EFFECT OF HEAVY METALS ON PHYSIOLOGY AND ANATOMY OF *ARTEMISIA NILAGIRICA* (CLARKE) PAMP.

Thesis submitted to the
University of Calicut in partial fulfilment of the requirements
for the award of the Degree of

DOCTOR OF PHILOSOPHY IN BOTANY
Under the Faculty of Science

By

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Under the Guidance of

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This is to certify that the thesis entitled **"EFFECT OF HEAVY METALS ON PHYSIOLOGY AND ANATOMY OF *ARTEMISIA NILAGIRICA* (CLARKE) PAMP"** submitted by Ms. **Fathimath Zuhra P.** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in Botany of the **University of Calicut**, is a bona fide record of the research work undertaken by her in this department under my supervision and guidance during the period 2018- 2026 and no part thereof has been submitted for the award of any other degree.

S.N.G.S College, Pattambi
17/01/2026.

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DECLARATION

I, **Fathimath Zuhra P.** hereby declare that the work presented in the thesis entitled **"EFFECT OF HEAVY METALS ON PHYSIOLOGY AND ANATOMY OF *ARTEMISIA NILAGIRICA* (CLARKE) PAMP"** is based on the original work done by me under the guidance of **Dr. Hussain K.** 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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Fathimath Zuhra P

ABSTRACT

Artemisia nilagirica (Clarke) Pamp., commonly known as Indian wormwood, is a tall aromatic perennial shrub belonging to the family Asteraceae and is widely recognized for its therapeutic properties. The present study investigates heavy metal-induced stress responses and tolerance mechanisms in *A. nilagirica* following exposure to aluminium (80 µM), chromium (70 µM), copper (50 µM), iron (70 µM) and mercury (10 µM) under Hoagland nutrient conditions. Roots, stems and leaves were analysed to assess morphological, anatomical, biochemical and metabolic alterations associated with metal toxicity.

Metal exposure resulted in significant reductions in root and shoot length and leaf area compared to control plants. Plants treated with mercury exhibited increased stomatal density and wider stomatal apertures, indicating an adaptive response likely facilitating mercury phytovolatilization. Copper exposure induced the development of glandular and non-glandular trichomes on both leaf surfaces, suggesting a sequestration-based detoxification strategy. Anatomical analyses revealed increased cell wall thickness as a key tolerance mechanism for immobilizing toxic metal ions and preventing cytoplasmic damage. Structural abnormalities, including disrupted epidermal layers and altered xylem vessel diameter, were observed in plants exposed to chromium, copper, iron and mercury, whereas aluminium treatment caused minimal anatomical alterations, indicating comparatively lower toxicity at the tested concentration.

Biochemical analyses demonstrated a significant decline in tolerance index across all metal treatments. Chlorophyll content decreased, while carotenoid levels increased, reflecting a protective response against photooxidative stress. Elevated levels of proteins, phenolics, proline, malondialdehyde, and enhanced activities of antioxidant enzymes such as catalase and superoxide dismutase confirmed the induction of oxidative stress and activation of antioxidant defence mechanisms. SEM and SEM-EDX analyses revealed ultrastructural changes and confirmed the

localization and accumulation of metal ions within plant tissues, while ICP-OES analysis validated metal bioaccumulation.

GC-MS profiling revealed substantial alterations in the composition of bioactive secondary metabolites under heavy metal stress, including the emergence of compounds potentially involved in metal detoxification, alongside a reduction in metabolites associated with medicinal efficacy. FTIR analysis further demonstrated metal-specific modifications in functional groups of proteins and polysaccharides, indicating significant metabolic perturbations. Cytotoxicity studies using Dalton's lymphoma ascites (DLA) cells showed that metal contamination significantly influenced the anticancer activity of *A. nilagirica* leaf extracts; although cytotoxicity increased with extract concentration, overall anticancer efficacy was attenuated due to metal-bioactive compound interactions.

Collectively, this study elucidates the complex physiological, biochemical, and molecular responses of *A. nilagirica* to heavy metal stress, highlighting its adaptive tolerance mechanisms, potential role as a bioindicator of metal pollution, and the need for careful evaluation of metal contamination in medicinal and therapeutic applications.

Keywords: *Artemisia nilagirica*, Bioaccumulation, Cytotoxicity, Glandular-trichomes, Secondary metabolites, Tolerance mechanisms.

സംഗ്രഹം

ആർട്ടെമിസിയ നീലഗിരിക്ക (ക്ലാർക്ക്) പാംപ്., സാധാരണയായി *ഇന്ത്യൻ വോംവുഡ്* എന്നറിയപ്പെടുന്ന, ആസ്റ്ററേസി കുടുംബത്തിൽപ്പെട്ട, ഉയരമുള്ളതും സുഗന്ധമുള്ളതുമായ ബഹുവർഷ കുറ്റിച്ചെടിയാണ്. വ്യാപകമായ ചികിത്സാ ഗുണങ്ങൾക്കായി പ്രശസ്തമായ ഈ സസ്യത്തിൽ ഘനലോഹങ്ങൾ പ്രേരിപ്പിക്കുന്ന സമ്മർദ്ദവും അതിനോടനുബന്ധിച്ച സഹിഷ്ണുതാ സംവിധാനങ്ങളും വിലയിരുത്തുകയാണ് നിലവിലെ പഠനത്തിന്റെ ലക്ഷ്യം. ഹോഗ്‌ലാൻഡ് പോഷക മാധ്യമത്തിൽ വളർത്തിയ ആർട്ടെമിസിയ നീലഗിരിക്കയുടെ വേരുകൾ, തണ്ടുകൾ, ഇലകൾ എന്നിവയെ 80 µM അലുമിനിയം, 70 µM ക്രോമിയം, 50 µM ചെമ്പ്, 70 µM ഇരുമ്പ്, 10 µM മെർക്കുറി എന്നിവയ്ക്ക് വിധേയമാക്കി. ലോഹ എക്സ്പോഷറിനെത്തുടർന്നുണ്ടായ മോർഫോളജിക്കൽ, ശരീരഘടനാപര, ബയോകെമിക്കൽ, ഉപാപചയ മാറ്റങ്ങൾ സമഗ്രമായി വിശകലനം ചെയ്തു.

