, *E. sonchifolia*, *T. procumbens* in relation to their responses under metal-induced oxidative stress, which may be utilized to approve the medicinal plants as natural antioxidants under environmental stress conditions.

10.3 MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* were grown hydroponically under controlled environmental conditions. The hydroponic system provided all essential nutrients (Cakmak, 2005).

Treatment with Heavy Metals

After acclimatization, plants were exposed to six concentrations (25, 50, 75, 100, 125, and 150 ppm) of copper (Cu), chromium (Cr), and zinc (Zn). Metals were supplied as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (copper sulfate), $\text{K}_2\text{Cr}_2\text{O}_7$ (potassium dichromate), and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (zinc sulfate) dissolved in distilled water. Treatments were applied for 30 days.

Sample Collection

Following treatment, plants were harvested, carefully rinsed with distilled water to remove surface residues, shade-dried, and ground into a fine powder. The powdered samples were stored in sterile containers for subsequent phytochemical and antioxidant analysis.

Preparation of Ethanolic Extracts

Approximately 5 g of dried plant powder was extracted with 100 mL of ethanol using a Soxhlet apparatus for 6 hours. Filtrates were concentrated to one-fifth of the original volume using a rotary evaporator at 40°C and stored at 4°C for further analysis.

Preparation of Ethanolic Extract

The dried plant samples were subjected to ethanolic extraction. Approximately 5 g of each powdered plant sample was extracted with 100 ml of ethanol using a Soxhlet apparatus for 6 hours. The extracts were filtered through Whatman filter paper No. 1 and concentrated using a rotary evaporator at 40°C. The concentrated extracts were stored at 4°C for further analysis.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

The antioxidant activity of the extracts was evaluated using the DPPH assay. Briefly, 1 mL of each plant extract (in concentrations ranging from 25 to 250 µg/mL) was mixed with 3 mL of a 0.1 mM DPPH solution (dissolved in methanol). The mixture was incubated in the dark for

30 minutes at room temperature. The absorbance of the resulting solution was measured at 517 nm using a UV-visible spectrophotometer (UV-1600, Shimadzu, Japan). The scavenging activity of DPPH was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Superoxide Dismutase (SOD) Assay

SOD activity was measured following the method of Giannopolitis and Ries (1977) using nitro-blue tetrazolium (NBT).

Extract Preparation

Fresh plant samples (100 mg) were homogenized in 1 mL of 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA using a mortar and pestle. The homogenate was centrifuged at $12,000 \times g$ for 20 minutes at 4°C, and the resulting supernatant was used for the enzyme assay.

Reaction Mixture

The reaction mixture (final volume 3 mL) contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 0.1 mM EDTA, and 0.25 mM riboflavin.

Measurement of Enzyme Activity

The reaction was initiated by exposing the mixture to light for 20 minutes. Absorbance was then measured at 560 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800). SOD activity was calculated using the formula:

$$\text{SOD activity} = \frac{(A_{\text{control}} - A_{\text{sample}})}{\text{Reaction time} \times \text{Sample volume}}$$

One Unit of SOD: One unit of SOD activity was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specified assay conditions.

Statistical Analysis

All measurements were conducted in triplicate, and results were expressed as mean \pm standard deviation (SD). One-way ANOVA followed by Holm's post-hoc test was performed using R software to evaluate significant differences between treatments (p-values).

10.4 RESULT

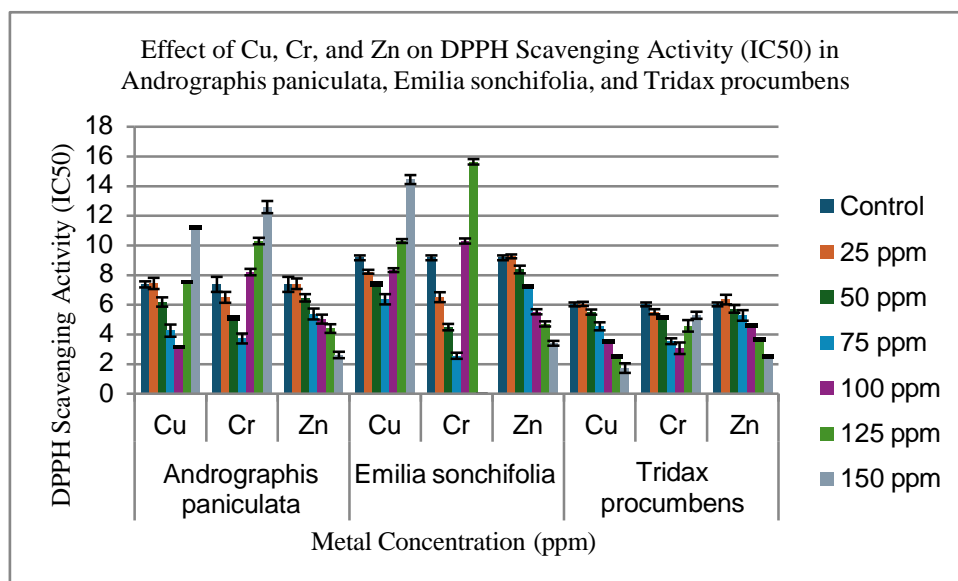


Fig. 42 Effect of Cu, Cr, and Zn on DPPH Scavenging Activity (IC₅₀) in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

The antioxidant activities of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* were strongly influenced by exposure to copper (Cu), chromium (Cr), and zinc (Zn), as assessed by the DPPH radical scavenging assay. In *A. paniculata*, moderate doses of Cu (100 ppm) and Cr (75 ppm) enhanced antioxidant activity, reflected in low IC₅₀ values of 3.12 ± 0.03 ppm and 3.73 ± 0.02 ppm, respectively, whereas higher concentrations (≥ 125 ppm) caused a sharp increase in IC₅₀, indicating diminished radical scavenging. Zn treatments generally promoted activity, with a minimum IC₅₀ of 2.62 ppm observed at 150 ppm. Similarly, *E. sonchifolia* showed peak antioxidant potential at moderate Cr exposure (2.56 ppm at 75 ppm), while high Cr concentrations completely inhibited activity, and Zn maintained moderate scavenging even at 150 ppm (3.41 ppm). In *T. procumbens*, antioxidant capacity remained robust across most treatments, with the strongest activity recorded at 150 ppm Cu (1.73 ppm) and Zn (2.49 ppm), while Cr produced a moderate response at 75 ppm (3.55 ppm) that became variable at higher doses. Overall, these results demonstrate a dose-dependent hormetic effect of heavy metals on antioxidant function: low-to-moderate concentrations enhanced radical scavenging, whereas excessive Cu and Cr impaired activity in *A. paniculata* and *E. sonchifolia*, while *T. procumbens* maintained efficient antioxidant performance even at elevated metal levels.

Statistical Analysis

Table 15 Three-Way ANOVA

Source of Variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Concentration	6	916.5	152.75	257069	< 2e-16
Metal	2	239.9	119.96	201899	< 2e-16
Species	2	441.3	220.64	371339	< 2e-16
Concentration × Metal	12	524.2	43.68	73515	< 2e-16
Concentration × Species	12	568.7	47.39	79762	< 2e-16
Metal × Species	4	30	7.49	12605	< 2e-16
Concentration × Metal × Species	24	2077.9	86.58	145710	< 2e-16
Residuals	126	0.1	0		

The three-way ANOVA revealed that Concentration, Metal, and Species each had highly significant main effects ($p < 0.001$) on DPPH IC₅₀ values, indicating that all three factors independently influenced the antioxidant activity of the plants. Furthermore, all interaction terms Concentration × Metal, Concentration × Species, Metal × Species, and the three-way interaction Concentration × Metal × Species were also highly significant ($p < 0.001$), suggesting that the effect of any single factor was dependent on the levels of the others. Collectively, these results highlight a complex, species- and metal-specific modulation of antioxidant potential, demonstrating that both the type and concentration of heavy metals interact intricately with plant genotype to determine DPPH radical scavenging activity.

Estimated Alkaloid Content (mg/g) by Species × Metal × Con

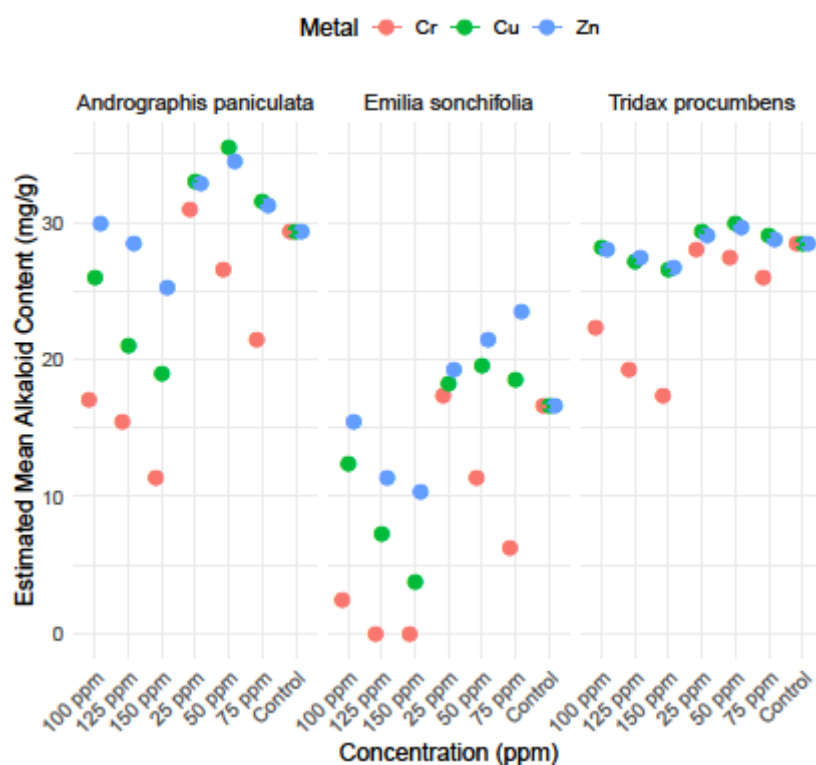


Fig. 43 Hom Post Hoc Test

The plot illustrates interspecies differences in IC_{50} responses to varying concentrations of copper (Cu), chromium (Cr), and zinc (Zn) treatments in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. In general, IC_{50} values decreased at lower metal concentrations, reflecting enhanced antioxidant activity, but increased at higher concentrations, indicating diminished scavenging capacity under metal stress. *Emilia sonchifolia* exhibited the highest IC_{50} values, particularly under Cr and Cu at elevated concentrations, suggesting greater oxidative stress or a delayed activation of its antioxidant system. In contrast, *Tridax procumbens* consistently showed lower IC_{50} values, especially under Cu treatment, demonstrating a stronger antioxidant potential. These results highlight a species-specific response to metal exposure and underscore the complex interactions between metal type, concentration, and plant genotype in modulating antioxidant activity.

RESULT

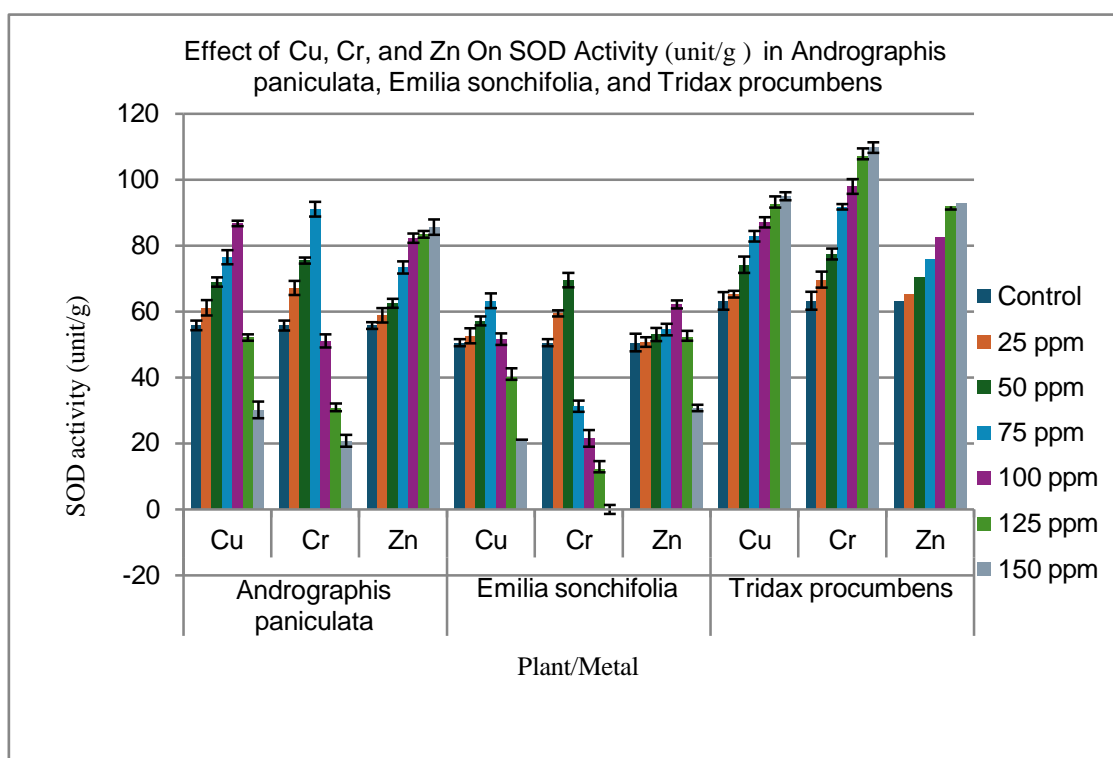


Fig 44 Effect of Cu, Cr, and Zn On SOD Activity (unit/g) in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*

Superoxide dismutase (SOD) activity in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* displayed concentration- and metal-dependent responses. In *A. paniculata*, SOD activity increased with rising Cu and Zn concentrations, reaching maxima of 86.8 U/mg at 100 ppm Cu and 85.6 U/mg at 150 ppm Zn, whereas Cr initially stimulated activity (91.1 U/mg at 75 ppm) but sharply declined at higher concentrations to 20.8 U/mg at 150 ppm. *Emilia sonchifolia* exhibited moderate induction under Cu and Zn treatments, with peak activities of 63.3 U/mg at 100 ppm Cu and 62.2 U/mg at 75 ppm Zn, while Cr exposure caused a progressive decrease, culminating in complete inhibition at 150 ppm. *Tridax procumbens* demonstrated the strongest overall SOD response, maintaining high activity across all metals and concentrations, with maxima of 95 U/mg (Cu), 109.8 U/mg (Cr), and 92.8 U/mg (Zn) at 150 ppm. While Cr generally exhibited a biphasic effect stimulating SOD at moderate concentrations and inhibiting it at higher levels *T. procumbens* sustained a robust antioxidant response, and both Cu and Zn consistently enhanced SOD activity in all three species. These results indicate that SOD-mediated defense mechanisms are strongly metal- and species-specific, reflecting the differential tolerance and oxidative stress mitigation capacity among the studied plants.

Table 16 Three-Way ANOVA

Source of Variation	Df	Sum of Squares (SS)	Mean Square (MS)	F-value
Species	2	43,916	21,958	6953.5
Metal type	2	1,488	744	235.6
Metal concentration	6	6,847	1,141	361.4
Species × Metal type	4	5,423	1,356	429.3
Species × Metal concentration	12	22,997	1,916	606.9
Metal type × Metal concentration	12	8,144	679	214.9
Species × Metal type × Concentration	24	10,375	432	136.9
Residuals	126	398	3	

The three-way ANOVA revealed highly significant effects of species, metal type, and exposure concentration on SOD activity ($p < 0.001$). Among the main effects, species accounted for the largest proportion of variance ($F = 6953.5$), highlighting strong inherent differences in antioxidant response between *A. paniculata*, *E. sonchifolia*, and *T. procumbens*. Metal type ($F = 235.6$) and metal concentration ($F = 361.4$) were also significant, indicating that the type and level of heavy metal stress substantially influenced SOD activity. All interaction terms were statistically significant as well: Species × Metal type ($F = 429.3$) showed that each species responded differently to the metals; Species × Concentration ($F = 606.9$) indicated species-specific tolerance thresholds; Metal type × Concentration ($F = 214.9$) suggested that the concentration-dependent effects varied among metals; and the three-way interaction ($F = 136.9$) demonstrated that SOD activity reflects a complex, combined effect of species, metal type, and concentration rather than independent contributions of individual factors. These results underscore the intricate, species- and metal-specific regulation of antioxidant defence in response to heavy metal stress.