ലോഹ ചികിത്സകൾ കൺട്രോൾ സസ്യവുമായി താരതമ്യപ്പെടുത്തുമ്പോൾ വേരിന്റെയും തണ്ടിന്റെയും നീളത്തിലും ഇല വിസ്തീർണ്ണത്തിലും ഗണ്യമായ കുറവുകൾ ഉണ്ടാക്കി. മെർക്കുറിക്ക് വിധേയമായ സസ്യങ്ങളിൽ സ്റ്റോമറ്റൽ സാന്ദ്രതയും സ്റ്റോമറ്റൽ അപ്പർച്ചറുകളുടെ വീതിയും വർദ്ധിച്ചതായി കണ്ടെത്തി. ഇത് മെർക്കുറി-പ്രേരിത വിഷാംശം ലഘൂകരിക്കുന്നതിനായി ഫൈറ്റോവോളാറ്റിലൈസേഷൻ പ്രോത്സാഹിപ്പിക്കുന്ന ഒരു അഡാപ്റ്റീവ് പ്രതികരണമായി വ്യാഖ്യാനിക്കുന്നു. ചെമ്പ് ചികിത്സ ഇലയുടെ അഡാക്സിയൽ, അബാക്സിയൽ പ്രതലങ്ങളിൽ ഗ്ലാൻഡുലാർ കൂടാതെ നോൺ-ഗ്ലാൻഡുലാർ ട്രൈക്കോമുകളുടെ രൂപീകരണം പ്രേരിപ്പിച്ചു, ഇത് ലോഹ അയോണുകളെ വേർതിരിച്ചുവയ്ക്കുന്ന ഒരു സംരക്ഷണ തന്ത്രത്തെ സൂചിപ്പിക്കുന്നു.

ശരീരഘടനാപരമായ പരിശോധനയിൽ, വിഷലോഹ അയോണുകളെ അചഞ്ചലമാക്കുന്നതിനും സൈറ്റോപ്ലാസ്മിക് നാശം തടയുന്നതിനും സഹായിക്കുന്ന പ്രധാന സഹിഷ്ണുതാ സംവിധാനമായി കോശഭിത്തിയുടെ കനം വർദ്ധിച്ചതായി കണ്ടെത്തി. ക്രോമിയം, ചെമ്പ്, ഇരുമ്പ്, മെർക്കുറി എന്നിവയ്ക്ക് വിധേയമായ സസ്യങ്ങളിൽ എപ്പിഡർമൽ പാളികളുടെ വിഘടനം, സൈലം വസലുകളുടെ വലുപ്പമാറ്റങ്ങൾ തുടങ്ങിയ ഘടനാപരമായ അസാധാരണതകൾ വ്യക്തമായിരുന്നു. ഇതിന് വിരുദ്ധമായി, അലുമിനിയം ചികിത്സയ്ക്ക് വിധേയമായ സസ്യങ്ങൾ, പരീക്ഷിച്ച സാന്ദ്രതയിൽ താരതമ്യേന കുറഞ്ഞ വിഷാംശം കാണിച്ചതിനാൽ വളരെ കുറവ് ശരീരഘടന മാറ്റങ്ങൾ മാത്രമേ പ്രകടിപ്പിച്ചുള്ളൂ.

എല്ലാ ലോഹ ചികിത്സകളിലും ടോളറൻസ് ഇൻഡക്സിൽ ഗണ്യമായ കുറവ് രേഖപ്പെടുത്തി. ക്ലോറോഫിൽ പോലുള്ള ഫോട്ടോസിന്തറ്റിക് പിഗ്മെന്റുകളുടെ അളവ് കുറഞ്ഞപ്പോൾ, കരോട്ടിനോയിഡുകളുടെ സാന്ദ്രത വർദ്ധിച്ചതായി കണ്ടെത്തി, ഇത് ഫോട്ടോ-ഓക്സിഡേറ്റീവ് നാശത്തിനെതിരെയുള്ള ഒരു സംരക്ഷണ പ്രതികരണത്തെ സൂചിപ്പിക്കുന്നു. പ്രോട്ടീനുകൾ, ഫിനോളിക് സംയുക്തങ്ങൾ, പ്രോലിൻ, മലോൻഡിയാൽഡിഹൈഡ്

എന്നിവയുടെ അളവും കാറ്റലേസ്, സൂപ്പർഓക്സൈഡ് ഡിസ്മ്യൂട്ടേസ് പോലുള്ള ആന്റിഓക്സിഡന്റ് എൻസൈമുകളുടെ പ്രവർത്തനവും വർദ്ധിച്ചതായി കണ്ടെത്തി, ഇത് ലോഹ-പ്രേരിത ഓക്സിഡേറ്റീവ് സമ്മർദ്ദത്തോട് പ്രതികരിക്കുന്ന സസ്യത്തിന്റെ പ്രതിരോധ സംവിധാനങ്ങളെ വ്യക്തമാക്കുന്നു.

സ്കാനിംഗ് ഇലക്ട്രോൺ മൈക്രോസ്കോപ്പി (SEM)യും SEM-എനർജി ഡിസ്പേഴ്സീവ് എക്സ്-റേ സ്പെക്ട്രോസ്കോപ്പി (SEM-EDX) യും സസ്യ അവയവങ്ങളിലെ സൂക്ഷ്മ ഘടന മാറ്റങ്ങളും കോശങ്ങളിലെ ലോഹ അയോണുകളുടെ സ്ഥാനവും വിതരണവും സ്ഥിരീകരിച്ചു. ഇൻഡക്റ്റീവ്ലി കപ്പിൾഡ് പ്ലാസ്മ-ഒപ്റ്റിക്കൽ എമിഷൻ സ്പെക്ട്രോസ്കോപ്പി (ICP-OES) ഉപയോഗിച്ച് ഘനലോഹങ്ങളുടെ ബയോഅക്യുമുലേഷനും സ്ഥിരീകരിച്ചു.

GC-MS വിശകലനം ഘനലോഹ സമ്മർദ്ദം ആർട്ടെമിസിയ നീലഗിരിക്കയിലെ ബയോആക്റ്റീവ് സെക്കൻഡറി മെറ്റബോളൈറ്റുകളുടെ ഘടനയിൽ ഗണ്യമായ മാറ്റങ്ങൾ സൃഷ്ടിക്കുന്നതായി വെളിപ്പെടുത്തി. ലോഹ വിഷാംശം ലഘൂകരിക്കാൻ സഹായിക്കുന്ന ചില പുതിയ സംയുക്തങ്ങൾ കണ്ടെത്തിയെങ്കിലും, സസ്യത്തിന്റെ ഔഷധ മൂല്യത്തിന് നിർണായകമായ ചില ദ്വിതീയ മെറ്റബോളൈറ്റുകൾ കുറയുന്നതായി കണ്ടെത്തി. FTIR പഠനം വിവിധ ലോഹങ്ങൾ പ്രോട്ടീനുകളുടെയും പോളിസാക്രൈഡുകളുടെയും പ്രവർത്തന ഗ്രൂപ്പുകളിൽ ലോഹ-നിർദ്ദിഷ്ട വ്യതിയാനങ്ങൾ സൃഷ്ടിക്കുന്നതായി സൂചിപ്പിച്ചു, ഇത് സസ്യ കോശങ്ങളിലെ ഉപാപചയ തടസ്സങ്ങളെ പ്രതിഫലിപ്പിക്കുന്നു.