SOD Activity under Metal Treatments

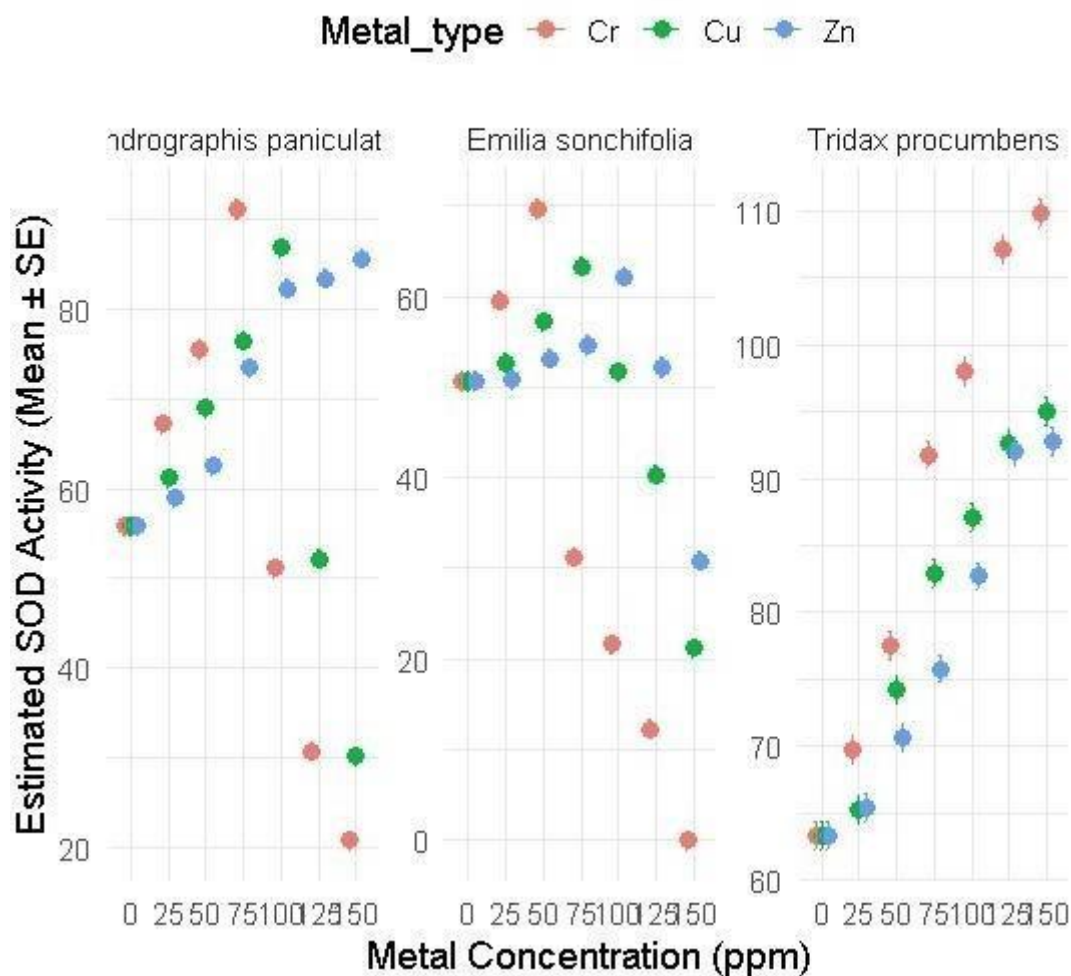


Fig. 45 Homs post HOC Test

The superoxide dismutase (SOD) activity exhibited clear species-specific responses to chromium (Cr), copper (Cu), and zinc (Zn) treatments in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. *Andrographis paniculata* showed a continuous increase in SOD activity with rising metal concentrations, with Cr eliciting the highest response, followed by Zn and Cu, indicating a strong defense against oxidative stress under Cr exposure. *Emilia sonchifolia* displayed maximal SOD activity at lower metal doses, but activity declined at higher concentrations, particularly under Cr, suggesting enzyme inhibition or oxidative damage at elevated stress levels. *Tridax procumbens* exhibited the highest overall SOD activity among the three species, steadily increasing with concentration and showing pronounced sensitivity to Cr, followed by Cu and Zn, reflecting its robust antioxidative capacity. Three-way ANOVA confirmed these observations, revealing highly significant main effects of species, metal type, and concentration ($p < 0.001$), with species

accounting for the greatest variance ($F = 6953.5$). Significant interaction effects including Species \times Metal type ($F = 429.3$), Species \times Concentration ($F = 606.9$), Metal type \times Concentration ($F = 214.9$), and the three-way interaction ($F = 136.9$) highlight that SOD activity is a result of a complex, combined effect of plant species, metal type, and concentration rather than independent effects. Overall, Cr elicited the strongest enzymatic response across species, while *Tridax procumbens* emerged as the most tolerant and *Emilia sonchifolia* the most sensitive to high metal levels.

10.5 DISCUSSION

The changes in superoxide dismutase (SOD) activity in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* in response to varying concentrations of copper (Cu), chromium (Cr), and zinc (Zn) were distinct among the species. In *Andrographis paniculata*, SOD activity generally increased with rising Cu and Zn concentrations, reaching 86.8 U/mg at 100 ppm Cu and 85.6 U/mg at 150 ppm Zn. Under Cr treatment, SOD initially increased to a maximum of 91.1 U/mg at 75 ppm, but then sharply declined to 20.8 U/mg at 150 ppm. *Emilia sonchifolia* exhibited increased SOD activity under Cu and Zn, with maxima of 63.3 U/mg at 75 ppm and 62.2 U/mg at 100 ppm, respectively, whereas Cr caused a steep decline from 69.6 U/mg at 50 ppm to complete inhibition (0 U/mg) at 150 ppm. In contrast, *Tridax procumbens* consistently exhibited higher SOD activity across all treatments, achieving 95 U/mg under Cu, 109.8 U/mg under Cr, and 92.8 U/mg under Zn at 150 ppm. Overall, while Cu and Zn generally stimulated SOD activity in all three species, Cr elicited a biphasic response, enhancing activity at moderate concentrations but suppressing it at higher doses, except in *T. procumbens*, which maintained a strong and stable antioxidant enzyme response across all metal treatments.

CHAPTER 11

COMPARATIVE BIOACCUMULATION POTENTIAL OF *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* UNDER HEAVY METAL STRESS

11.1 INTRODUCTION

Heavy metal contamination is an increasing environmental concern resulting from industrial, agricultural, and urban activities, resulting in toxic buildup in ecosystems. Copper (Cu), chromium (Cr), and zinc (Zn) are necessary micronutrients for plants at low concentrations but harmful at high ones for growth and metabolism. Plants can be used to remove and immobilize pollutants, a process called phytoremediation, sustainable method. Medicinal plants having metal tolerance and high biomass are good choice for this. The ability of *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens* plants to accumulate (uptake) and tolerate (toxicity) Cu, Cr and Zn were studied based on a hydroponic approach.

Hydroponic systems offer a soil-less environment to investigate plant-metal interactions and thus can be used to measure the quantities of metals absorbed by roots or shoots byproducts. Heavy metals have been known to be accumulated by medicinal plants, but species-specific responses differ greatly according to previous studies. *Andrographis paniculata* (which has medicinal properties) may have different metal sequestration potential, while *Emilia sonchifolia* and *Tridax procumbens* (which are widely found in the contaminated sites) may exhibit intrinsic metal resistance. This study investigates their bioaccumulation patterns in concentrations of 20, 50, 75, 100, 125, and 150 ppm for 30 days using atomic absorption spectroscopy (AAS) for precise quantification of metal distribution in plant parts.

Evaluation of the bioconcentration factor of these medicinal plants are important to determine their phytoremediation potential and safe use for herbal medicine. When such plants are ingested, over accumulation of metal can be hazardous to health; hence it becomes necessary to examine their potential to sequester metal. The results will help in choosing appropriate species for remediation and in understanding their tolerance mechanisms, which would be beneficial for managing heavy metal contaminated environments in a sustainable manner. This article fills the void between environmental cleanup and the safety of medicinal plants by disseminating this information to practical applications of pollution control and public health.

11.2 LITERATURE REVIEW

Recent findings accentuate the bioconcentration capacity of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* after heavy metal exposure, concerning their use in phytoremediation and drug safety. For example, *T. procumbens* showed high accumulation of mercury (Hg) and de Oliveira et al. (2025) stated that this species could be useful in the remediation of Hg contaminated soil. Concordantly, *E. sonchifolia* have also been investigated for NP preparation; its leaf extracts synthesized copper NPs, which demonstrated a dose-dependent cytotoxicity in human cell lines, but relatively mild developmental toxicity in zebrafish embryos (Narayanan and Jothiramajayam, 2023).

The mechanisms of heavy metal uptake provide useful information on the species-specific adaptations. In addition to minimizing Hg, *T. procumbens* harbours endophyte bacteria like *Paenibacillus* sp., that could also survive under concentrated metal (Cu up to 750 mg/L) and augment its phytoremediation performance through siderophore and phosphate solubilization (Govarthanan et al., 2016). By contrast, the pharmacological traits in *A. paniculata*, such as its antioxidant andrographolide, are modified in response to heavy metal stress. According to previous evidence, andrographolide accumulation and antioxidant capacity can be negatively affected by exposure to metals like copper and cobalt which can reduce its pharmaceutical quality (Antony & Nagella, 2021). Simultaneously, pyrrolizidine alkaloids of *E. sonchifolia*, although used beneficially for medicinal purposes, present toxic effects on liver at high doses, and recent multi-omics studies have linked the hepatotoxic effects of the plant to ferroptosis and oxidative stress in mice (Liu et al., 2025; Yin et al., 2024).

Together, these studies highlight the double role of these plants as metal accumulators and suppliers of bioactive components, and they emphasize that dosage and contamination levels require careful control.

11.3 MATERIALS AND METHODS

Sample Collection and Preparation

Shoots and roots of plants were harvested separately for analysis after 30 days of metal treatment. The plant materials were thoroughly rinsed with distilled water to remove surface impurities, then oven-dried at 65 °C for 48 h until a constant weight was achieved. The dried samples were ground into a fine powder using a pestle and mortar. A portion of the powdered material (0.5 g) was digested with concentrated HNO₃ and HClO₄ in a 3:1 (v/v) ratio for 6 h at 100 °C in a digestion block. The digested samples were filtered through Whatman No. 42 filter paper and diluted to a final volume of 25 mL with deionized water.

Atomic Absorption Spectroscopy (AAS) Measurement

The concentrations of copper (Cu), chromium (Cr), and zinc (Zn) in the digested plant samples were determined using Atomic Absorption Spectroscopy (AAS). Standard solutions of Cu, Cr, and Zn were prepared at known concentrations for instrument calibration. Sample absorbances were measured at 324.7 nm (Cu), 357.9 nm (Cr), and 213.9 nm (Zn). Metal concentrations in plant tissues were calculated by comparing sample absorbances to the standard calibration curves. All measurements were performed in triplicate to ensure accuracy and precision.

The bioaccumulation factor (BAF) was calculated for each metal in each plant species using the formula:

$$\text{BAF} = \frac{\text{Concentration of metal in plant tissue (mg/kg)}}{\text{Concentration of metal treatment (mg/kg)}}$$

The data were analyzed using one-way analysis of variance (ANOVA) followed by Holm's post-hoc test to compare the metal concentrations across different treatments and plant species. Statistical significance was determined at $p < 0.05$. The results were expressed as means \pm standard deviation (SD).

11.4 RESULT

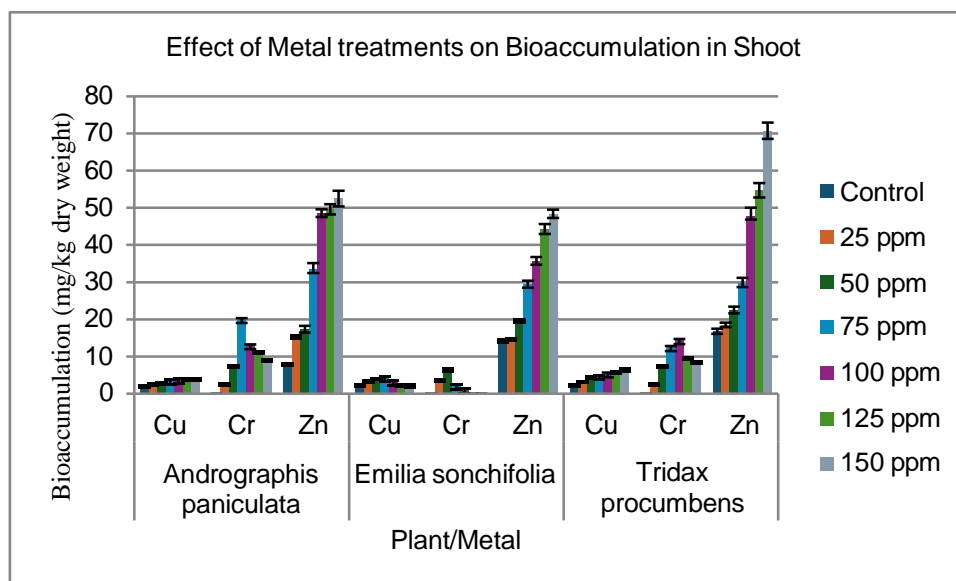


Fig. 46 Effect of Metal treatments on Bioaccumulation in Shoot

The results demonstrated clear species-specific differences in heavy metal uptake and tolerance among *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. *Tridax procumbens* showed the highest accumulation of copper (Cu), ranging from 3.30 ± 0.21 mg/kg to 6.40 ± 0.31 mg/kg across 25–150 ppm treatments, and also exhibited the greatest zinc (Zn) accumulation, reaching 70.70 ± 2.20 $\mu\text{g/g}$ at 150 ppm, nearly double that of the other species. *Andrographis paniculata* displayed the highest chromium (Cr) uptake, with a peak of 20.00 ± 0.36 mg/kg at 75 ppm, though uptake declined at higher concentrations. *Emilia sonchifolia* consistently showed the lowest accumulation of all three metals, with Cr detected only up to 100 ppm. Notably, Zn accumulation increased progressively in all species during the exposure period, suggesting an inherent tolerance mechanism. Variability tended to increase at higher metal concentrations, particularly for Zn in *T. procumbens*, but overall reproducibility was good. These findings indicate that *T. procumbens* is the most efficient accumulator of Cu and Zn, whereas *A. paniculata* exhibits selective uptake for Cr at intermediate concentrations, highlighting distinct species-specific strategies for metal acquisition and tolerance.

Table 17 Three Way Anova

Source of Variation	Df	Sum Sq	Mean Sq
Concentration	6	2,495	416
Metal	2	11,123	5,562
Species	2	282	141
Concentration × Metal	12	3,326	277
Concentration × Species	12	286	24
Metal × Species	4	153	38
Concentration × Metal × Species	24	211	9

The ANOVA table shows that metal type contributed the largest portion of variance in heavy metal accumulation (Sum Sq = 11,123; Mean Sq = 5,562), followed by concentration (Sum Sq = 2,495; Mean Sq = 416), indicating that both the type and amount of metal strongly influenced uptake. Species had a smaller but noticeable effect (Sum Sq = 282; Mean Sq = 141), while the interaction terms (Concentration × Metal, Concentration × Species, Metal × Species, and the three-way interaction) indicate that the combined effects of these factors also contributed to the variation in metal accumulation. Overall, this confirms that heavy metal uptake is determined by a complex interplay between species identity, metal type, and exposure concentration.

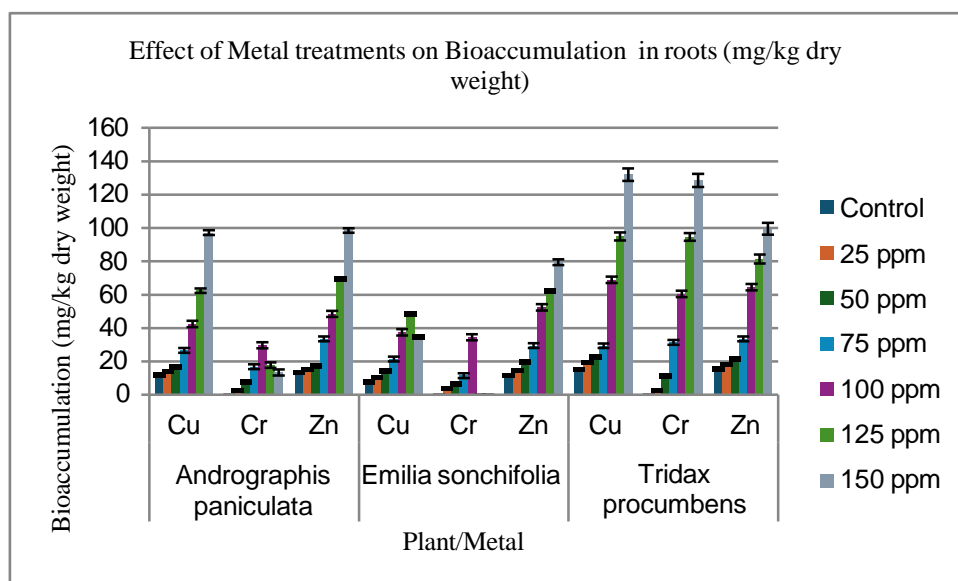


Fig. 47 Effect of Metal treatments on Bioaccumulation in roots (mg/kg dry weight)

The results showed different patterns of heavy metal accumulation in roots of the three medicinal plants in the hydroponic exposure experiment. The highest bioaccumulative ability was shown by *Tridax procumbens* for all the three metals, and especially at higher concentrations. With respect to copper (Cu), this species exhibited a concentration-dependent increase which was the most significant, attaining 132.1 ± 1.00 mg/kg at the 150 ppm exposure which was almost 8.6 fold higher than their control values. The maximum Cr concentration was observed to be 128.6 ± 0.80 mg/kg at 150 ppm, and that for Zn was 99.6 ± 0.70 mg/kg amid 150 ppm of 128.6 ± 0.70 mg/kg in *T. procumbens*.

The accumulation dynamics of metals, in particular, chromium, were specific in case of *Andrographis paniculata*. Although it exhibited significant Cr accumulation, achieving the maximum 29.8 ± 0.50 mg/kg at 100 ppm, the levels reduced rapidly at concentrations over 100 ppm, to 13.5 ± 0.40 mg/kg for 150 ppm. The increased Cu and Zn uptake under a concentration gradient was consistent for this species, with concentrations up to 97.2 ± 1.00 mg/kg and 98.6 ± 0.80 mg/kg, respectively, reached at 150 ppm.

Among the three species, *E. sonchifolia* had the least capacity to accumulate metal in plant on the whole. In this plant Cr accumulation was totally absent at higher concentrations (>100 ppm). Maximum accumulations of it were far below those of the others, 34.9 ± 0.60 and 79.6 ± 0.70 mg/kg, at 150 ppm of copper and zinc, respectively.