ഡാൽട്ടൺിറെ ലിംഫോമ അസൈറ്റ്സ് (DLA) കോശങ്ങൾ ഉപയോഗിച്ചുള്ള സൈറ്റോടോക്സിസിറ്റി പഠനം, ലോഹങ്ങളുടെ സാന്നിധ്യവും സാന്ദ്രതയും ആർട്ടെമിസിയ നീലഗിരിക്ക ഇല എക്സ്ട്രാക്റ്റിന്റെ കാൻസർ വിരുദ്ധ ഫലപ്രാപ്തിയെ ഗണ്യമായി സ്വാധീനിക്കുന്നതായി തെളിയിച്ചു. എക്സ്ട്രാക്റ്റിന്റെ സാന്ദ്രത വർദ്ധിക്കുന്നതനുസരിച്ച് സൈറ്റോടോക്സിസിറ്റി ഉയർന്നുവെങ്കിലും, ലോഹ-ബയോആക്റ്റീവ് സംയുക്ത ഇടപെടലുകൾ മൂലം മൊത്തത്തിലുള്ള കാൻസർ വിരുദ്ധ ഫലപ്രാപ്തി കുറയാൻ സാധ്യതയുണ്ടെന്ന് പഠനം സൂചിപ്പിക്കുന്നു.

സമഗ്രമായി, ഈ പഠനം ആർട്ടെമിസിയ നീലഗിരിക്ക ഘനലോഹ സമ്മർദ്ദത്തോട് പ്രതികരിക്കുന്നതിനുള്ള ഘടനാപര, ശാരീരിക, ബയോകെമിക്കൽ, തന്മാത്രാതല അഡാപ്റ്റീവ് സംവിധാനങ്ങളെ വ്യക്തമാക്കുന്നു. പാരിസ്ഥിതിക ലോഹ മലിനീകരണത്തിന്റെ ബയോഇൻഡിക്കേറ്ററായി ഈ സസ്യത്തിന്റെ സാധ്യതയും, ചികിത്സാ പ്രയോഗങ്ങൾക്ക് മുൻപ് ലോഹ മലിനീകരണത്തിന്റെ സ്വാധീനം സൂക്ഷ്മമായി വിലയിരുത്തേണ്ടതിന്റെ ആവശ്യകതയും ഈ കണ്ടെത്തലുകൾ അടിവരയിടുന്നു.

കീവേഡുകൾ: ആർട്ടെമിസിയ നീലഗിരിക്ക, ബയോഅക്യുമുലേഷൻ, സൈറ്റോടോക്സിസിറ്റി, ഗ്ലാൻഡുലാർ ട്രൈക്കോമുകൾ, ദ്വിതീയ മെറ്റബോളൈറ്റുകൾ, സഹിഷ്ണുതാ രീതികൾ.

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ABBREVIATIONS

Al	-	Aluminium
As	-	Arsenic
BCF	-	Bio Concentration Factor
$Ca(NO_3)_2.4H_2O$	-	Calcium Nitrate Tetrahydrate
CAT	-	Catalase
Cd	-	Cadmium
Chl	-	Chlorophyll
Chl a	-	Chlorophyll a
Chl b	-	Chlorophyll b
Co	-	Cobalt
Cr	-	Chromium
Cu	-	Copper
$CuSO_4.5H_2O$	-	Copper (II) Sulfate Pentahydrate.
DW	-	Dry Weight
EDTA	-	Ethylene Diamine Tetra Acetate
EDX	-	Energy Dispersive X-ray analysis
Fe	-	Iron
FTIR	-	Fourier Transform Infrared Spectroscopy
FW	-	Fresh Weight
GCMS	-	Gas Chromatography and Mass Spectrometry
GPX	-	Guaiacol Peroxidase
H_3BO_3	-	Boric Acid
Hg	-	Mercury
H_2MoO_4	-	Molybdic Acid
H_2O_2	-	Hydrogen Peroxide
ICP-OES	-	Inductively Coupled Plasma Optical Emission Spectroscopy
KCl	-	Potassium Chloride
KNO_3	-	Potassium Nitrate
MC	-	Moisture Content
MDA	-	Malon Di Aldehyde
MDHA	-	Mono De Hydro Ascorbate
$MgSO_4.H_2O$	-	Magnesium Sulfate Monohydrate
Mn	-	Manganese
$MnSO_4.H_2O$	-	Manganese (II) Sulfate Monohydrate

MT	-	Metallo Thionein
NaFeEDTA	-	Sodium Iron (III) Ethylene Diamine Tetra Acetate
$NH_4H_2PO_4$	-	Ammonium Dihydrogen Phosphate
Ni	-	Nickel
O^{2-}	-	Superoxide
Pb	-	Lead
PC	-	Phyto Chelatin
PSI	-	Photosystem I
PSII	-	Photosystem II
r	-	Pearson's correlation coefficient
ROS	-	Reactive Oxygen Species
RWC	-	Relative Water Content
Se	-	Selenium
SEM	-	Scanning Electron Microscope
SOD	-	Super Oxide Dismutase
TBA	-	Thio Barbituric Acid
TCA	-	Tri Chloroacetic Acid
TF	-	Translocation Factor
Zn	-	Zinc
$ZnSO_4.7H_2O$	-	Zinc Sulfate Heptahydrate

CHAPTER 1
INTRODUCTION

Heavy metals are naturally occurring elements that cannot be broken down or eliminated by biological processes. Excessive exposure of heavy metal can disrupt ecosystems, damage plant growth, and negatively affect human health. Growing environmental pollution due to urbanization, industrialization, and agricultural practices exacerbates the risks posed by the metals, making ongoing research vital to understanding and managing their impact. As such, the study of heavy metals in natural biological systems and their environmental interactions remains an important area of scientific inquiry.

Heavy metals are typically classified based on their density and atomic weight. Naturally present in the Earth's crust, they enter ecosystems through processes like volcanic eruptions and rock weathering. However, human activities such as mining, industrial waste disposal, and the excessive use of pesticides and fertilizers have significantly increased their environmental levels. The rapid industrialization and urbanization have aggravated heavy metal pollution, leading to long-term impacts on plant ecosystems, water quality, and soil health.

Soil and water contamination by heavy metals is an environmental crisis by which, humans and the environment are permanently harmed. As industry develops globally, hazardous heavy metal pollution of the environment is increasing. The typical growth and development of plants is altered by exposure to elevated metal concentrations. Certain plants take in and collect relatively large amounts of heavy metals from their contaminated surroundings; as a result, they experience structural changes that limit their ability to thrive.

The two key factors governing phytoextraction and the ability of plants to accumulate metals are heavy metal solubility and speciation in soil solution (Krishnamurti and Naidu, 2002). One of the primary causes of failed phytoextraction has been identified is the low solubility of several heavy metals, such as lead (Pb)

(Whiting *et al*., 2001; Hettiarachchi and Pierzynski, 2004), because different species, such as free hydrated metals and metal ligand complexes, can be absorbed by plant roots to varying degrees. The quantity of heavy metals that plants absorb depends on the concentration of metal ions in solution as well as the species that are present.

Toxic levels of heavy metals can seriously hinder the growth and development of plants. The change in root architecture, which includes retarded growth and development of root as one of the first obvious indications. Root systems become impaired due to the effect of metals like aluminium (Al) and mercury (Hg) that prevent cell divisions and elongation. Furthermore, heavy metals impair photosynthetic efficiency by degrading photosynthetic pigments and interfering with the formation of chlorophylls. Under metal stress, the production of reactive oxygen species (ROS) worsens cellular structure by altering enzyme activity and membrane integrity (Rao, *et al*., 2025).

Heavy metals like iron (Fe) and copper (Cu) are essential for plant metabolism in trace amounts. Iron is essential for electron transport and chlorophyll synthesis, while copper is a vital component of enzymes involved in photosynthesis and respiration. Higher amounts of these metals, however, cause oxidative stress and interfere with enzyme processes, making them hazardous. Plants have developed complex systems to control the intake of heavy metals and preserve equilibrium. These include transporter proteins like the ABC (ATP-binding cassette) and ZIP (Zinc-regulated transporter/Iron-regulated transporter-like protein) families. Gaining knowledge of these processes helps us understand how plants manage heavy metal stress and preserve metabolic balance (Abou Seeda, *et al*., 2022).

The most reactive metal in everyday use is Al, which has a high resistance to corrosion and weathering. Aluminium rapidly develops an imperceptible oxide coating on its surface when exposed to air, shielding the metal from additional corrosion. Aluminium toxicity is one of the major abiotic stress problems where acidic soil (pH 4.5 - 5.5) is present. It adversely affects the plant growth and development which ultimately reduces the yield (Rahman and Upadhyaya, 2021).

The main consequence of Al is decrease in root development. It has been thoroughly studied how different mononuclear hydroxyl-Al and polynuclear Al species, particularly the "Al^{13}" complex, might cause rhizotoxicity. A number of recent reviews have proposed other mechanisms of Al tolerance and detailed numerous other Al-related physiological problems in plants in addition to rhizotoxicity (Rengel, 2022, Ofoe, 2023).

Chromium (Cr) is regarded as a significant environmental contaminant because of its extensive industrial use. In addition to altering the germination process and the growth of roots, stems and leaves, Cr has toxic effects on plant growth and development that also negatively impact physiological processes (Shanker *et al*., 2005).

It is well known that Cr, is a poisonous element that can harm both plants and animals. Lipid peroxidation is induced in plants as a result of Cr-induced oxidative stress, which seriously damages cell membranes. Cr-induced oxidative stress starts the breakdown of photosynthetic pigments, which slows growth. A high concentration of Cr can disrupt the ultrastructure of the chloroplasts, which will disrupt the photosynthetic process (Hayat *et al*., 2012).

In plants, Cr has been reported to induce growth retardation, chlorosis, wilting, decreased photosynthesis and eventually plant death. Furthermore, plants cultivated in Cr-stressed environments also produce more ROS, which can disrupt mitochondrial respiration and carbohydrate metabolism by damaging the synthesis of biomolecules like proteins, lipids, and nucleic acids (Gill and Tuteja 2010, Gill *et al*., 2015). Cr stress is one of the key variables influencing photosynthesis, including electron transport, CO2 fixation, photophosphorylation, and enzyme activity.

Plant systems are particularly vulnerable to Cr pollution, which is mostly caused by industrial sources. Because it can pass through cell membranes and cause oxidative stress and genotoxic consequences, hexavalent Cr (Cr^{6+}) is extremely toxic. Reduced plant growth is the result of disruption of chloroplast structure and inhibition of photosynthetic activities. In order to lessen oxidative damage, plants react by

triggering antioxidant defence mechanisms, which include enzymes like catalase and superoxide dismutase (Wakeel *et al*., 2020).

A high concentration of Cu is poisonous to humans, animals and plants and pollutes the environment. Both natural and man-made sources contribute to Cu pollution, primarily factories that produce or use Cu metal or related compounds, Cu mining, burning fossil fuels, home waste water, landfills, phosphate fertilizer production, volcanoes, windblown dust, sea spray, forest fires and vegetation decay. In previous decades, 939,000 metric tons of Cu were released annually into the environment (Singh *et al*., 2003).

Numerous physiological activities, including oxidation, photosynthesis, and the metabolism of carbohydrates, proteins, and cell walls, as well as symbiotic N_2 fixation, depend on Cu, a micronutrient (Quartacci *et al*., 2000; Kabata-Pendias 2000). Copper levels in cells must be kept low, though, as too much Cu can alter DNA, cell membrane integrity, respiration, photosynthesis, and enzyme activity, which can stunt plant growth and plant survival (Zvezdanovic *et al*., 2007; Chaffai *et al*., 2007; Zhao *et al*., 2010, Yruela, 2013).

At elevated Cu concentrations, changes occur in photosynthetic pigment synthesis, cell membrane permeability, homeostasis, and plant nutritional balance (Adrees *et al*., 2015; Gautam *et al*., 2016). Increased ROS, decreased carbon fixation, and decreased or inhibited plant development are all consequences of oxidative stress caused by excess Cu ions in plants, which disrupt photosynthesis (Kupper and Andresen 2016). Since the root is the first organ to come into touch with the pollutant, exposure to Cu stress causes modification in the morphology of the root (Cai *et al*., 2014). To deal with Cu stress, plants use a variety of detoxifying techniques, including sequestration in vacuoles and chelation by metallothioneins. These processes demonstrate dual nature of Cu as a possible poison and a micronutrient.

Known since 1844 (Gris), chlorosis due to Fe deficiency was the first plant nutrient deficiency to be studied. Whether it is Fe-toxicity or Fe-deficiency, everyone interested in any element of plant nutrition must be aware of iron nutrition issues because they continue to be significant in important crops around the world.

For plants, Fe is a vital nutrient. it has a major role in plant metabolism, especially in the activation of enzymes and electron transport. Excess iron, however, can cause oxidative damage to biological components by catalysing the Fenton processes that produce hydroxyl radicals. Through a system of storage proteins and transporters, plants maintain Fe homeostasis keeping levels within a safe range. It is essential to comprehend these molecular processes in order to create mitigation methods for Fe toxicity. In the electron-transport pathways of respiration and photosynthesis, it plays a crucial role in accepting and donating electrons. However, excessive iron accumulation might be harmful. Through the Fenton reaction, it can catalytically produce hydroxyl radicals that harm DNA, proteins, and lipids. Therefore, plants need to react to iron stress by addressing both Fe overload and Fe shortage. Stunted roots and bronzing of the leaves are classic signs of Fe toxicity. Plants have evolved morphological and physiological avoidance and/or tolerance methods to deal with and survive hazardous soil conditions and excessive Fe accumulation in tissue (Harish *et al*., 2023).