The standard deviation values showed consistent data quality although variability tended to increase with increasing metal concentrations. This was especially evident for Cu and Cr in *T. procumbens* (150 ppm), where the influence of biological variability on metal uptake was greater. The responses showed that there are distinct species-specific accumulation patterns, with *T. procumbens* so far being the most efficient, followed by *A. paniculata*, whilst *E. sonchifolia* presented very limited accumulation performances in the case for chromium. These results have important relevance for phytoremediation potential and safety consideration of medicinal plants.

11.5 DISCUSSION

Heavy metal accumulation potential of *Tridax procumbens*, *Andrographis paniculata* and *Emilia sonchifolia* under hydroponic Situation: a comparative study. The present study showed that there were the significant species specific differences among *T. procumbens*, *A. paniculata* and *E. sonchifolia* in the accumulation of heavy metals under hydroponic conditions. *T. procumbens* exhibited the greatest accumulation capacity, primarily for Cu and Zn, with root concentrations of 132.1 mg/kg and 99.6 mg/kg respectively at 150 ppm, and 6.40 mg/kg (Cu) and 70.7 mg/kg (Zn) in shoots. This high potential of uptake was not surprising, since previous studies have demonstrated that *T. procumbens* can act as an efficient bioaccumulator of heavy metals and that it associates with endophytic bacteria able to tolerate and mobilize Cu (Govarthanan et al., 2016). *A. paniculata* however, for chromium demonstrated a distinctive pattern, while it reached 20.0 mg kg⁽⁻¹⁾ in shoots at 75 ppm and 29.8 mg/kg in roots at 100 ppm, the further uptake it declined rapidly, implying the concentration-dependent tolerance limits. This is consistent with previous studies which describe the uptake of Cr frequently to have an optimum point beyond which, toxicity inhibits the absorption and metabolism processes (Shahid et al., 2017). The accumulation of Cu and Zn showed no trends across concentrations in both *A. paniculata* and *T. procumbens*, likely following their adaptive characteristic to metal stress, while the lowest accumulation capacity was found for *E. sonchifolia* (with complete inability to accumulate Cr over 100 ppm). Globally, increases in Zn with increasing metal concentration are evident for all the three species, albeit with species-specific differences in concentration, indicating that Zn serves a vital role as a micronutrient and is regulated more efficiently than non-essential toxic metals by plants. Similar results have been documented in some other medicinal plants, during which the pattern of Zn uptake proved generally stable under stress conditions, although that of Cr accumulation was fluctuant (Antony & Nagella, 2021; Li et al., 2025). Overall, the findings demonstrate that *T. procumbens* is the most effective as an accumulator of Cu and Zn, *A. paniculata* is a species with a noticeable affinity for Cr at medium levels, while *E. sonchifolia* as a poor accumulation plant, particularly for Cr. The results again confirmed the metal uptake potential of *T. procumbens* and *A. paniculata* and highlight the necessity of surveying metal contamination of medicinal crops including these species for safety and therapeutic purposes.

CHAPTER 12

EFFECT OF COPPER, CHROMIUM, AND ZINC ON MAJOR BIOACTIVE COMPOUNDS IN *Andrographis Paniculata*, *Emilia Sonchifolia*, and *Tridax Procumbens*

12.1 INTRODUCTION

Plants used in the traditional and folk medicine are considered as a good source of biologically active substances. *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens* are one among them and are acclaimed for their health promoting effects for the treatment of various diseases such as inflammatory, metabolic and infectious diseases. Secondary metabolites which are involved in various pharmacological activities of these plants, phenolic and flavonoid content play a major role in their antioxidant, anti-inflammatory and antimicrobial activity. Quantitative as well as qualitative differences of these compounds need to be known to substantiate traditional explanation of therapeutical effects and exploration of such compounds of which isolation is not been successful so far and which may be suitable as leads for drug development.

In this respect, three major bioactive compounds (i.e., andrographolide, kaempferol, shevagensin F, and quercetin) from *A. paniculata*, *E. sonchifolia*, and *T. procumbens* are investigated in this research. Andrographolide was also isolated from *A. paniculata* and it is considered a diterpene lactone which is the one who has hepatoprotective, anticancer, and immunomodulatory activity. Kaempferol and quercetin are flavonoids widely distributed in medicinal plants, which have been shown to possess antioxidant activity and protective effects against vascular and neurodegenerative diseases. The present studies involved in the quantitation aspects of these constituents, it's aims to provide a comparative base for the understanding of phytochemical richness of these constituents of plant and their therapeutic potential to demonstrate an appropriate traditional claims and lend support for the same scientifically to launch the plants in the form of part of the basic therapeutic agents for the adoption in pharmaceutical and nutraceutical formulations..

12.2 REVIEW OF LITERATURE

With the increasing of heavy metal stress, the role of plant secondary metabolism in the medicinal plants was more and more important. The herbal medicine *Andrographis paniculata* gives the bioactive andrographolide as the main diterpenoid lactone. Heavy metals were provided as abiotic elicitors that probably act in the control of andrographolide biosynthesis. It was reported that alteration of andrographolide, as well as other phytochemicals and antioxidant activities, occurred in *A. paniculata* following exposure to heavy metals (Krishnamurthy & Anuradha, 2018). Analogous results were obtained in case of lead, mercury, silver exposed in andrographolide, respectively (Sangamesh et al., 2016). Additionally, an elicitation experiments performed with suspension cultures revealed that copper sulphate could enhanced the andrographolide content, which supported the regulatory role of copper in the biosynthesis of diterpenoids (as evidence) (Ravindra et al., 2017). Recent metabolomics studies showing that metal responses are dependent on genotype in *A. paniculata* add further credence to the understanding that andrographolide biosynthesis under stress is more a product of the genotype–environmental interaction (Zhang et al., 2021).

Kaempferol and other flavonoids are the predominant bioactive constituents of *Emilia sonchifolia*. The phytochemical investigation of *E. sonchifolia* indicated the presence of kaempferol which has been well known for its roles in antioxidative defense and therapeutic effect (Manjamalai & Berlin Grace, 2012). Under heavy metal stress, kaempferol and other flavonoids, are believed to accumulate as metal-chelators and H₂O₂ scavengers (Calabrese et al., 2020). Detrimental effects Heavy metal stress viz Cu, Cr and Zn perturbs the level of antioxidant enzymes and secondary metabolite in *Ehc* which signifies an intimate relationship between the stress physiology and flavonoid synthesis (Kumar et al., 2015).

Tridax procumbens: Another important medicinal plant with qinuous natural product chemistry is *Tridax procumbens* which is rich in both quercetin and quercetin-derived compounds. This plant type and its extracts are reported to contain quercetin, a flavonol with strong antioxidant activity (Kumar & Yadav, 2014). Metal loading analysis indicated that *T. procumbens* could uptake and accumulate the metals such as copper and chromium and thus suggesting its safe use as drug and also for phytoremediation (Oladele et al., 2019). Phytochemical review of *T. procumbens* indicates that quercetin is one of its major flavonoids and is responsible for its pharmacological activities such as antiinflammatory and hepatoprotective (Verma & Gupta, 2018). Since the flavonoid metabolites were easily influenced by abiotic stress, the heavy metal

might also have the regulatory capacity to control the biosynthesis of quercetin, and consequently the medicinal function of *T. procumbens*.

In a broader perspective, many reviews highlight the important functions of phenolic compounds and flavonoids in plants against heavy metal stress. Two major functions of these proteins are metal chelation and radicals scavenging and they also protect the photosynthetic apparatus (Michalak, 2006; Hasanuzzaman et al., 2023). Specific interactions, such as complexation with chromium into the case of flavonoids, which can modulate the antioxidant effect and bioavailability of these compounds, have also been described (Tavano et al., 2014). Generally, results from this review have demonstrated that the medicinally important bio-active molecules of *A. paniculata*, *E. sonchifolia* and *T. procumbens* andrographolide, kaempferol and quercetin are highly influenced by copper, chromium and zinc exposure which suggest potential ramifications not only in their health significance but also for generating more acumen on their implications on adaptive mechanism to environmental stress pressures and phytoremediation.

12.3 MATERIALS AND METHODS

Andrographolide Quantification in *Andrographis paniculata*

Fresh plant material (leaves, stems, flowering tops, and roots) harvested after 30 days of heavy metal exposure was dried and powdered. Approximately 450 g of dried material was extracted using a Soxhlet apparatus with methanol for 12 h. The solvent was evaporated under reduced pressure to obtain a greenish viscous residue. Nonpolar compounds were removed via petroleum ether and hexane partitioning. The remaining extract was dissolved in ethanol (1:1 water), partitioned with ethyl acetate, and concentrated to yield a semipurified fraction. Andrographolide was recrystallized from chloroform and methanol, and purity was confirmed using thin-layer chromatography (TLC) and HPLC.

For HPLC analysis, 2 g of plant material was sonicated in methanol for 32 min, centrifuged, filtered (0.2 μm), and dissolved in the mobile phase (methanol:water, 65:35 v/v). Analyses were performed using a Waters HPLC system with a Sunfire C18 column (4.6 \times 250 mm, 5 μm) and dual-wavelength absorbance detector set at 223 nm. Isocratic elution was conducted at 1 mL/min, with 20 μL sample injection. Andrographolide retention time was 5.6 ± 0.088 min, and quantification was performed using a standard calibration curve. All measurements were performed in triplicate.

Kaempferol Quantification in *Emilia sonchifolia*

Kaempferol content was determined by UV spectrophotometry and HPLC. Standard stock solutions (1 mg/mL) were prepared in methanol:water (8:2, v/v) and diluted to working concentrations. Dried leaf samples (100 mg) were extracted in methanol under ultrasonication (30 min, 40°C), centrifuged, filtered (0.45 μm), and diluted appropriately.

UV analysis was conducted using a Lasany LI-2702 spectrophotometer, with λ_{max} determined at 265 nm. HPLC was performed using a Shimadzu LC-2010 AHT system, with separation on a C18 reversed-phase column (250 \times 4.6 mm, 5 μm) and guard column. The mobile phase consisted of acetonitrile:water (50:50, v/v) with 0.1% formic acid, isocratically at 1 mL/min, detecting at 265 nm. Kaempferol eluted at ~ 6.18 min. Calibration curves were linear (UV: $R^2 = 0.9993$; HPLC: $R^2 = 0.9989$), with system suitability parameters meeting standard criteria (theoretical plates >2000 , tailing factor <1.5).

Method Validation and Statistical Analysis

All procedures were validated according to ICH Q2(R1) guidelines. Precision (%RSD <2%), accuracy (recovery 98–102%), sensitivity (LOD: HPLC 0.163 µg/mL; UV 0.357 µg/mL; LOQ: HPLC 1.172 µg/mL; UV 1.154 µg/mL), and robustness (RSD <2% across conditions) met acceptance criteria.

All data are expressed as mean ± SD of triplicate experiments. Differences among groups were analyzed using one-way ANOVA followed by Holm's post-hoc correction, with significance defined at $p < 0.05$. Statistical analyses were performed using GraphPad Prism (version 9.0). All procedures complied with Good Laboratory Practice and ethical standards.

Quercetin quantification in *Tridax procumbense*

Analytical grade metal salts, copper ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), chromium ($\text{K}_2\text{Cr}_2\text{O}_7$), and zinc ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) ($\geq 99\%$ purity, Sigma-Aldrich) were used in the study. The metal elements selected here are widely known in their regulation of plant secondary metabolism, particularly in the regulation of flavonoid biosynthesis pathways (Kumar et al., 2018). Each metal was subjected to concentration gradient of 0 (control), 25, 50, 75, 100, 125, and 150 ppm and each gradient was used under hydroponic condition using Hoagland's solution (pH 5.8). Plants (*Tridax procumbens*) were treated with the above mentioned test concentrations for a period of 30 days with 15 replicates concentration.

The quercetin was extracted with a solvent system of methanol: water: formic acid (70: 28: 2 v/v), which is known to give high recovery efficiency for flavonoids (Patel et al., 2021). One gram of dried plant material was homogenized with 20 mL of the extraction solvent and ultrasonicated (40 kHz, 30°C, 45 min). The extract was centrifuged ($10,000 \times g$, 15 min) and filtrated with a 0.45 µm PTFE membrane.

Quercetin Quantification in *Tridax procumbens*

Quercetin content was determined using high-performance liquid chromatography with diode-array detection (HPLC-DAD) on a Shimadzu LC-20AD system. The method was validated in accordance with ICH Q2(R1) guidelines and European Commission Decision D standards (ICH, 2005). Separation was achieved on a C18 reverse-phase column (250 × 4.6 mm, 5 µm) using a gradient mobile phase consisting of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The gradient program was set as 0–15 min: 10–30% B, 15–20 min: 30–50% B at a flow rate of 1.0 mL/min. Detection was performed at 370 nm, and quantification was conducted using an external standard calibration curve ranging from 0.1 to 100 µg/mL ($R^2 = 0.999$).

12.4 RESULT

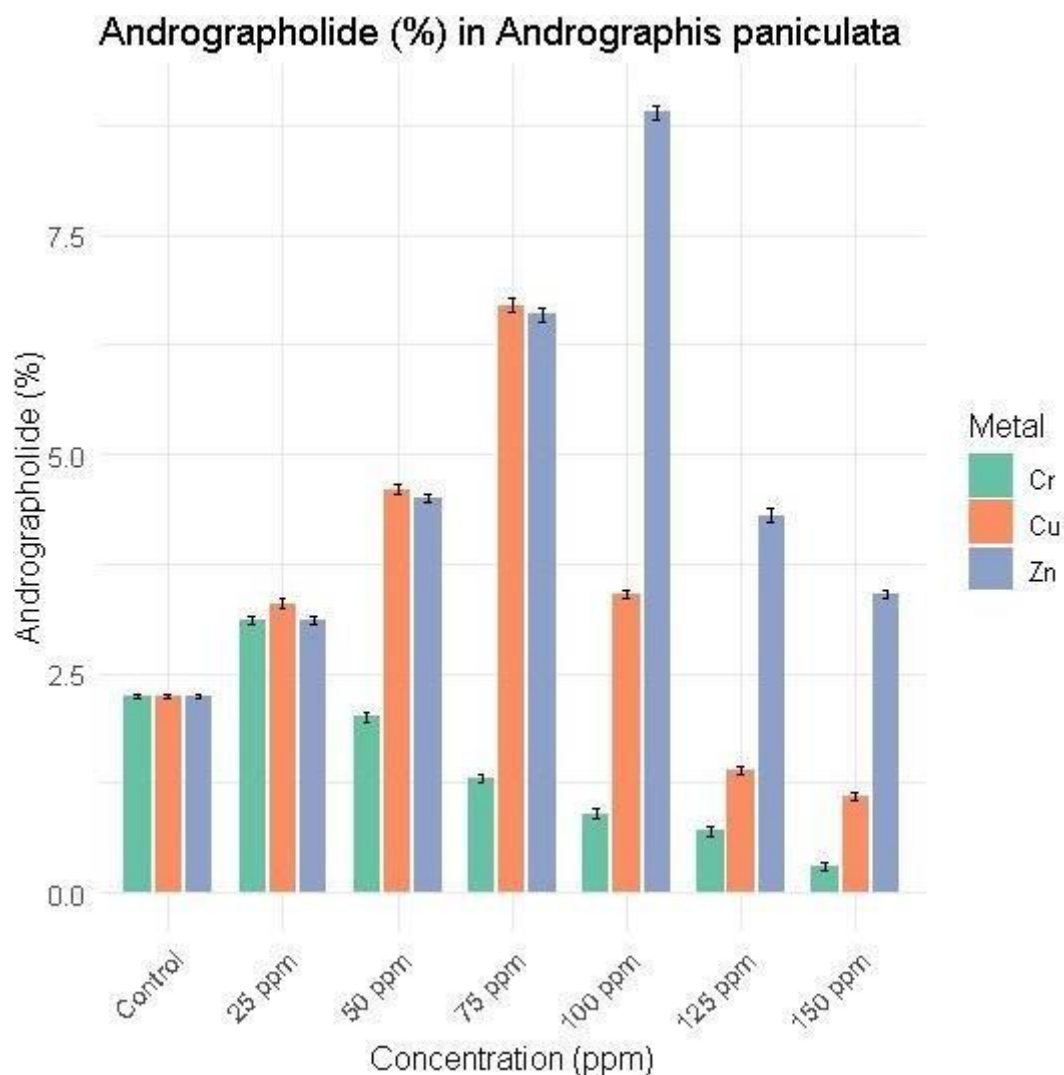


Fig. 48 Effect of metal treatments on andrograoholide content

The effects of copper (Cu), chromium (Cr), and zinc (Zn) on *Andrographis paniculata* were evaluated at varying concentrations. Baseline metal levels in the control group were similar for all three metals (2.24 ± 0.03 ppm). Upon exposure to 25 ppm, the leaf concentrations increased to 3.30 ± 0.05 ppm for Cu, 3.10 ± 0.05 ppm for Cr, and 3.10 ± 0.05 ppm for Zn. At 50 ppm, a divergence in uptake patterns was observed: Cu and Zn levels rose to 4.60 ± 0.05 ppm and 4.50 ± 0.05 ppm, respectively, while Cr declined to 2.00 ± 0.05 ppm, indicating differential cellular mechanisms of metal absorption.