The amount of Hg in plants varies greatly and is dependent on the capacity of plant to absorb mercury. According to Rothenberg *et al*. (2012), there may be notable variations in Hg uptake even across subspecies cultivated at the same location. When plants are cultivated in contaminated areas, they may also become tolerant to elevated Hg levels (Raj and Maiti, 2020).

Three oxidation states of Hg are possible: Hg (I), Hg (II), and Hg (0). The oxidation state of Hg affects its characteristics and chemical behaviour. The two primary oxidation states of mercury are Hg (0) and Hg (II). However, a variety of inorganic and organic chemical compounds are formed by both Hg (I) and Hg (II) (Zheng *et al*., 2012).

The accumulation of Hg in plants is thought to be significantly influenced by a variety of particular local environmental conditions, which may even outweigh the significance of local soil Hg concentrations (Overesch *et al*., 2007; Hang *et al*., 2016). Mushrooms have been discovered to have extremely high levels of Hg (Melgar *et al*., 2009; Falandysz *et al*., 2015).

One of the most harmful heavy metal, Hg has a negative impact on the physiology of plants. By attaching itself to thiol groups in proteins and enzymes, it interferes with cellular respiration, photosynthesis, and protein synthesis. The utilization of plants to detoxify soils tainted with Hg has been investigated by advances in phytoremediation. To improve Hg tolerance and sequestration, these tactics employ genetic engineering and hyperaccumulators (Falandysz, 2017).

Agriculture, environmental health, and pharmaceutical safety are all significantly impacted by the problem of heavy metal pollution in therapeutic plants. These issues are intended to be addressed by regulatory frameworks like the WHO guidelines on medicinal plant quality. Standardizing safety procedures and guaranteeing adherence, however, continue to be major challenges. Promising answers are provided by developments in biotechnology, such as the creation of genetically edited plants with increased metal tolerance. Furthermore, the dangers of heavy metal contamination can be reduced by combining soil remediation methods with sustainable farming methods.

It is well known that medicinal plants can help treat illnesses in the conventional medical system. These days, the development of herbal products as dietary supplements has been aided by growing consumer demand and scientific interest. Oriental herbal remedies have a significant place in the 21st-century pharmaceutical and health sectors due to resurgence of interest. While, the increased heavy metal pollution will adversely affect the consumers. Accumulation and amplification of heavy metals in human tissues through the consumption of medicinal plants can have hazardous health outcomes (Hlihor *et al*., 2022).

Artemisia nilagirica (Clarke) Pamp. Locally known as “Indian wormwood” belongs to Asteraceae. In India the plant is distributed in Assam, Bihar, Madhya Pradesh, Maharashtra, Manipur, Uttar Pradesh, Tamil Nadu, and Kerala. In Kerala it is found distributed in hilly areas of Idukki, Kannur, Kollam, Kottayam, Malappuram, Palakkad, Pathanamthitta, Thiruvananthapuram, Wayanad.

Over the course of a decade, this medicinal plant has reportedly been utilized to cure a wide range of microbiological ailments, including diabetes, stress,

depression, and inflammation. The phytochemical analysis of *A. nilagirica* recorded the presence of effective biological compounds like alkaloids, amino acids, flavonoids, phenols, tannins and terpenoids. These derivatives could be potential alternatives to the traditional chemical control of clinical pathogen and phytopathogenic bacteria (Ahameethunisa and Hopper, 2010).

The hairy, frequently half-woody plant is upright. They have branching, leafy stems. Astringent, bitter, and fragrant are the leaves and flowering tops. The fragrant shrub *A. nilagirica* has a variety of volatile oils as well as biologically significant substances like coumarins, acetylenes, sesquiterpene lactones, and numerous other phytochemicals like flavonoids, phenols, and alkaloids (Puri & Kesavan, 2018). However, the safety and effectiveness of medicinal plants are seriously threatened by the bioaccumulation of heavy metals. High concentrations of heavy metals can impair plant development and morphology, interfere with metabolic processes, and induce oxidative stress, all of which reduce the potential of plants as medicines.

In experimental biological models, including humans, plants, and animals, several pharmaceutical effects have been validated. Numerous intriguing pharmacological properties of this therapeutic herb, including insecticidal, antibacterial, antiulcer, anticancer, antioxidant, and anti-asthmatic properties, have been reported. It is regarded as an effective alternative, emmenagogue, stomachic, and antispasmodic. It is administered to the head to stop convulsions, indicated for skin conditions, hysteria and blocked menstruation. In cases of asthma and brain disorders, leaves and stem apices are also used to treat nervous and spasmodic illnesses. An infusion is administered as a tonic and a potent decoction as a vermifuge. Due to their carminative and haemostatic properties, leaves are used as a treatment for headaches, haemorrhages, blood vomiting and haematuria. Additionally, eczema, herpes, purulent scabies, intestinal and urinary issues, dysentery and metrorrhagia are all advised to use it. Volatile oil of the plant functions as a weak pesticide and an effective larvicide.

Different ethnic groups in Bangladesh use this plant in different ways. In Sylhet, Lalong and Patra utilize a leaf decoction for colds and coughs. Fresh leaves

and *Momordica charantia* are mashed and turned into a paste to be used as "voron" to relieve headaches. The Khaisa people of the greater Sylhet district apply a paste made from young leaves to their livers to relieve liver and body discomfort and to reduce inflammation. Leaf juice is used to treat leprosy by the Garo ethnic group in the districts of Sunamganj, Netrokona, Mymensingh, Tangail, and Sherpur. Because *Artemisia nilagirica* contains secondary metabolites, it has a significant therapeutic value.

Medicinal plants are particularly susceptible to heavy metal contamination. Studies have suggested that bioaccumulation could alter the secondary metabolite profile of plant, reducing their therapeutic value. Heavy metal stress might limit the formation of sesquiterpene lactones, which are essential therapeutic compounds, in *A. nilagirica*. In addition, individuals who use contaminated medicinal herbs run the risk of developing severe health problems.

The disparate effects of various heavy metals have been uncovered by extensive research, highlighting the necessity of better understanding how they affect plant systems. For instance, Gill and Tuteja (2010) concentrated on oxidative stress as a plant response mechanism, whereas Kabata-Pendias (2015) described the function of trace elements in plant growth and toxicity. Critical bioaccumulation pathways and processes, such as metal transporters and chelation pathways, have also been found by studies on plant heavy metal uptake (Clemens *et al*., 2002; Hall, 2002).

The primary goal of the present study is to assess how heavy metals such as Al, Cr, Cu, Fe and Hg affect growth, structural integrity, and biochemical characteristics of *A. nilagirica*. The study intends to clarify the physiological adaptations and bioaccumulation capacity of plant by simulating contaminated conditions, which will support sustainable usage of medicinal plants by the pharmaceutical industry.

Research on the effects of heavy metals on physiology and morphology of *A. nilagirica* is particularly significant because this plant is a valuable therapeutic one. *A. nilagirica* can be widely used in medicine because of its antibacterial, anti-inflammatory, and antioxidant properties. However, the accumulation of heavy metals

in plants may alter their metabolic processes, affecting the synthesis of secondary metabolites that are critical to their medicinal benefits. Given the increasing levels of environmental pollution, particularly soil contamination with metals such as Al, Cr, Cu, Fe and Hg, it is imperative to understand how heavy metals impact the physiological processes and anatomical structure of *A. nilagirica*. To assess the potential risks of using this plant in traditional medicine, particularly in areas where heavy metal contamination is common, such research is required.

Additionally, examining the physiological and anatomical responses of *A. nilagirica* to heavy metals can provide crucial information regarding its metal uptake, detoxifying, and translocation mechanisms. These findings are pivotal for ensuring the safety of plant and therapeutic efficacy as well as for its potential use in phytoremediation. Because heavy metals often accumulate in plant roots, it is important to evaluate how this buildup affects root architecture, nutrient absorption, and overall plant health. By knowing how these pollutants affect the anatomical structure of plant, such as changes to the cell wall, root structure, and leaf morphology-strategies for enhancing the plant resistance to metal toxicity can be developed, guaranteeing the long-term use of *A. nilagirica* for environmental and medicinal applications.

The bioaccumulation of heavy metals possesses a number of health risks to users of Ayurvedic medications, and the therapeutic qualities of plant may be diminished or negatively impacted. The scope of this inquiry was to analyse the bioaccumulation potential of *A. nilagirica* and to clarify the effects of Al, Cr, Cu, Fe and Hg on structural and functional alterations. The growth performance under both controlled and artificially contaminated conditions is revealed by means of simulation trials. The following primary goals are being pursued by the study.

Objectives of the Study

1. To analyse the effects of heavy metals (Al, Cr, Cu, Fe, Hg) on structural and functional aspects of *Artemisia nilagirica* (Clarke) Pamp. under nutrient culture method.

2. To evaluate growth pattern on the basis of morphological parameters.

3. To examine anatomical details using histochemical staining, light microscopy, scanning electron microscopy and EDX analysis to point out the structural changes as well as translocation and accumulation pattern of heavy metals in the plant body.

4. To assess the impact of heavy metals on physiological processes including synthesis and distribution of primary metabolites - carbohydrates, proteins and pigments.

5. To reveal the bioaccumulation potential and tolerance mechanisms of *A. nilagirica* under controlled and artificially contaminated environments, by analysing the absorption and translocation of Al, Cr, Cu, Fe and Hg in root, stem and leaves using ICP-OES analysis.

6. To disclose the impact of heavy metals on the medicinal properties and secondary metabolite production in the plant for pharmaceutical safety.

CHAPTER 2
REVIEW OF LITERATURE

Effects of Heavy Metal Stress on Plant Growth and Morphology

Environmental pollution by heavy metals comes mostly by anthropogenic activities including, mining, smelting, power transmission, electroplating, ore refining, intensive agricultural practices and waste disposal on land (Orcutt and Nilsen,2000; Cseh, 2002; Pilon-Smits, 2005; Li *et al*., 2015). Growing of aromatic and medicinal plants in heavy metal contaminated soil, resulted in significant changes in quality and quantity of its medicinal properties. Even the plants can absorb and accumulate these contaminants in its harvestable foliage. Plants act differentially to different environmental stresses and they tolerate this condition by altering physiology and metabolism. Hence it is preferable to evaluate the biological activity of the plants before using for medicinal purposes.

Human activity affects differentially on environment and as a result metal contamination are becoming increasingly common (Mourinha *et al*., 2022). Metals occur naturally in terrestrial ecosystem and is found in various parts such as soil, rock, air, water and in organisms. Plants require these metals in trace amounts but in excess these are toxic to plants. The unplanned disposal of municipal waste, mining, use of extensive pesticides, insecticides, fungicides, and use of other agrochemicals ended up in significant causes of environment pollution and causes of most concern.

Metal toxicity rate depends on many factors and also the bioavailability of the metals in the soil system i.e., if the metal is in absorbable or non-absorbable form (Sauve *et al*., 1996; McBride *et al*., 1997; Peijnenburg *et al*., 2000). Heavy metals have different transfer rates from soil to plants depending upon their transfer coefficients (Saha *et al*., 2015). Cadmium and zinc have high transfer coefficients and are readily taken up by the plants, whereas copper, chromium, cobalt and lead have low transfer coefficients (Wang *et al*., 2006; Melo *et al*., 2014; Chang *et al*., 2014; Mirecki *et al*., 2015). Heavy metals, such as cadmium, copper, lead, chromium,

manganese, iron and mercury are major environmental pollutants, notably in zones with high human influence. Asati *et.al*. (2016) reported the heavy metal accumulation in soils and its concern in agricultural production due to the adverse effects on food safety, marketability and crop growth due to phytotoxicity, and environmental health of soil organisms.

Heavy metal accumulation in plants is affected by various factors such as atmospheric deposition of metals from mining and smelting operation, addition of heavy metals in soil through sewage sludges, and use of pesticides (Baye and Hymete 2010; Nwoko and Mgbeahuruike 2011). Numerous plants are able to grow in metal-contaminated soils. These plants have the ability to accumulate heavy metals in their biomass (Prasad and Freitas 2003; Tangahu *et al*., 2011; Matache *et al*., 2013; Vymazal and Brezinova 2015; Irshad *et al*., 2015) and have been classified as hyperaccumulators (Peer *et al*., 2005), e.g., *Brassica* sps, *Euphorbia macroclada, Centaurea virgata*, *Alnus nepalensis,* etc. (Jing *et al*., 2014). On the other hand, some plants are no accumulators but may be used for phytoremediation of heavy metals in soils for their more biomass production capability, e.g., sunflower, jack bean, velvet bean, castor bean, willow, poplar, leguminous trees, etc. as reported by Bothe, 2011; Souza *et al*., 2013.