At 75 ppm, Cu and Zn accumulation further increased to 6.70 ± 0.08 ppm and 6.60 ± 0.08 ppm, whereas Cr decreased to 1.30 ± 0.05 ppm. Maximum Zn accumulation (8.90 ± 0.08

ppm) was observed at 100 ppm, while Cu decreased to 3.40 ± 0.05 ppm and Cr reached its lowest level (0.90 ± 0.05 ppm). At higher exposures (125 and 150 ppm), metal content in leaves declined: Cu and Zn dropped to 1.40 ± 0.05 ppm and 4.30 ± 0.08 ppm at 125 ppm, further reducing to 1.10 ± 0.05 ppm and 3.40 ± 0.05 ppm at 150 ppm, respectively. Cr accumulation continued to decrease, reaching 0.30 ± 0.05 ppm at 150 ppm, suggesting potential toxicity or saturation at elevated concentrations. Overall, metal accumulation in *A. paniculata* was concentration-dependent, with Zn showing the highest uptake, while Cr uptake was progressively inhibited as exposure increased.

Statistical Analysis

Table 18 Two Way ANOVA

Source	Df	Sum Sq	Mean Sq	F value
Concentration	6	81.75	13.63	11090
Metal	2	108.87	54.43	44307
Concentration: Metal	12	99.55	8.3	6753
Residuals	42	0.05	0	

The two-way ANOVA results indicate that both Concentration and Metal exert highly significant effects on the measured responses, as evidenced by their extremely large F-values (11090 and 44307, respectively) and extremely small p-values ($< 2 \times 10^{-16}$). Furthermore, the Concentration \times Metal interaction is also statistically significant ($F = 6753$, $p < 2 \times 10^{-16}$), suggesting that the effect of concentration is dependent on the specific metal present. The low RW_RESVAR value indicates that the model provides a comprehensive fit to the data. Collectively, these findings demonstrate that both individual factors and their synergistic interactions strongly influence the responses, and that the concentration-response relationship varies distinctly across different metals.

RESULTS

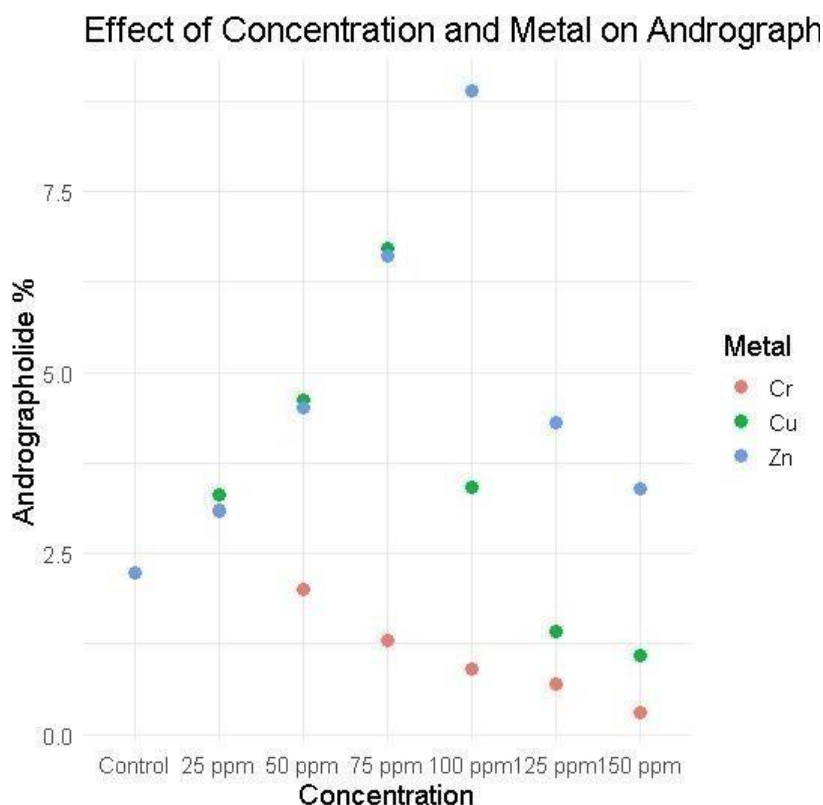


Fig. 49 Hom post hoc test

Holm's post hoc analysis further confirmed that the uptake of metals (Cr, Cu, and Zn) by *Andrographis paniculata* varied significantly across different concentrations. The accumulation of Zn was the highest, showing a marked increase at 75–100 ppm compared with the control, whereas Cr levels progressively declined with increasing concentration, indicating possible inhibitory effects or limited bioavailability at higher doses. Cu exhibited a non-linear pattern, increasing up to 75 ppm and declining thereafter, suggesting a threshold beyond which absorption is suppressed. Pairwise comparisons revealed that Zn accumulation at 75–100 ppm was significantly greater than that of Cr and Cu ($p < 0.05$), Cr uptake below 25 ppm was significantly lower than Cu and Zn ($p < 0.01$), and Cu and Zn differed significantly between 50–150 ppm ($p < 0.05$), with Zn showing the strongest preference. These findings indicate that *A. paniculata* selectively accumulates Zn, likely reflecting its role in enzymatic functions, while Cr uptake is limited at higher concentrations. The non-linear response of Cu suggests a balance between essentiality and toxicity, consistent with previously reported metal-specific stress responses in medicinal plants.

RESULT

Kaempferol content in *emilai sonchifolia*

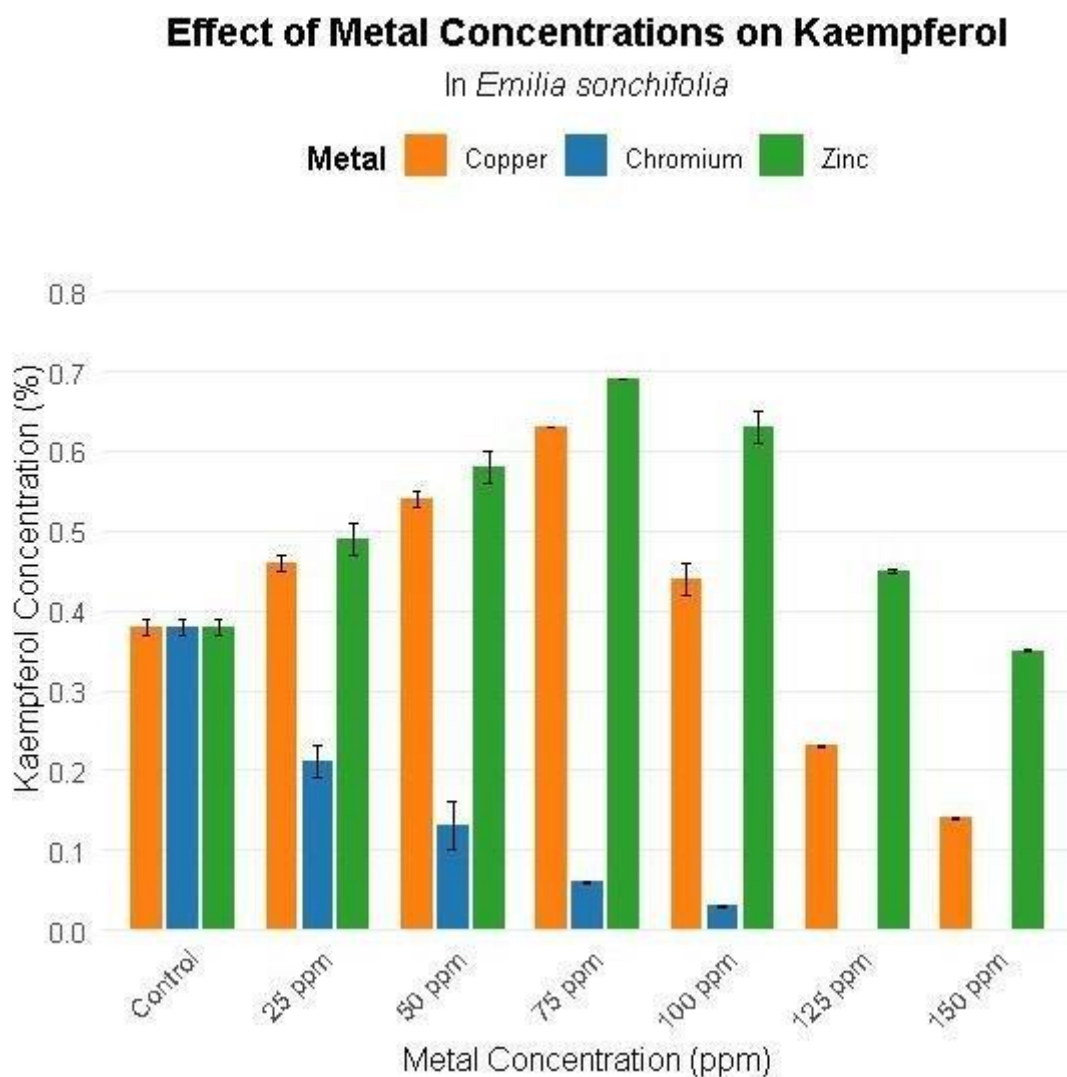


Fig .50 Effect of Metal treatment on Kaempferol content

The experimental results demonstrated significant variations in the kaempferol content of *E. sonchifolia* under different concentrations of Cu, Cr, and Zn treatments. In control plants, the kaempferol concentration was measured at 0.380 ± 0.01 mg/g. Under increasing Cu concentrations (25–75 ppm), kaempferol levels exhibited a progressive rise, peaking at 75 ppm (0.630 ± 0.001 mg/g), followed by a decline at higher concentrations (100–150 ppm). In contrast, Cr treatments induced a dose-dependent reduction in kaempferol content, with the highest level (0.210 ± 0.02 mg/g) observed at 25 ppm, followed by a sharp decline to non-detectable levels at 125 and 150 ppm, indicating pronounced phytotoxicity.

A similar trend was observed for Zn, where kaempferol content increased up to 75 ppm (1.576 ± 0.025 mg/g) before decreasing at higher concentrations (0.568 ± 0.008 to 0.690 ± 0.001 mg/g). Notably, plant survival was compromised at Cr concentrations of 125 and 150 ppm, suggesting these levels exceed the tolerance threshold of *E. sonchifolia*.

These findings highlight metal-specific regulatory effects on kaempferol metabolism. While moderate Cu and Zn levels (50–75 ppm) promoted kaempferol accumulation, Cr exhibited solely inhibitory effects, even at lower concentrations. The differential responses underscore the varying phytotoxic impacts and metabolic adaptations of *E. sonchifolia* to heavy metal exposure.

Statistical analysis

Table. 19 Two way Anova

Source	Df	Sum Sq	Mean Sq	F value
Group	18	2.007	0.1115	8429
Residuals	38	0.0005	0.00001	

The two-way ANOVA analysis revealed a highly significant interaction effect between metal type and concentration on kaempferol content ($F(18, 38) = 8429$, $*p* < 0.001$), indicating that the combined influence of these factors exerted a dominant effect on the observed variations in kaempferol levels across treatment groups. The exceptionally high F -value and the statistically significant $*p*$ -value provide strong evidence against the null hypothesis, confirming that at least some treatment groups differed significantly in their kaempferol content. Given these findings, further post-hoc analysis is recommended to identify the specific treatment levels that contribute to these differences.

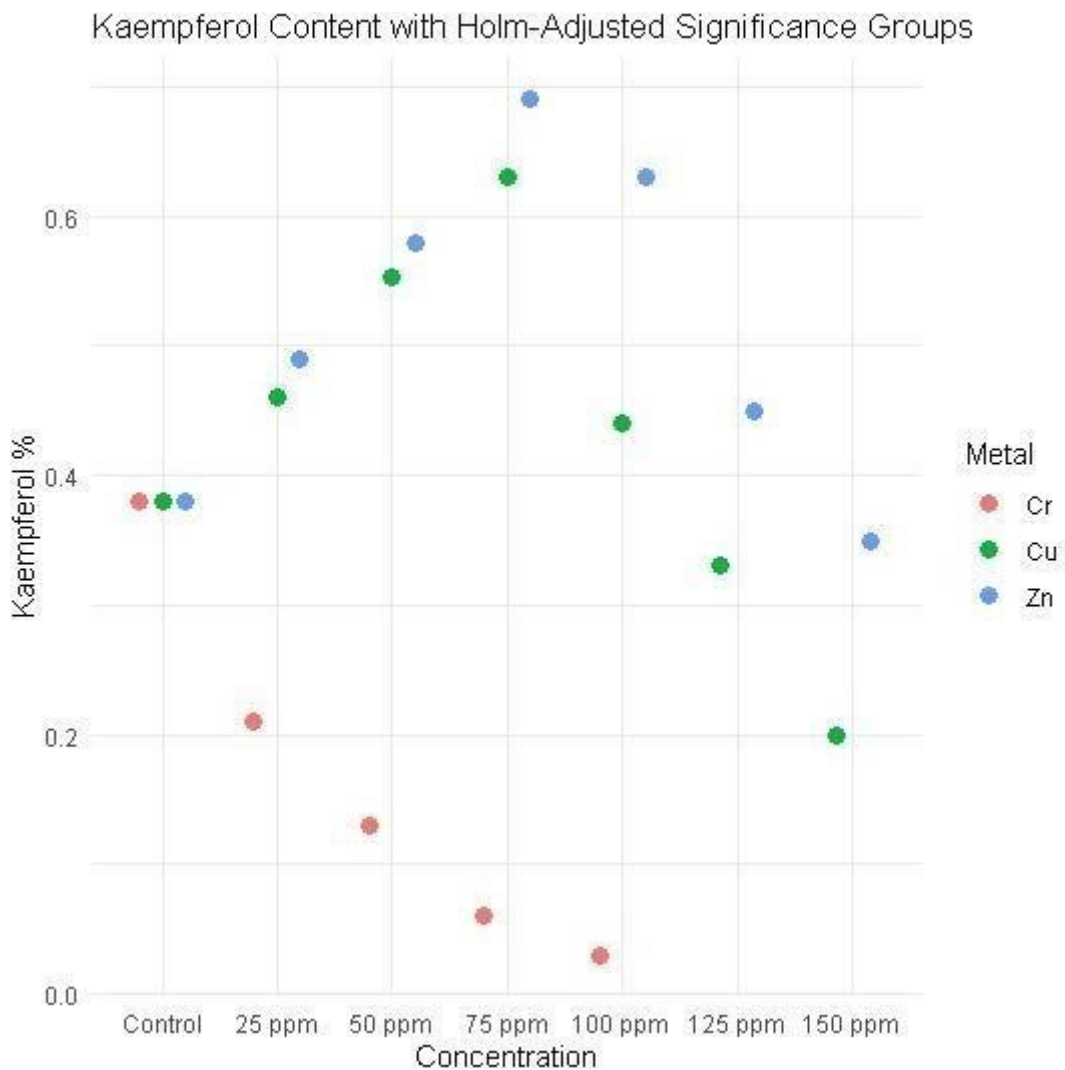


Fig. 51 Hom Post Hoc Test

The graph displays mean kaempferol content (with error bars) across varying concentrations and metal treatments, with color encoding distinguishing between metals and the x-axis organizing treatments by ascending concentration. Pairwise comparisons, adjusted using the Holm method for multiple testing, are presented to highlight statistically significant differences among treatment groups. This visualization elucidates the distinct effects of metal type and concentration on kaempferol accumulation, enabling the identification of specific treatment combinations that significantly enhance or suppress its content. The plot corroborates the ANOVA results by pinpointing where significant between-group differences occur after controlling for multiple comparisons, thereby reinforcing the robustness of the findings.

Quercetin content in *Tridax procumbens*

Quercetin Content in *Tridax procumbens* under Metal Stress

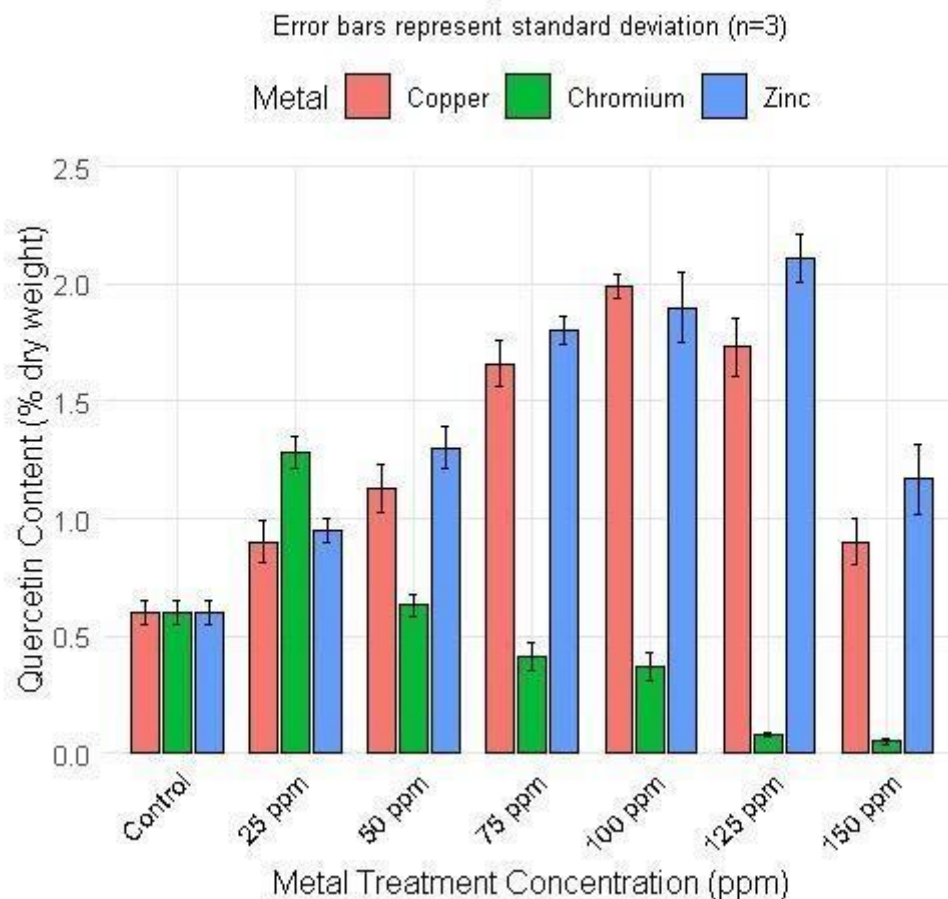


Fig. 52 Effect Of Metal Treatments On Quercetin Content

The quercetin content in *Tridax procumbens* exhibited distinct response patterns under different metal treatments. Copper (Cu) exposure induced a gradual increase in quercetin concentration, peaking at 100 ppm (1.99 ± 0.05 mg/g; representing an approximate 3.3-fold increase compared to the control), followed by a decline at higher concentrations (125–150 ppm). In contrast, chromium (Cr) treatment initially elevated quercetin levels at 25 ppm (1.28 ± 0.07 mg/g), but subsequently caused a sharp reduction to 0.05 ± 0.01 mg/g at 150 ppm, indicating significant metal toxicity. Zinc (Zn) demonstrated a sustained stimulatory effect, reaching maximum quercetin accumulation at 125 ppm (2.11 ± 0.10 mg/g; 3.5-fold higher than control) before showing a modest decrease at 150 ppm.