Since the roots are the primary places where metals are absorbed by plants, the root system is most impacted by heavy metals. Metal accumulation in the rhizosphere can impede the uptake of nutrients and water by causing reduced root hair development, altered root architecture, and stunted root growth. A study conducted by Ghosh *et al*., (2022) showed that exposure to cadmium produced root tip swelling and greatly decreased root growth in *Trifolium repens*. Because the plant finds it more difficult to get vital nutrients, the metal-induced alterations in root morphology may have a detrimental effect on shoot growth. Further contributing to reduced growth, heavy metals can also cause callose plugs to form in the roots, which block the passage of nutrients and water (Ghosh *et al*., 2022). Heavy metals can cause necrosis, chlorosis, and decreased leaf area on the shoot system, which can impact the biomass of the plant as a whole.

Biochemical and Physiological Responses

In essence, heavy metals harm cellular macromolecules such as proteins, lipids, and nucleic acids by generating free radicals and reactive oxygen species (ROS), which in turn promote unchecked oxidation and radical chain reactions (Phaniendra *et al*., 2015). Because they are primary producers and make up the base of the ecological pyramid, heavy metals that enter the body of a plant go up the food chain through the trophic levels. For heavy metals that are bioaccumulative - that is, neither readily digested by plants nor broken down in the environment-this issue is made worse. There is a risk of food web contamination since certain heavy metal-tolerant plant species, such as *B. napus* and *B. Juncea*, have an innate capacity to store heavy metals in their bodies (Gall *et al*., 2015; Mourato *et al*., 2015).

In the same way that heavy metals interact with nuclear proteins and DNA, they also produce reactive oxygen species (ROS). For plants, this results in anomalies related to morphology, metabolism, and physiology (Manara, 2012). Therefore, in order to cope with heavy metals, plants have developed defence systems. Among these are two general approaches: avoiding or tolerating the toxicity of heavy metals. Thick cuticle, trichomes, cell walls, plasma membranes, and mycorrhiza are examples of physical barriers that are the first line of defence against heavy metals in plants (Hall, 2002; Wong *et al*., 2004; Harada *et al.*, 2010). To lessen the adverse effects of heavy metals, plants produce and release certain compounds if the metals are able to get through these biophysical barriers (Viehweger 2014; Dalvi and Bhalerao 2013; Sharma and Dietz 2006).

Recent years have seen an abundance of research on the effects of heavy metals on plant physiology and biochemistry. Essential functions including respiration, cell division, and photosynthesis are all hampered by heavy metals. For example, exposure to lead and cadmium lowers transpiration, photosynthetic rate, and chlorophyll content, which impacts plant growth and productivity. According to recent research, oxidative stress brought on by cadmium increases, oxygen radicals which may cause protein denaturation, DNA damage, and lipid degradation. According to a study by Sharma *et al*. (2023), Brassica juncea under Cd and Pb stress

showed a considerable decrease in stomatal conductance and chlorophyll content, which was linked to elevated ROS and oxidative damage. Likewise, exposure to arsenic has been demonstrated to change metabolic processes, such as the production of proteins and amino acids, which results in chlorosis and growth inhibition in a variety of crops, including wheat and rice (Sharma *et al*., 2022). By disrupting ion channels and transporters, the buildup of heavy metals in plant tissues also impairs the uptake of vital nutrients, resulting in deficits in calcium, potassium, and magnesium.

Bioaccumulation and Translocation of Heavy Metals

A characteristic of certain plant species that is being investigated for phytoremediation applications is their capacity to collect heavy metals in their tissues. These plants can help clean up polluted surroundings by absorbing and concentrating metals from contaminated soils. Investigation done on Ali *et al*. (2021) capacity of *Salvadora persica* for bioaccumulation revealed that the plant could store large amounts of lead and chromium in its roots, which could make it a viable option for soil decontamination. The water hyacinth, *Eichhornia crassipes*, has also been found to be a hyperaccumulator of lead and cadmium, and studies have demonstrated how effective it is at eliminating these metals from soil and water (Ali *et al*., 2021). In addition to aiding in the cleanup of polluted areas, the ability of this plant to bioaccumulate provides chances for the creation of inexpensive and sustainable methods of removing harmful heavy metals from the environment.

Tolerance Mechanisms to Heavy Metal Toxicity

Plants have evolved various detoxification and compartmentalization approaches to combat the harmful effects of metals. The chelation and sequestration of heavy metals in vacuoles, where they are less likely to damage cellular structures, is one of the most popular tactics. Additionally, plants generate metal-binding proteins including phytochelatins (PCs) and metallothioneins (MTs), which combine with metals to form complexes that lessen their toxicity. The function of these proteins in heavy metal stress tolerance was highlighted by research by Liu *et al*. (2022) that revealed improved tolerance to cadmium and zinc in *Arabidopsis thaliana*

overexpressing MTs. Additional mechanisms include the control of ion transporters, which are involved in vacuole sequestration or the removal of metals from the cytoplasm (Liu *et al*., 2022). According to Sharma *et al*. (2023), plants also activate antioxidant defence systems, such as peroxidases, catalase, and superoxide dismutase (SOD), to lessen oxidative stress brought on by metal exposure. In order for plants to survive in contaminated settings, these defence mechanisms are essential for preserving cellular integrity.

Trichomes, the hair-like epidermal appendages, which are primarily recognized for their protective role against herbivorous insects, but they also contribute to heavy metal tolerance. Trichomes either serve as a depository site for heavy metals or secrete various secondary metabolites that lead to detoxification of toxic heavy metals as well explained by Emamverdian *et al*., (2015). The first mechanism of heavy metal tolerance has been reported for trichomes of *Alyssum lesbiacum*, which store significant amount of Nickel (Kramer *et al*., 1997); and trichomes of *Arabidopsis halleri* that store higher concentrations of zinc (Sarret *et al*., 2002). Choi *et al*., 2001 reported that, increased number of trichomes were found associated with cadmium toxicity in tobacco seedlings.

Medicinal Implications of Heavy Metal Exposure

A crucial, yet often neglected, aspect of phytoremediation involves recovery or disposal of heavy metals (accumulated in plants) in such a manner that the plant biomass can be properly handled and the associated environmental risks could be reduced (Goyal & Mamta 2020). The effectiveness of medicinal plants for therapeutic reason is often accounted for by their organic constituents like flavonoids, alkaloids, essential oils, vitamins, glycosides, etc., and little attention has been paid to their inorganic constituents (Desideri, & Domenico 2010). Excess doses or prolonged intake of medicinal plants can lead to accumulation of trace elements which cause various health problems (WHO, 1992; Sharma *et al*.,2009). At earlier, Arceusz *et al*., (2010) had informed that, the elemental contents of the medicinal plants are very important and herbal preparations need to be screened for toxic trace elements. Several such studies on the elemental constituents of medicinal plants have enhanced

the awareness of trace elements in products (Caldas and Machado, 2004 in Brazil; Sharma *et al*., 2009 in India; Koe and Sari, 2009, Basgel and Erdemoglu, 2006 in Turkey; Ajasa *et al*., 2004 in Nigeria). In humans, the essential metals cause toxic effects on high ingestion although non-essential trace metals cause even at very low concentrations.