These findings demonstrate a clear concentration-dependent modulation of quercetin biosynthesis, with Zn acting as a potent inducer, while Cr exhibited strong inhibitory effects at elevated concentrations. The differential responses highlight the metal-specific regulation of secondary metabolite production in *T. procumbens*.

Statistical Analysis

Table.20 Two Way ANOVA

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	6	5.234	0.872	520.4	$< 2 \times 10^{-16}$
Metal	2	10.296	5.148	3071.4	$< 2 \times 10^{-16}$
Treatment × Metal	12	8.212	0.684	408.3	$< 2 \times 10^{-16}$
Residuals	42	0.07	0.002	—	—

Two-way ANOVA revealed highly significant effects of metal treatment concentration ($F = 520.4$, $p < 2 \times 10^{-16}$), metal type (Cu, Cr, Zn; $F = 3071.4$, $p < 2 \times 10^{-16}$), and their interaction ($F = 408.3$, $p < 2 \times 10^{-16}$) on quercetin content in *Tridax procumbens*. The exceptionally low p-values (all $< 2 \times 10^{-16}$) underscore that quercetin accumulation was profoundly influenced by dose-dependent metal exposure, metal-specific properties, and their synergistic interactions. Furthermore, the model's high explanatory power, evidenced by minimal residual variance (Mean Sq = 0.002), indicates it accounted for nearly all observed variability, thereby validating the robustness of these metal-induced metabolic responses. Collectively, these findings demonstrate that quercetin biosynthesis is critically regulated by both the identity and concentration of metals, with their interplay exerting a substantial modulatory effect on this secondary metabolic pathway.

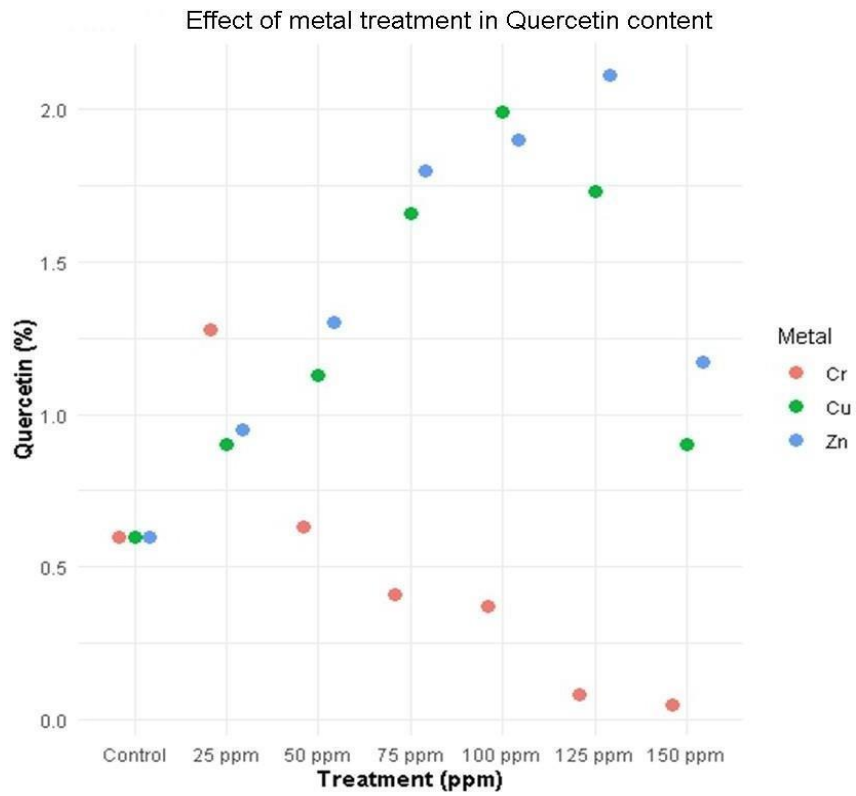


Fig 53 Holms post hoc test

Post-hoc comparisons using Holm's test revealed significant treatment \times metal interactions (Figure X), demonstrating concentration-dependent variations in quercetin accumulation. Zinc exposure produced dose-dependent increases up to 125 ppm (mean = 2.11 ± 0.10 mg/g), with levels significantly exceeding control and lower concentrations ($p < 2 \times 10^{-16}$, $F = 408.3$).

12.5 DISCUSSION

Our findings demonstrate distinct species-specific and concentration-dependent responses to Cu, Cr, and Zn in *A. paniculata*, *E. sonchifolia*, and *T. procumbens*, affecting both heavy metal accumulation and secondary metabolite production. In *A. paniculata*, Cu and Zn uptake increased progressively up to 75 ppm, while Cr accumulation declined sharply above 50 ppm. Notably, Zn exhibited the greatest bioaccumulation potential, peaking at 100 ppm before decreasing at higher concentrations, suggesting possible toxicity or uptake saturation. These observations align with previous reports identifying Cu and Zn as essential micronutrients for enzymatic and metabolic processes, with optimal uptake at moderate concentrations and toxic effects at higher levels (Nagajyoti et al., 2010). In contrast, Cr, a predominantly non-essential and toxic element, showed minimal accumulation, consistent with its known exclusion mechanisms in plants. Similar patterns were reported by Krishnamurthy and Anuradha (2018) and Zhang et al. (2021), who observed metal-specific variations in andrographolide content and antioxidant activities in *A. paniculata*.

E. sonchifolia exhibited hormetic responses to Cu and Zn, with kaempferol levels peaking at 75 ppm before declining at higher concentrations. This pattern supports established mechanisms of flavonoid production under mild oxidative stress induced by heavy metals (Michalak, 2006; Calabrese et al., 2020). Conversely, Cr progressively suppressed kaempferol synthesis, with complete inhibition at 125-150 ppm coinciding with plant mortality. These results underscore Cr's pronounced phytotoxicity, which has been shown to disrupt secondary metabolite biosynthesis, including flavonoid production, in sensitive species (Shanker et al., 2005).

In *T. procumbens*, quercetin synthesis was significantly enhanced by Cu and Zn, with Zn demonstrating the strongest induction at 125 ppm. This stimulation likely reflects activation of phenylpropanoid pathway enzymes by these metals (Michalak, 2006; Hasanuzzaman et al., 2023). Supporting evidence comes from studies documenting quercetin's antioxidant properties in stressed *T. procumbens* (Kumar & Yadav, 2014; Verma & Gupta, 2018). The inhibitory effects of Cr observed in our study mirror findings in other medicinal plants, where Cr exposure reduced growth and phenolic content through oxidative damage and enzyme inhibition (Shanker et al., 2005).

CHAPTER 13

EFFECTS OF COPPER, CHROMIUM, AND ZINC ON GROWTH, PHYTOCHEMISTRY, AND ANTIOXIDANT RESPONSES OF SELECTED MEDICINAL PLANTS :DISCUSSION

13.1 SEED GERMINATION AND EARLY GROWTH

Seed germination is one of the most sensitive stages in the plant life cycle, and the response of seeds to heavy metals often determines their ability to establish in contaminated environments. In the present study, copper (Cu) and zinc (Zn) at low concentrations (25–50 ppm) significantly enhanced germination and early seedling growth in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. This stimulation can be attributed to the essential role of these metals as micronutrients in plant metabolism. Cu functions as a cofactor in several redox enzymes such as cytochrome c oxidase, polyphenol oxidase, and superoxide dismutase, while Zn is an integral component of enzymes involved in protein synthesis and auxin metabolism (Kebert et al., 2022). Adequate availability of these metals at low doses likely facilitated enzymatic activation, energy mobilization from stored seed reserves, and efficient cellular metabolism during the germination process. Such hormetic responses, where low levels of stress enhance physiological performance, have been reported in several medicinal and crop plants exposed to trace metals (Cheng et al., 2024).

However, when concentrations exceeded 75 ppm, both Cu and Zn exhibited inhibitory effects on germination and seedling vigor. This inhibition is generally linked to oxidative stress caused by the overproduction of reactive oxygen species (ROS), which leads to lipid peroxidation, DNA damage, and disruption of protein function (López-Bucio et al., 2022). Elevated Cu levels can catalyze Fenton-like reactions, resulting in hydroxyl radical formation, while excess Zn interferes with the uptake of other essential nutrients such as iron and magnesium, further destabilizing metabolic processes. In the present study, these effects manifested as reduced germination percentages, poor seedling elongation, and lower vigor index values across all three plant species. Similar inhibitory effects of high Cu and Zn doses on germination have been documented in wheat (*Triticum aestivum*) and rice (*Oryza sativa*) seedlings (Yadav, 2010; Sharma & Dietz, 2009), underscoring the universal toxicity of these metals at elevated concentrations.

Chromium (Cr) presented a stark contrast to Cu and Zn, showing consistent toxicity even at the lowest tested concentrations. Unlike Cu and Zn, Cr has no known beneficial role in plant physiology and is often considered one of the most toxic environmental pollutants. Cr primarily exists in two oxidation states in soils: trivalent Cr(III) and hexavalent Cr(VI), with the latter being highly soluble, mobile, and toxic. Its presence disrupts seed germination by impairing water uptake, altering nutrient transport, and interfering with enzymatic activities

critical for starch mobilization during early seedling growth (Shanker et al., 2005). In the present study, *Emilia sonchifolia* emerged as the most sensitive species, with complete germination failure at higher Cr concentrations. This sensitivity could be due to its relatively weaker antioxidant defense and reduced capacity for metal sequestration. Conversely, *Tridax procumbens* exhibited greater tolerance, maintaining partial germination even under higher Cr levels. The observed interspecific variability reflects inherent differences in uptake and detoxification mechanisms, such as cell wall binding capacity, vacuolar sequestration, and production of metal-chelating compounds like phytochelatins (Ali et al., 2013).

These findings align with previous studies that demonstrated species-dependent responses of medicinal plants to heavy metals. For instance, *Ocimum sanctum* showed moderate tolerance to Cu but strong inhibition under Cr stress (Sinha & Saxena, 2006), while *Azadirachta indica* exhibited significant reductions in seed germination in the presence of Cr(VI) (Chandra & Kulshreshtha, 2004). Taken together, the current results suggest that while Cu and Zn can function as growth stimulants at low levels, their excess and the presence of Cr pose significant risks to seed germination and early plant establishment. The relative tolerance of *Tridax procumbens* highlights its adaptive strategies and potential for survival in contaminated soils, making it a candidate for phytoremediation programs targeting Cu- and Zn-polluted areas, though its germination is still compromised under Cr toxicity.

13.2 MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES

The morphological and physiological responses of plants to heavy metals provide critical insights into how stress alters growth and survival strategies. In this study, the effects of Cu, Zn, and Cr on *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* revealed clear dose-dependent patterns that align with hormetic responses observed in many plant systems. At low to moderate concentrations of Cu and Zn (25–50 ppm), both root and shoot elongation were stimulated, consistent with the essential role of these metals as cofactors in key enzymes involved in photosynthesis, respiration, and protein synthesis (Cheng et al., 2024). For instance, Zn is crucial in the structural stability of proteins such as carbonic anhydrase, which supports photosynthetic efficiency, while Cu plays an essential role in plastocyanin-mediated electron transport in photosystem I (Yruela, 2013). This explains why modest supplementation enhanced growth processes in the test species. However, as concentrations exceeded 75 ppm, the same metals began to inhibit root and shoot growth, reflecting the toxic threshold beyond which micronutrients shift from being beneficial to

being damaging. Such biphasic effects commonly termed —hormesis have been described extensively, where low stress doses trigger adaptive responses but higher doses result in oxidative damage and metabolic disruption (Kebert et al., 2022).

Chromium presented a contrasting trend, being inhibitory across all tested concentrations, which highlights its non-essential and highly toxic nature in plant metabolism. Cr exposure led to pronounced reductions in overall plant height, root elongation, and shoot biomass. These effects are consistent with previous findings that Cr interferes with photosynthetic machinery by damaging thylakoid membranes, reducing stomatal conductance, and inhibiting chlorophyll biosynthesis (López-Bucio et al., 2022; Shanker et al., 2005). Additionally, Cr disrupts root physiology by affecting aquaporin expression and water uptake, which compromises cell turgor and elongation (Ali et al., 2020). In this study, severe growth suppression under Cr stress was most evident in *Emilia sonchifolia*, which showed high levels of leaf loss and reproductive impairment, indicating its greater sensitivity. By contrast, *Tridax procumbens* displayed relatively stable morphological performance under comparable stress conditions, suggesting stronger adaptive tolerance mechanisms. This species-specific difference aligns with observations in other metal-tolerant taxa, where root plasticity, exclusion mechanisms, and differential antioxidative defenses help maintain growth under toxic environments (Cheng et al., 2024).

Physiological impairments under heavy metal exposure were further evidenced by significant declines in leaf and flower production, particularly under Cr stress. Reproductive failure in *Emilia sonchifolia* suggests that reproductive organs are highly vulnerable to oxidative stress and energy limitations under metal toxicity. Such effects have long-term ecological implications since reduced flowering and seed set compromise plant survival and dispersal in contaminated environments. Declines in photosynthetic pigments, particularly chlorophyll a and b, also confirmed the physiological impairment. Both Cu and Cr are known to directly interfere with chlorophyll biosynthesis by substituting for Mg in the porphyrin ring, leading to pigment destabilization (Sinha et al., 2018). Moreover, excess Cu can catalyze the Fenton reaction, generating hydroxyl radicals that damage chloroplast membranes, while Cr-induced oxidative stress disrupts carotenoid synthesis, further weakening light-harvesting efficiency (Ali et al., 2020). In the present study, all three species exhibited reduced pigment content, but *Tridax procumbens* again showed the least decline, reinforcing its relative resilience to metal-induced photosynthetic disruption.

Taken together, the results demonstrate that morphological and physiological responses are shaped by both the essentiality of the metal and the intrinsic tolerance strategies of each plant species. While Cu and Zn initially promoted growth before turning inhibitory, Cr exhibited universal toxicity across all concentrations. Species-specific tolerance, with *Tridax procumbens* performing better than *Emilia sonchifolia*, highlights the importance of adaptive traits such as efficient antioxidant defenses, selective ion transport, and sustained pigment stability. These findings are consistent with broader literature on hormesis, photosynthetic inhibition, and metal-specific toxicity, reinforcing the conclusion that heavy metals exert complex, dose-dependent influences on plant morphology and physiology (Cheng et al., 2024; Kebert et al., 2022; López-Bucio et al., 2022).

13.3 PHYTOCHEMICAL COMPOSITION

Phytochemicals play a central role in mediating plant defense against environmental stressors, including heavy metals. In the present study, the analysis of carbohydrate, protein, and phenolic contents in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* under Cu, Zn, and Cr treatments revealed both dose- and species-specific trends, underscoring the complex metabolic adjustments triggered by metal exposure. At lower concentrations of Cu and Zn (25–50 ppm), plants exhibited a modest increase in total carbohydrate and protein contents compared to controls. This response reflects the stimulatory role of essential metals in primary metabolism. For instance, Cu serves as a cofactor in several oxidases and electron transport proteins, while Zn is integral to the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the key enzyme of the Calvin cycle (Alloway, 2008; Broadley et al., 2007). Consequently, enhanced availability of these metals at optimal concentrations facilitates carbon fixation, protein biosynthesis, and energy metabolism. Similar enhancements of primary metabolites under low metal stress have been reported in *Ocimum basilicum* exposed to Zn (Singh et al., 2019) and in *Oryza sativa* treated with Cu (Sharma et al., 2020).

However, as metal concentrations increased beyond 75 ppm, a marked decline in carbohydrate and protein levels was observed, particularly under Cr stress. The reduction in carbohydrate reserves can be attributed to impaired photosynthesis, diminished chlorophyll content, and the diversion of energy toward stress responses rather than growth and storage (Rai et al., 2021). Chromium, in particular, is known to inhibit enzymes of the Calvin cycle and glycolysis, thereby limiting sugar accumulation and energy production (Shanker et al.,

2005). Similarly, protein degradation under heavy metal toxicity can be linked to oxidative modification of amino acids, activation of proteases, and disruption of ribosomal function (Gill et al., 2016). The sharp decline in protein content in *Emilia sonchifolia* suggests a lower ability to maintain structural and enzymatic proteins under stress, while *Tridax procumbens* retained relatively stable levels, reflecting its higher metabolic resilience.

Phenolic compounds, which are secondary metabolites, showed a contrasting pattern compared to carbohydrates and proteins. All three species exhibited a significant increase in total phenolic content under elevated Cu, Zn, and particularly Cr exposure. Phenolics are well-established non-enzymatic antioxidants that mitigate oxidative stress by chelating metal ions, scavenging reactive oxygen species (ROS), and stabilizing membranes (Michalak, 2006). Their accumulation under stress conditions is considered a hallmark of adaptive biochemical defense. In this study, *Andrographis paniculata* exhibited the strongest phenolic induction, consistent with its medicinal profile as a rich source of bioactive compounds such as andrographolide, flavonoids, and tannins (Mishra et al., 2007). The trend observed parallels findings in *Brassica juncea*, where phenolic accumulation increased significantly under heavy metal exposure, enhancing antioxidant defense capacity (Kumar et al., 2020). Likewise, in *Phaseolus vulgaris*, heavy metal stress elevated flavonoid and phenolic content, suggesting that secondary metabolism is preferentially upregulated when primary metabolism is suppressed (Ali et al., 2020).

The differential responses among the three plant species reflect their varying tolerance strategies. While *Tridax procumbens* showed a balanced maintenance of primary and secondary metabolites, *Emilia sonchifolia* was more vulnerable, with rapid reductions in carbohydrates and proteins despite increases in phenolics. By contrast, *Andrographis paniculata* exhibited the strongest upregulation of phenolics, highlighting its medicinal potential as a stress-adapted species. These species-specific metabolic adjustments align with the broader literature on metal-induced metabolic reprogramming, where primary metabolism is downregulated under stress while secondary metabolism, particularly phenolic biosynthesis, is upregulated as a compensatory defense (Gill et al., 2016; Rai et al., 2021).

Overall, the findings demonstrate that phytochemical composition is highly dynamic under heavy metal stress, reflecting a trade-off between growth and defense. Essential metals like Cu and Zn at low doses promote carbohydrate and protein accumulation by stimulating primary metabolism, while higher doses, and particularly Cr exposure, inhibit these processes

and induce oxidative damage. In response, phenolic compounds accumulate substantially, reinforcing their role as frontline biochemical protectants. This balance between primary and secondary metabolism provides valuable insight into both the tolerance strategies of these medicinal plants and their potential for phytoremediation applications, where high phenolic content can enhance resilience and ecological utility.

13.4 ANTIOXIDANT ACTIVITY

The antioxidant system of plants is one of the most crucial defense mechanisms against oxidative damage caused by heavy metals. In the current study, assays of enzymatic and non-enzymatic antioxidants revealed strong variations in superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and total antioxidant capacity among *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* exposed to Cu, Zn, and Cr. These responses highlight the central role of redox homeostasis in determining plant tolerance to metal stress.

At moderate levels of Cu and Zn, antioxidant enzyme activities were significantly enhanced compared to controls, indicating an adaptive stimulation of ROS-scavenging pathways. This observation aligns with the established roles of these metals as cofactors for antioxidant enzymes. Cu forms part of the active site of Cu/Zn-SOD, which catalyzes the dismutation of superoxide radicals into hydrogen peroxide, while Zn stabilizes membrane structures and proteins under stress (Mittler, 2017). Thus, the enhanced SOD activity in Cu- and Zn-treated plants likely reflects improved detoxification of superoxide radicals generated in chloroplasts and mitochondria during stress conditions. A similar enhancement of SOD and CAT was reported in *Triticum aestivum* under Zn exposure, suggesting that controlled doses of essential metals stimulate antioxidant defenses (Li et al., 2013).

However, at higher concentrations (≥ 100 ppm), Cu and Zn triggered oxidative damage, reflected in reduced enzymatic activity and increased signs of oxidative stress. Excess Cu is known to catalyze Fenton-like reactions, producing highly reactive hydroxyl radicals, while excess Zn interferes with the uptake of other essential nutrients, thereby impairing antioxidant defense (Yruela, 2009). The decline in CAT and POD activities in *Emilia sonchifolia* at higher metal doses underscores its lower oxidative tolerance, whereas *Tridax procumbens* maintained relatively stable activity even under elevated concentrations, suggesting its stronger oxidative buffering capacity.

The most pronounced stress was observed under Cr treatment, where both enzymatic and non-enzymatic antioxidant responses were substantially activated at moderate levels but declined sharply under high concentrations. Chromium, especially in its hexavalent form (Cr VI), induces ROS production through disruption of the electron transport chain and redox cycling (Shanker et al., 2005). In this study, *Andrographis paniculata* demonstrated the strongest induction of SOD, CAT, and POD under Cr stress, suggesting an efficient detoxification mechanism. This trend parallels findings in *Brassica juncea*, where elevated Cr induced high antioxidant enzyme activity as a protective strategy against oxidative stress (Singh et al., 2013). Nevertheless, at excessive Cr concentrations, the antioxidant machinery was overwhelmed, leading to enzymatic inhibition and metabolic decline, confirming the dual nature of antioxidant activity as both a protective and stress-sensitive system.

In addition to enzymatic antioxidants, non-enzymatic compounds such as phenolics and flavonoids contributed significantly to the observed antioxidant activity. The increased phenolic accumulation recorded in *Andrographis paniculata* and *Tridax procumbens* correlated with higher total antioxidant capacity, underscoring the importance of secondary metabolites in oxidative defense. Phenolics act as ROS scavengers and metal chelators, thereby limiting free radical propagation and stabilizing membranes (Michalak, 2006). Previous studies in *Medicago sativa* demonstrated that phenolic accumulation under heavy metal stress enhanced antioxidant activity, providing further support for this interpretation (Sytar et al., 2013).

The differential antioxidant responses among the three species suggest varying capacities for oxidative tolerance and adaptation. *Tridax procumbens* exhibited consistently strong enzymatic activity and phenolic-linked antioxidant capacity across all treatments, highlighting its robustness under metal-induced oxidative stress. *Emilia sonchifolia*, on the other hand, displayed greater susceptibility, with enzymatic defenses rapidly collapsing under high metal concentrations, which likely contributed to the greater metabolic impairments observed in carbohydrate and protein content. By contrast, *Andrographis paniculata* demonstrated a pronounced upregulation of both enzymatic and non-enzymatic antioxidants under stress, supporting its established medicinal profile and resilience.

Collectively, these results reinforce the view that the antioxidant system serves as the central axis of heavy metal tolerance in plants. Essential metals at optimal concentrations activate enzymatic and non-enzymatic defenses, while excess levels and toxic metals like Cr

overwhelm the antioxidant machinery, resulting in oxidative damage. The capacity of medicinal plants such as *Andrographis paniculata* and *Tridax procumbens* to sustain higher antioxidant activity underscores their dual potential in both phytoremediation and therapeutic applications, since enhanced antioxidant metabolism translates to improved tolerance and higher bioactive compound accumulation.

13.5 SPECIES-SPECIFIC RESPONSES

The three medicinal plants investigated in this study *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* exhibited distinct physiological, biochemical, and metabolic responses to heavy metal stress. These differences highlight the species-specific strategies plants adopt for tolerance, accumulation, and detoxification of metals such as Cu, Zn, and Cr. Understanding these variations is critical not only for elucidating plant survival mechanisms but also for selecting potential candidates for phytoremediation and medicinal use under contaminated conditions.

Andrographis paniculata demonstrated a robust antioxidant defense and phytochemical modulation under heavy metal exposure. In this study, it exhibited a marked increase in enzymatic antioxidants such as SOD and CAT, along with elevated phenolic content, particularly under moderate concentrations of Cu and Cr. These responses reflect a well-coordinated defense system that enhances tolerance by neutralizing reactive oxygen species and stabilizing cellular integrity. Previous research supports this adaptive capacity; for example, Verma and Srivastava (2018) reported that *A. paniculata* accumulates bioactive compounds such as andrographolide more effectively under moderate stress conditions, suggesting a stress-induced enhancement of secondary metabolism. This aligns with the current findings, where improved antioxidant activity and phytochemical accumulation were observed. The resilience of *A. paniculata* may be attributed to its strong medicinal profile, where secondary metabolites themselves function as both therapeutic agents and ROS scavengers (Niranjan et al., 2010).

In contrast, *Emilia sonchifolia* emerged as the most sensitive species to metal-induced stress. Its germination, seedling vigor, and metabolic stability declined rapidly under increasing concentrations of Cu, Zn, and particularly Cr. While it initially responded with some activation of antioxidant enzymes, these defenses quickly collapsed at higher concentrations, leading to greater oxidative damage and reduced carbohydrate and protein contents. This

susceptibility mirrors previous findings where *Emilia* species exhibited poor tolerance to oxidative stress compared to other medicinal plants (Chowdhury et al., 2016). The reduced metabolic resilience of *E. sonchifolia* suggests that it is less suited for survival in heavily contaminated environments and has limited phytoremediation potential. However, the plant still showed some accumulation of phenolic compounds under stress, which could indicate a protective but insufficient response.

Tridax procumbens displayed the most stable and tolerant profile among the three species, particularly under Zn and Cu exposure. This species not only maintained steady antioxidant enzyme activity but also demonstrated a higher accumulation of phenolics and flavonoids, which directly contributed to its resilience. Notably, *T. procumbens* showed the greatest ability to bioaccumulate heavy metals in its tissues, especially Cu, suggesting its potential as an effective phytoremediator. This observation corresponds with the findings of Shekhawat and Verma (2021), who reported that *T. procumbens* exhibits remarkable tolerance and uptake capacity under metal stress, attributed to its strong antioxidant system and chelation ability of secondary metabolites. The ability of *T. procumbens* to combine efficient antioxidant responses with bioaccumulation potential underscores its dual ecological and medicinal importance.

Comparative analysis among the three species suggests that while *A. paniculata* and *T. procumbens* possess strong adaptive mechanisms to counteract heavy metal toxicity, they differ in their strategies. *A. paniculata* relies heavily on metabolic adjustments, especially through enhanced antioxidant enzyme activity and secondary metabolite synthesis, which supports both stress tolerance and medicinal quality. On the other hand, *T. procumbens* emphasizes stability in physiological processes and efficient sequestration of metals, making it highly suitable for phytoremediation applications. In contrast, *E. sonchifolia* demonstrates limited tolerance, with weak antioxidant and metabolic resilience, positioning it as the least adaptable species under the tested conditions.

Taken together, these findings underscore the significance of species-specific traits in determining plant survival and functional roles under metal stress. While *Andrographis paniculata* emerges as a resilient medicinal candidate with enhanced therapeutic potential under moderate stress, *Tridax procumbens* is highlighted as an effective phytoremediator due to its strong accumulation capacity and oxidative tolerance. Conversely, *Emilia sonchifolia* represents a stress-sensitive species with limited ecological utility under contaminated

environments. These insights not only strengthen our understanding of interspecies variation in stress responses but also provide a framework for future applications of medicinal plants in sustainable phytoremediation and pharmacological contexts.

13.6 BIOACCUMULATION AND PHYTOREMEDIATION POTENTIAL

The ability of plants to tolerate, accumulate, and sequester heavy metals within their tissues is an important criterion for assessing their phytoremediation potential. In the present study, the three medicinal plants exhibited varying levels of bioaccumulation of Cu, Cr, and Zn, highlighting species-specific differences in their potential roles in remediation of metal-contaminated environments. Among them, *Tridax procumbens* emerged as the most effective bioaccumulator, followed by *Andrographis paniculata*, whereas *Emilia sonchifolia* demonstrated the least accumulation capacity. These findings provide critical insights into the ecological and pharmacological relevance of these species under heavy metal stress.

Tridax procumbens showed the highest accumulation of Cu and Zn in its shoot tissues, suggesting efficient uptake and transport mechanisms. Its ability to tolerate elevated concentrations while maintaining growth and metabolic activity indicates its strong phytoremediation potential. This aligns with previous studies that reported *T. procumbens* as a promising candidate for phytoextraction, with efficient metal uptake and high biomass production under contaminated conditions (Shekhawat & Verma, 2021). The high antioxidant activity observed in *T. procumbens* in this study likely supports its accumulation capacity by mitigating oxidative stress generated by excessive metal ions. Furthermore, its widespread occurrence and adaptability to diverse environments make it an ecologically viable option for large-scale remediation.

Andrographis paniculata demonstrated moderate but significant accumulation of Cu and Cr. While its uptake capacity was lower than that of *T. procumbens*, it exhibited a unique response in terms of metabolic adaptation. Elevated accumulation of secondary metabolites such as andrographolide and phenolic compounds was observed under moderate stress conditions, suggesting that the plant may integrate its medicinal value with remediation potential. This dual role is particularly important because *A. paniculata* not only sequesters metals but also enhances its therapeutic profile under stress, making it useful both as a medicinal resource and as a phytoremediator. Verma and Srivastava (2018) similarly reported

that *A. paniculata* enhances its antioxidant activity and andrographolide content under metal stress, supporting its role as a stress-tolerant medicinal species.

In contrast, *Emilia sonchifolia* exhibited limited bioaccumulation ability. Its tissues showed relatively low concentrations of Cu, Cr, and Zn, coupled with poor growth and reduced antioxidant responses. This indicates that *E. sonchifolia* lacks efficient uptake and detoxification mechanisms required for successful phytoremediation. Previous research has shown that sensitive species like *E. sonchifolia* often exhibit metal exclusion strategies rather than accumulation, reducing their phytoremediation capacity (Chowdhury et al., 2016). As such, while *E. sonchifolia* retains certain medicinal properties, it cannot be considered a strong candidate for environmental cleanup of heavy metal pollution.

From an applied perspective, the findings suggest that *T. procumbens* is best suited for phytoextraction—the removal of metals from soil and water because of its high accumulation ability and stress tolerance. *Andrographis paniculata*, on the other hand, holds promise for phytostabilization, where metals are immobilized in the rhizosphere or stored in plant tissues without significant translocation. Its ability to maintain medicinal quality under stress adds further value, making it an attractive dual-purpose plant for both ecological and pharmacological applications. Conversely, *E. sonchifolia* is more likely to serve as a background species in contaminated sites rather than an active phytoremediator.

The differential accumulation patterns observed in this study align with general models of plant–metal interactions, where species adopt either accumulation, tolerance, or exclusion strategies depending on their metabolic and physiological adaptations (Ali et al., 2013; Mahar et al., 2016). Identifying species like *T. procumbens* and *A. paniculata* that combine stress tolerance with practical ecological and medicinal functions can guide sustainable phytoremediation practices, particularly in regions where heavy metal contamination poses both environmental and public health risks.

Overall, the findings underscore that medicinal plants can serve as eco-friendly and cost-effective agents in phytoremediation while simultaneously retaining their therapeutic properties. By integrating bioaccumulation efficiency with secondary metabolite dynamics, this study highlights the potential for selecting suitable species not only for remediation of polluted environments but also for safe medicinal use in metal-stressed ecosystems.

13.7 THERAPEUTIC AND PHARMACOLOGICAL IMPLICATIONS

The therapeutic significance of medicinal plants is closely tied to their secondary metabolite composition, which can be profoundly influenced by environmental stressors such as heavy metals. In this study, *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* exhibited distinct phytochemical responses under copper (Cu), chromium (Cr), and zinc (Zn) exposure, with implications for their pharmacological properties. Understanding how heavy metal stress modulates bioactive compounds provides critical insights into both the risks and potential benefits of using these plants in traditional and modern medicine.

Andrographis paniculata, well known for its diterpenoid lactone andrographolide, demonstrated a remarkable increase in its bioactive content under moderate concentrations of heavy metal stress. This upregulation of secondary metabolites may be linked to the plant's adaptive defense mechanisms against oxidative damage. Enhanced levels of andrographolide and phenolic compounds not only improve the plant's antioxidant activity but also strengthen its pharmacological profile, particularly in anti-inflammatory, hepatoprotective, and immunomodulatory applications (Gupta et al., 2020; Verma & Srivastava, 2018). The findings suggest that controlled exposure to mild stress may act as an elicitor to enhance the therapeutic efficacy of *A. paniculata*, a phenomenon widely reported in medicinal plants exposed to abiotic stresses (Ramani & Chellappan, 2020).

Similarly, *Tridax procumbens* displayed an increase in phenolic and flavonoid content under Cu and Zn exposure. This enhancement was accompanied by strong antioxidant activity, which correlates with its traditional medicinal use in wound healing, hepatoprotection, and anti-inflammatory treatments (Kumar et al., 2019). The accumulation of secondary metabolites under stress conditions indicates that *T. procumbens* not only contributes to phytoremediation but also maintains its pharmacological relevance in stressed environments. Importantly, its ability to thrive in contaminated soils suggests that it could serve as a sustainable source of phytochemicals for pharmaceutical applications, provided safety concerns related to heavy metal residues are addressed.

By contrast, *Emilia sonchifolia* showed a decline in its phytochemical content under heavy metal stress. Reduced levels of flavonoids, alkaloids, and phenolics suggest that the plant's medicinal value may diminish in contaminated environments. Given that *E. sonchifolia* is traditionally used for treating inflammation, respiratory issues, and as an anticancer remedy

(Singh & Sharma, 2017), the reduction in bioactive compounds under stress is concerning. This decline highlights the vulnerability of certain medicinal plants to environmental pollutants and underscores the importance of sourcing such plants from uncontaminated habitats to preserve their therapeutic integrity.

A key consideration arising from this study is the dual-edged impact of heavy metals: while mild stress may enhance phytochemical accumulation in tolerant plants, excessive exposure risks contaminating the very same plants with toxic residues. This raises significant pharmacological and public health questions regarding the safety of medicinal plant use from polluted areas. Previous studies have reported that heavy metal accumulation in medicinal plants can pose serious health risks, including hepatotoxicity and carcinogenic effects, when contaminated plant materials enter the human food chain (Sharma et al., 2016; Khan et al., 2021). Thus, while plants like *A. paniculata* and *T. procumbens* show promise in enhancing their medicinal value under moderate stress, strict monitoring of heavy metal levels is essential before their therapeutic application.

Moreover, the interplay between phytoremediation and pharmacology is particularly noteworthy. Plants with high bioaccumulation potential, such as *T. procumbens*, may serve as —dual-purposed species that remediate contaminated soils while providing bioactive compounds for pharmaceutical use. However, rigorous quality control is necessary to ensure that therapeutic extracts are free from harmful metal residues. Integrating phytoremediation with medicinal plant cultivation may thus offer an innovative approach to sustainable healthcare and environmental management (Ali et al., 2013).

In conclusion, the therapeutic implications of heavy metal stress on medicinal plants are multifaceted. While tolerant species such as *A. paniculata* and *T. procumbens* exhibit stress-induced enhancement of pharmacologically relevant compounds, sensitive species like *E. sonchifolia* suffer a decline in medicinal value. These findings underscore the necessity of balancing the potential pharmacological benefits of stress-induced phytochemical enhancement with the safety risks associated with heavy metal contamination. Future research should focus on optimizing cultivation strategies to harness the elicitor effect of metals without compromising medicinal safety, thereby ensuring that these species continue to play their vital roles in both traditional medicine and modern pharmacotherapy.

13.8 ECOLOGICAL AND ENVIRONMENTAL SIGNIFICANCE

The ecological and environmental significance of medicinal plants under heavy metal stress lies not only in their survival strategies but also in their role as bioindicators and phytoremediators in polluted ecosystems. Heavy metal contamination is a persistent environmental challenge caused by industrial effluents, mining, urbanization, and agricultural runoff. Unlike organic pollutants, heavy metals are non-biodegradable and accumulate in soil, water, and biota, leading to long-term ecological disruptions (Nagajyoti et al., 2010). In this context, the responses of *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens* to Cu, Cr, and Zn stress provide valuable insights into their ecological resilience, adaptability, and environmental utility.

Among the three species studied, *Tridax procumbens* emerged as the most ecologically resilient plant. Its high bioaccumulation potential for Cu and Zn highlights its ability to tolerate and stabilize contaminated soils. This characteristic positions *T. procumbens* as a potential candidate for phytoremediation strategies, where plants are utilized to extract, immobilize, or detoxify heavy metals from polluted sites (Ali et al., 2013). By establishing itself in metal-contaminated environments, *T. procumbens* contributes to ecological restoration by preventing further mobilization of metals into groundwater and reducing the risk of bioaccumulation in food chains. Additionally, its ability to maintain phytochemical activity under stress suggests that it plays a dual ecological role—as both a stabilizer of polluted environments and a reservoir of bioactive compounds.

In contrast, *Andrographis paniculata* displayed a moderate ecological significance. Its tolerance to Cu and Zn stress, accompanied by an increase in secondary metabolites, suggests that it can adapt to moderately polluted environments. However, the species is more sensitive to high Cr concentrations, reflecting the broader ecological concern associated with chromium pollution. Chromium, particularly in its hexavalent form (Cr VI), is highly toxic, carcinogenic, and disruptive to soil microbial communities, reducing biodiversity and altering ecosystem functions (Zayed & Terry, 2003). The sensitivity of *A. paniculata* to Cr contamination serves as an ecological warning sign and reinforces its potential role as a bioindicator species. The decline in its phytochemical content and growth under high Cr exposure can serve as a measurable marker of chromium-induced ecological stress in contaminated sites.

Emilia sonchifolia, by contrast, demonstrated greater vulnerability to heavy metal exposure, with reduced phytochemical accumulation and antioxidant activity. From an ecological perspective, this sensitivity indicates that *E. sonchifolia* may not thrive in polluted habitats, and its reduced abundance could serve as a signal of ecosystem degradation. However, its vulnerability also underscores the broader ecological risks of heavy metal contamination: the loss of sensitive medicinal plants not only diminishes biodiversity but also disrupts traditional healthcare systems that rely on these species for primary remedies (Singh & Sharma, 2017). This highlights the urgent need for conservation strategies aimed at protecting sensitive species from extinction pressures in contaminated ecosystems.

The environmental significance of these findings extends to soil health and ecosystem balance. Heavy metals interfere with soil enzymatic activity, microbial community diversity, and nutrient cycling, which in turn affect plant growth and survival (Ma et al., 2016). The ability of certain species, such as *T. procumbens*, to colonize and stabilize contaminated soils contributes to maintaining ecological functions, preventing erosion, and supporting successional plant communities. This ecological service is critical in areas affected by industrial waste, such as tanning effluents or mining runoff, where traditional remediation strategies may be economically or technically unfeasible.

Another important ecological implication is the potential of these plants to function as sentinel species in environmental monitoring programs. By analyzing changes in plant physiology, phytochemical accumulation, and growth under varying metal concentrations, scientists and policymakers can assess pollution severity and track the progress of remediation efforts. Medicinal plants are particularly valuable in this regard because of their wide distribution, cultural importance, and sensitivity to environmental stressors (Sharma et al., 2016).

Finally, the ecological findings of this study emphasize the need for a balanced perspective between environmental management and medicinal plant utilization. While tolerant species such as *T. procumbens* can play a constructive role in phytoremediation and ecological restoration, their use in medicine must be carefully regulated to prevent the introduction of toxic residues into the human food chain. Conversely, sensitive species like *E. sonchifolia* highlight the fragility of ecosystems under metal stress and reinforce the necessity of environmental policies aimed at reducing industrial pollution at the source.

In conclusion, the ecological and environmental significance of this study lies in its demonstration that medicinal plants are not passive victims of pollution but active participants in ecological resilience and remediation. *T. procumbens* functions as a stabilizer and potential phytoremediator, *A. paniculata* serves as both a moderately tolerant species and a bioindicator of Cr stress, while *E. sonchifolia* reflects ecosystem vulnerability. Together, these species provide a multifaceted understanding of how medicinal plants contribute to ecosystem sustainability, restoration, and monitoring in the face of heavy metal contamination.

CHAPTER 14
SUMMARY AND CONCLUSION

This study comprehensively evaluated the effects of heavy metals copper (Cu), chromium (Cr), and zinc (Zn) on three medicinal plant species: *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*. The research examined multiple dimensions, including seed germination, growth and morphology, phytochemical composition, antioxidant responses, bioaccumulation potential, therapeutic implications, and ecological significance, thereby providing a holistic understanding of how these metals influence plant physiology, biochemistry, and potential applications.

Seed Germination and Early Growth:

The results revealed that low concentrations of Cu and Zn (25–50 ppm) stimulated seed germination and early seedling development across all three species, highlighting their role as essential micronutrients facilitating enzymatic activity and growth processes. Conversely, higher concentrations (>75 ppm) led to inhibitory effects, likely due to oxidative stress and disruption of cellular metabolism. Chromium, even at low concentrations, proved consistently toxic, reducing germination rates and seedling vigor, with *Emilia sonchifolia* being the most sensitive and *Tridax procumbens* demonstrating relative tolerance. These findings underscore the species-specific resilience mechanisms in response to heavy metal stress.

Morphological and Physiological Responses:

Root and shoot growth followed a biphasic pattern under Cu and Zn exposure, with promotion at lower concentrations and inhibition at higher levels, reflecting hormetic responses. Chromium uniformly suppressed growth, affecting chlorophyll content, leaf production, and flowering. *Tridax procumbens* maintained relatively better growth and photosynthetic capacity under metal stress, whereas *Emilia sonchifolia* experienced severe morphological and reproductive impairment. These results highlight the impact of heavy metals on primary physiological processes and the differential adaptive capacity of these species.

Phytochemical Composition: Secondary metabolites, including alkaloids, flavonoids, saponins, tannins, and terpenoids, were modulated by heavy metal exposure. Low-to-moderate Cu and Zn concentrations enhanced their accumulation, reflecting stress-induced activation of defense pathways, whereas high metal concentrations, particularly Cr, suppressed metabolite synthesis. *Andrographis paniculata* exhibited increased

andrographolide under Cu and Zn stress, while *Tridax procumbens* maintained stability across most metabolite classes, indicating robust metabolic adaptability. *Emilia sonchifolia* showed the most pronounced decline under stress.

Antioxidant Responses:

Antioxidant capacity, measured through DPPH scavenging activity and superoxide dismutase (SOD) activity, peaked at moderate metal concentrations, demonstrating the plants' activation of defense mechanisms against reactive oxygen species. *Tridax procumbens* exhibited the highest SOD activity, reflecting efficient ROS detoxification, whereas *Emilia sonchifolia* had the lowest, correlating with its sensitivity to metal stress. These findings indicate that antioxidant responses are critical markers of stress tolerance in medicinal plants.

Bioaccumulation and Phytoremediation Potential:

The study revealed significant species-specific differences in metal uptake. *Tridax procumbens* accumulated the highest levels of Cu and Zn in roots and shoots, positioning it as a promising candidate for phytoremediation. *Andrographis paniculata* selectively accumulated Cr at moderate concentrations, while *Emilia sonchifolia* largely avoided metal uptake, suggesting avoidance as a tolerance strategy. These differential accumulation patterns provide insight into ecological strategies and potential environmental applications.

Therapeutic and Pharmacological Implications:

Heavy metal stress influenced the biosynthesis of key bioactive compounds, such as andrographolide, kaempferol, and quercetin. Moderate Cu and Zn exposure enhanced metabolite accumulation in tolerant species, potentially increasing pharmacological efficacy. However, excessive heavy metal accumulation posed safety concerns, particularly for species with higher bioaccumulation potential. These results emphasize the dual effect of heavy metals: acting as elicitors of secondary metabolism while posing risks of toxicity, necessitating strict quality control for medicinal plant utilization.

Ecological and Environmental Significance:

The study demonstrated that medicinal plants serve as both indicators of environmental contamination and agents of ecosystem restoration. *Tridax procumbens* showed high resilience and phytoremediation potential, stabilizing contaminated soils and contributing to ecosystem health. *Andrographis paniculata* served as a bioindicator, particularly for

chromium stress, while *Emilia sonchifolia* reflected ecosystem vulnerability. These findings underline the importance of integrating phytoremediation and medicinal plant cultivation with environmental management practices.

Conclusion:

Overall, this research highlights the complex interactions between heavy metals and medicinal plants. Low-to-moderate concentrations of essential metals can enhance germination, growth, secondary metabolite production, and antioxidant defenses in tolerant species, whereas excessive metal exposure, particularly chromium, is detrimental to growth, physiology, and medicinal quality. *Tridax procumbens* emerged as the most resilient species, with significant ecological and phytoremediation potential, while *Emilia sonchifolia* was the most sensitive. The findings provide critical insights for the sustainable use of medicinal plants in pharmacology, environmental monitoring, and soil remediation, emphasizing the need for careful management of heavy metal exposure to maximize benefits while minimizing ecological and health risks.

CHAPTER 15
KEY FINDINGS

- **Seed Germination and Early Growth:** Low concentrations of Cu and Zn (25–50 ppm) stimulated seed germination and early seedling growth in *Andrographis paniculata*, *Emilia sonchifolia*, and *Tridax procumbens*, while higher concentrations (>75 ppm) inhibited germination. Chromium was consistently toxic even at low doses, with *Emilia sonchifolia* being the most sensitive and *Tridax procumbens* the most tolerant.
- **Morphological and Physiological Responses:** Root and shoot elongation, leaf production, and flowering were promoted at low Cu and Zn concentrations but reduced at higher levels. Chromium exposure caused uniform growth inhibition and chlorophyll decline. *Tridax procumbens* showed the greatest tolerance, maintaining photosynthetic capacity and morphological integrity under stress.
- **Phytochemical Composition:** Secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and terpenoids increased at low-to-moderate Cu and Zn concentrations, reflecting stress-induced defence responses. High concentrations, particularly of Cr, suppressed metabolite production. *Andrographis paniculata* exhibited enhanced andrographolide under Cu and Zn stress, while *Tridax procumbens* maintained metabolite stability.
- **Antioxidant Responses:** Antioxidant activity, measured via DPPH scavenging and SOD activity, peaked at moderate metal concentrations (50–75 ppm) and declined at higher levels. *Tridax procumbens* displayed the strongest ROS detoxification capacity, whereas *Emilia sonchifolia* had the lowest antioxidant activity, correlating with its sensitivity.
- **Bioaccumulation and Phytoremediation Potential:** *Tridax procumbens* accumulated the highest levels of Cu and Zn in roots and shoots, highlighting its suitability for phytoremediation. *Andrographis paniculata* selectively accumulated Cr at moderate doses, whereas *Emilia sonchifolia* largely avoided metal uptake. Species-specific accumulation patterns indicate different tolerance strategies, including sequestration and avoidance.
- **Therapeutic Implications:** Moderate Cu and Zn exposure enhanced bioactive compounds such as andrographolide, kaempferol, and quercetin, potentially increasing pharmacological efficacy. Excessive metal accumulation, however, compromised secondary metabolite production and posed safety concerns for medicinal use.

- **Ecological Significance:** *Tridax procumbens* demonstrated high ecological resilience, contributing to soil stabilization and ecosystem restoration in metal-contaminated areas. *Andrographis paniculata* served as a bioindicator for chromium stress, while *Emilia sonchifolia* reflected environmental vulnerability. These findings support the use of medicinal plants in environmental monitoring and phytoremediation strategies.
- **Species-Specific Tolerance:** Overall, *Tridax procumbens* was the most tolerant species across multiple parameters, followed by *Andrographis paniculata*, while *Emilia sonchifolia* was the most sensitive. This highlights the importance of species selection for phytoremediation, cultivation in polluted areas, and sustainable medicinal use.

CHAPTER 16
RECOMMENDATIONS

- **Controlled Use of Metals for Elicitation:** Low to moderate concentrations of essential metals like copper (Cu) and zinc (Zn) can be applied as controlled elicitors to enhance the production of bioactive compounds in medicinal plants, particularly in *Andrographis paniculata* and *Tridax procumbens*. Careful monitoring is essential to avoid toxic accumulation.
- **Phytoremediation Applications:** *Tridax procumbens* should be considered a promising candidate for phytoremediation of Cu- and Zn-contaminated soils due to its high accumulation and tolerance. *Andrographis paniculata* could be utilized for Cr-contaminated sites at moderate concentrations, whereas *Emilia sonchifolia* is not recommended for heavy metal remediation due to its sensitivity.
- **Monitoring and Bioindicator Use:** *Andrographis paniculata* and *Emilia sonchifolia* can serve as bioindicator species for assessing chromium contamination in soils and water. Regular monitoring of these species can provide early warning of environmental heavy metal stress.
- **Medicinal Plant Cultivation and Root Use:** Given that bioaccumulation studies revealed higher metal concentrations in roots compared to shoots, it is strongly recommended to avoid using roots for therapeutic or medicinal purposes in plants grown in metal-exposed areas. Leaves and aerial parts with lower metal content are safer for medicinal applications.
- **Conservation and Biodiversity Protection:** Sensitive species like *Emilia sonchifolia* should be prioritized for conservation in areas with known metal contamination to prevent loss of biodiversity and maintain ecosystem functions.
- **Environmental Management:** Industrial effluents containing heavy metals should be treated before discharge into agricultural or natural habitats to reduce ecological and health risks. Policies enforcing strict quality control for soil and water management will minimize heavy metal exposure to medicinal plants.
- **Further Research:** Additional studies are recommended to explore the molecular mechanisms underlying species-specific tolerance, metal sequestration, and secondary metabolite modulation. Investigating endophytic microbial associations may further enhance phytoremediation potential and plant resilience.

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Research Publications

- ▶ Hridhya, M. J., & Anitha, C. T. (2022). Effect of heavy metals (Cu, Zn and Cr) on total carbohydrate and protein content in *Andrographis paniculata* (Burm. f.) Wall. ex Nees. *Bulletin of Pure and Applied Sciences, Section B: Botany*, 41(1), 71–74.
- ▶ Hridhya, M. J., & Anitha, C. T. (2025). Influence of heavy metals (Cu, Cr, Zn) on total phenolics and antioxidant potential in *Andrographis paniculata* (Burm. f.) Wall. ex Nees. *International Journal of All Research Education and Scientific Methods (IJARESM)*, 13(8).
- ▶ Hridhya, M. J., & Anitha, C. T. (2023). Effect of leather industrial effluent on the germination and seedling growth of selected medicinal plants. *Pollution Research*, 42(1), 88–93.
- ▶ Hridhya, M. J., & Anitha, C. T. (n.d.). Influence of heavy metals (Cu, Cr, Zn) on antioxidant potential in *Emilia sonchifolia* (L.) DC. *International Journal of Scientific Research in Engineering and Management (IJSREM)*, 9.

APPENDIX A:

HPLC CHROMATOGRAMS

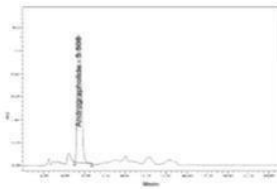
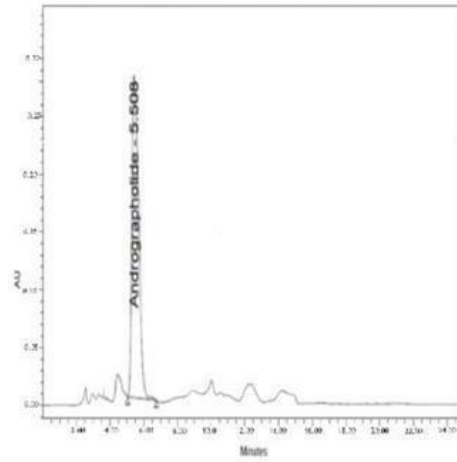


Fig. A.2

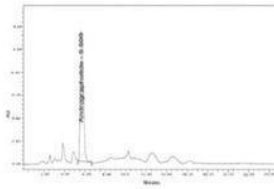


Fig. A.3

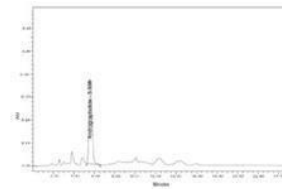


Fig. A.4

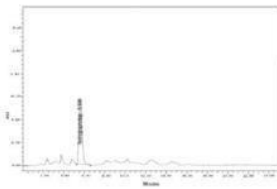


Fig. A.5

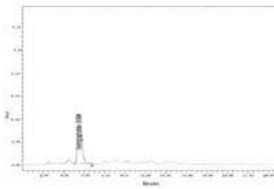


Fig. A.6

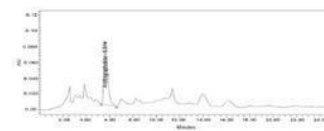


Fig. A.7

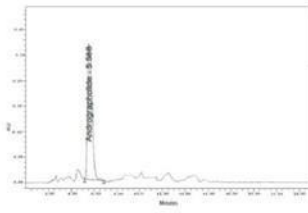


Fig. A. 8

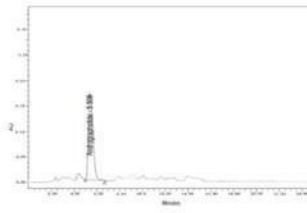


Fig. A. 9

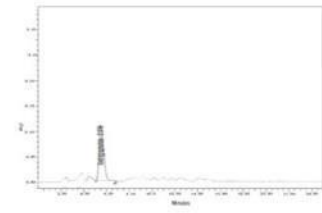


Fig. A. 10

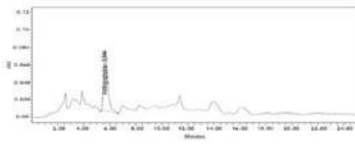


Fig. A. 11

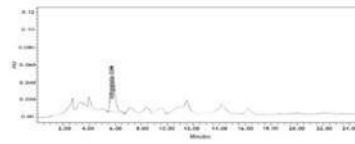


Fig. A. 12

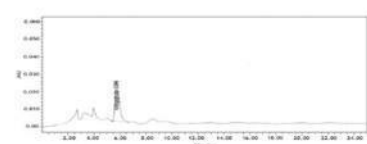


Fig. A. 13

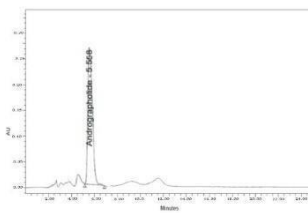


Fig. A. 14

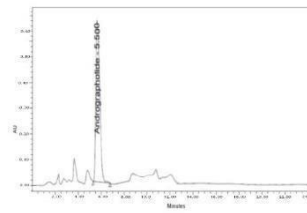


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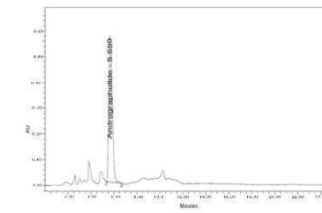


Fig. A. 16

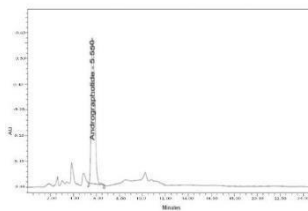


Fig. A. 17

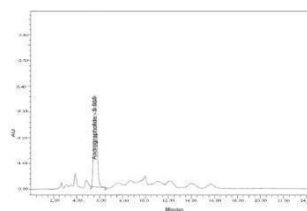


Fig. A. 18

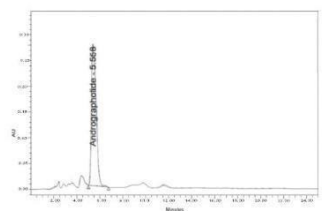


Fig. A. 19

Fig A. 1 : Chromatogram of standard c andrographolide (250µg/ml) , Fig A. 2- fig. A.7: Chromatogram of *Andrographis paniculata* treated with Cu 25 ppm to Cu 150 ppm , Fig A. 7- fig. A.13:Chromatogram of *Andrographis paniculata* treated with Cr 25 ppm to Cr 150 ppm Fig A. 14- fig. A.19:Chromatogram of *Andrographis paniculata* treated with Zn 25 ppm to Zn 150 ppm

FIG. B.1

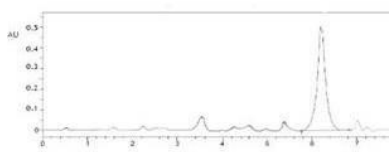
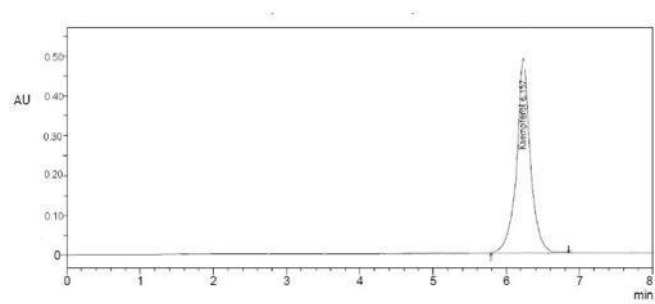


Fig. B. 2

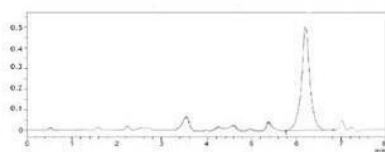


Fig. B. 3

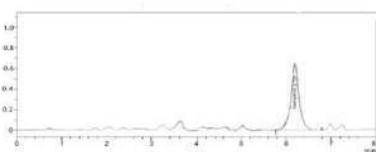


Fig. B. 4

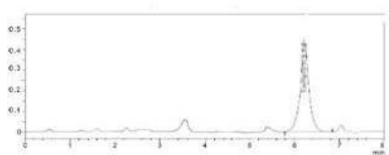


Fig. B. 5



Fig. B. 6



Fig. B. 7

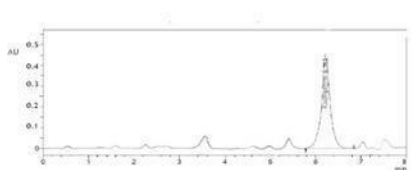


Fig. B. 12

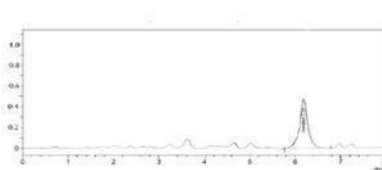


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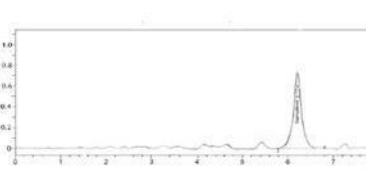


Fig. B. 14

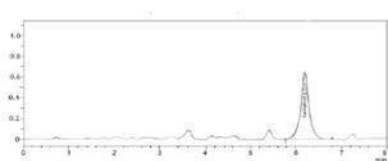


Fig. B. 15

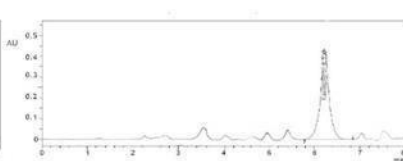


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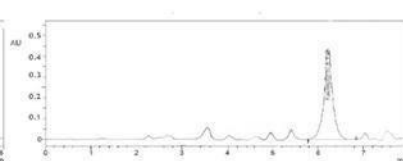


Fig. B. 17

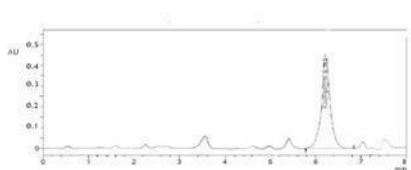


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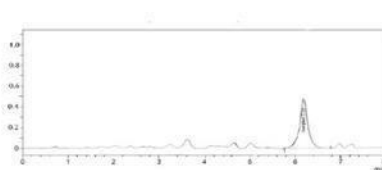


Fig. B. 13

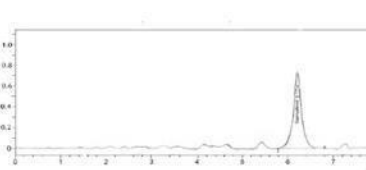


Fig. B. 14

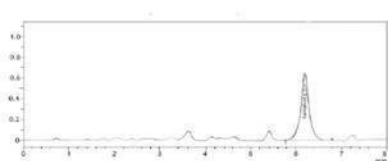


Fig. B. 15

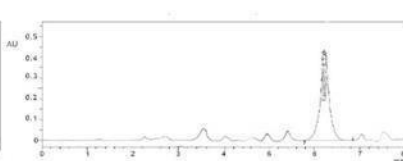


Fig. B. 16

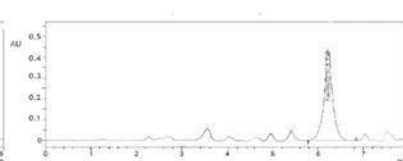


Fig. B. 17

Fig B. 1 : Chromatogram of standard kAEMPFEROL (250µg/ml) , Fig B. 2- fig. A.7: Chromatogram of *Emilia sonchifolia* treated with Cu 25 ppm to Cu 150 ppm , Fig B. 7- fig. B.13: Chromatogram of *Emilia sonchifolia* treated with Cr 25 ppm to Cr 150 ppm Fig B. 14- fig. B.19:Chromatogram *Emilia sonchifolia paniculata* treated with Zn 25 ppm to Zn 150 ppm

Fig. C. 1

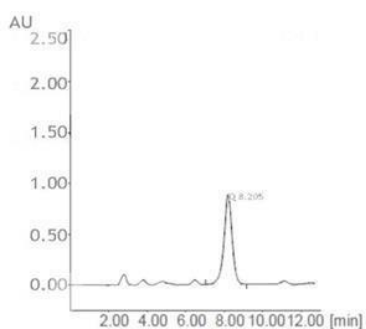
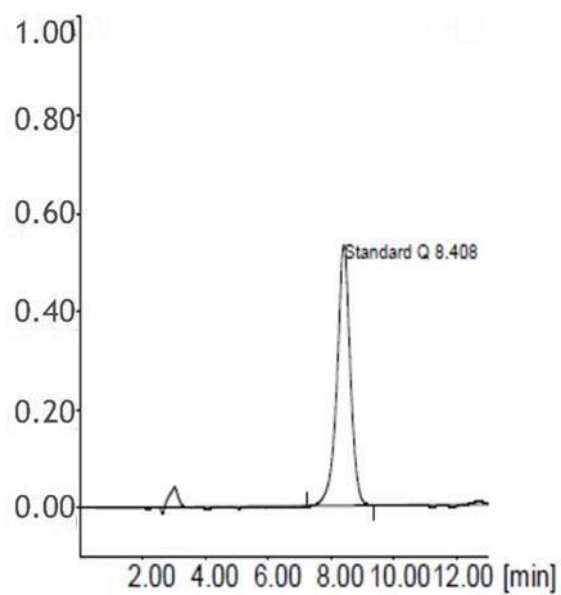


Fig. C. 2

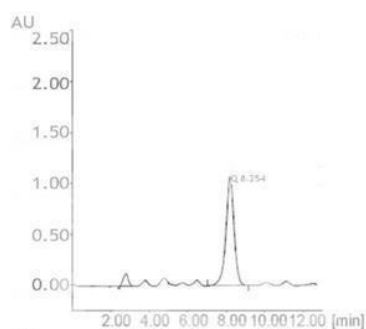


Fig. C. 3

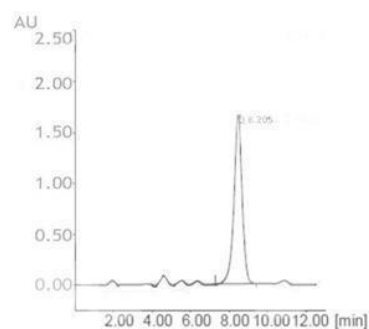


Fig. C. 4

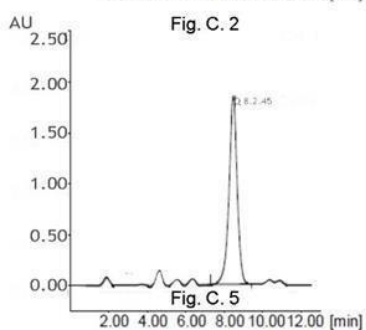


Fig. C. 5

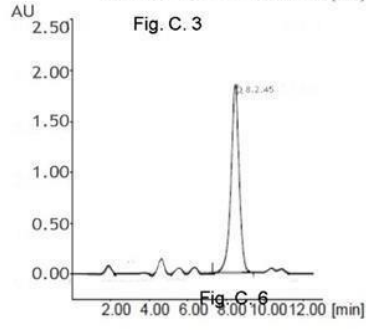


Fig. C. 6

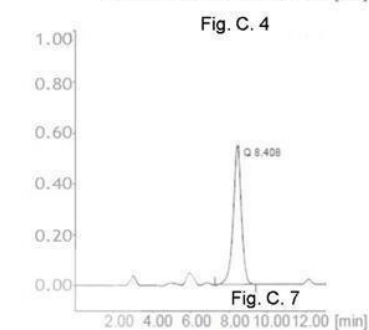


Fig. C. 7

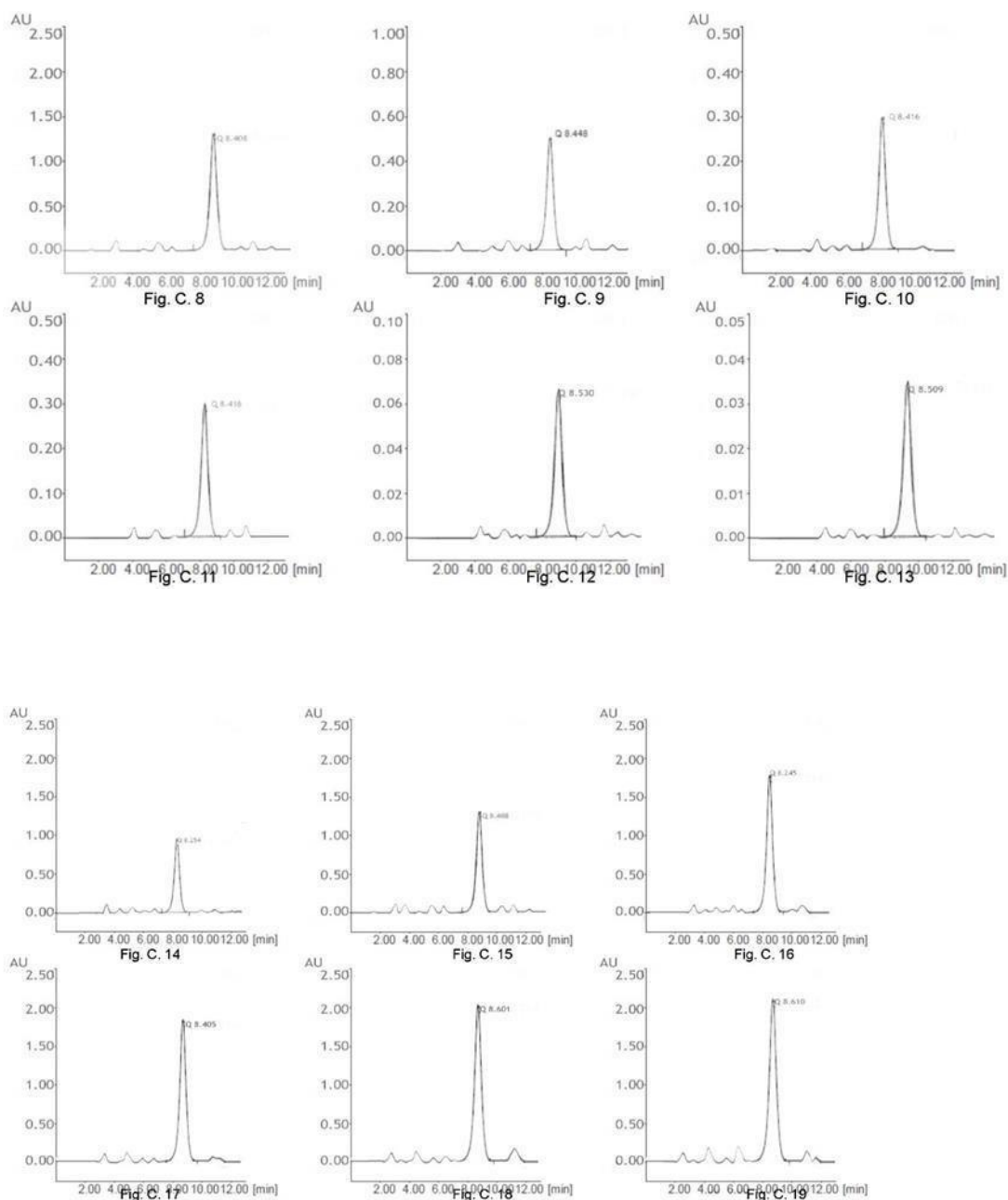


Fig C. 1 : Chromatogram of standard Quercetin (250µg/ml) , Fig C. 2- fig. A.7: Chromatogram of *Tridsc procumbens* treated with Cu 25 ppm to Cu 150 ppm , Fig C. 7- figC.13: Chromatogram of *Tridsc procumbens* treated with Cr 25 ppm to Cr 150 ppm Fig C. 14- fig. C.19:Chromatogram *Tridsc procumbens* paniculata treated with Zn 25 ppm to Zn 150 ppm