ALUMINIUM

Effects of Aluminium Toxicity on Plant Morphology and Anatomy

The primary effects of aluminium toxicity on root morphology are diminished root hair development, slowed root growth, and swelling at the terminals of the roots. Root meristem cells interruptions in mitotic activity and changes to cell wall characteristics are the causes of this (Kopittke *et al*., 2021). Al-induced cell wall stiffening is linked to increased lignin and hemicellulose deposition, which restricts the lengthening of roots (Sun *et al*., 2023). According to microscopic investigations, aluminium attaches itself to the cell wall, stiffening it and preventing the movement of water and nutrients (Yamamoto *et al*., 2022). In addition, the leaves exhibit altered mesophyll structure and increased stomatal density, which imply poor gas exchange and photosynthesis (Awasthi *et al*., 2020).

Impact on Plant Physiology

Toxic levels of aluminium interfere with physiological functions, mainly by preventing the absorption of nutrients and causing oxidative stress. Al causes deficits by competing with vital nutrients such potassium, magnesium, and calcium (Horst *et al*., 2019). According to Ma *et al*. (2021), Al also disrupts energy-dependent functions by interfering with ATP synthesis and cytoplasmic streaming. Lipid peroxidation, protein oxidation, and DNA damage are caused by the production of reactive oxygen species (ROS) during Al stress (Wang *et al*., 2022). According to Zhou *et al*. (2021), impacts of Al on stomatal control and chlorophyll production result in decreased photosynthetic efficiency.

Shoot and root responses to aluminium include cellular and structural alterations, increased diffusion resistance, reduced stomatal aperture, and decreased

photosynthetic rate (Mossor-pietrazecoska *et al*., 1997). According to Mossor-pietraszecoska, (2001) signal transduction pathway that transmits stress information within the cell and throughout the plant body due to aluminium stress as observed in yellow Lupine. A major consequence of aluminium toxicity is inhibition of root growth and this has been reported in numerous species (Ma *et al*., 2002).

Secondary Metabolite Production Under Aluminium Toxicity

Significant effects on plant defence and medicinal qualities result from the alteration of secondary metabolite profiles caused by aluminium poisoning. Research indicates that the production of phenolics, flavonoids, and tannins-which scavenge reactive oxygen species (ROS) and chelate aluminium ions-is increased under Al stress (Rahman *et al*., 2022). When plants like *Camellia sinensis* are stressed by Al, their increased lignin concentration suggests a defensive mechanism (Chen *et al*., 2020). Nevertheless, under extreme Al stress, the manufacture of some bioactive substances, such terpenoids and alkaloids, may be downregulated, which could impact the therapeutic potential of plants like *Ocimum basilicum* and *Withania somnifera* (Sharma *et al*., 2023).

Medicinal Potential of Plants Under Aluminium Stress

Certain plants maintain or even enhance specific medicinal properties through adaptive metabolic shifts; for example, phenolic accumulation in *Ocimum sanctum* under Al stress increases its antioxidant potential, while some species rich in flavonoids exhibit resilience, maintaining their medicinal properties despite Al toxicity (Ghosh *et al*., 2023). The secondary metabolite profiles of plants are closely linked to their medicinal efficacy and are altered under Al stress. In medicinal plants like *Centella asiatica* and *Artemisia annua*, decreased bioactive compound production under Al toxicity compromises therapeutic quality (Ahmad *et al*., 2021).

Bioaccumulation Patterns of Aluminium in Plants

Because of its poor mobility in the phloem, aluminium mostly builds up in roots. The formation of Al-organic acid complexes in the apoplast and Al binding to cell wall pectins are linked to accumulation (Poschenrieder *et al*., 2021). Al

bioaccumulation in leaves is high in plants like *Hydrangea macrophylla*, which may have uses in phytoremediation (Luo *et al*., 2022). Al is confined in vacuoles to reduce toxicity, according to X-ray imaging methods (Ma *et al*., 2023). The potential of some hyperaccumulators, such as *Melastoma malabathricum*, to flourish in surroundings high in Al is being investigated in order to gain knowledge about sustainable land management.

Tolerance Mechanisms Adopted by Plants

Plants employ several mechanisms to cope with aluminium toxicity:

Chelation and Detoxification: In order to chelate Al in the rhizosphere and decrease its bioavailability, organic acids such citrate, malate, and oxalate are released (Kochian *et al*., 2019).

Antioxidant Defence System: Reduced oxidative damage from Al is achieved by increased activity of enzymes such as glutathione reductase (GR), catalase (CAT), and superoxide dismutase (SOD) (Mittler, 2022).

Cell Wall Modifications: Stronger cell walls due to increased lignin synthesis prevent Al from penetrating the cytoplasm (Sun *et al*., 2023).

Vacuolar Compartmentalization: According to Yamamoto *et al*. (2022), Al sequestration in vacuoles reduces cytoplasmic exposure.

Regulation of Transporters: ALMT and MATE, two transporter proteins activated by aluminium, are essential for detoxification and Al exclusion (Ryan *et al*., 2021).

Recent Advances in Understanding Aluminium Stress Responses

Proteomics, transcriptomics, and genomics developments have revealed important Al-responsive genes and pathways. Al-tolerant crops are being engineered using CRISPR-Cas9 technology, which modifies transporter genes (Li *et al*., 2023). Proteomic investigations have uncovered Al-induced alterations in proteins that respond to stress, offering information on post-translational modifications (Cao *et al*., 2021).

CHROMIUM

Toxic heavy metal Cr reaches environment both naturally and also by anthropogenic activities. It is the seventh most abundant element in the environment and occurs mostly in trivalent and hexavalent forms. Comparatively hexavalent Cr is more toxic than trivalent Cr. Chronic exposure to Cr may result in liver, kidney and lung damage to human beings (Zayed and Terry, 2003). As per the report of FAO/WHO (1984) the permissible limit of Cr in edible plants was 0.02ppm. After comparison of the metal limit in the studied medicinal plants with those proposed by FAO/WHO (1984), it was found that all plants accumulate Cr above this limit. However, in medicinal plants, the WHO (2005) limits had not yet been established for Cr. Although in medicinal plants, permissible limits for Cr were set by the Canada which was 2 ppm in raw medicinal plant material and 0.02 mg/day in finished herbal products (WHO, 2005).

Effect of Chromium Toxicity on Plant Morphology and Anatomy

Plants that are toxically exposed to chromium experience drastic changes in their morphology and anatomy. According to Shanker *et al*. (2005), symptoms of Cr stress include reduced biomass, slowed development, delayed seed germination, and leaf chlorosis. Cr stress inhibited root elongation and shoot growth by interfering with cell division in meristematic tissues, according to a study on Triticum aestivum (wheat) (Kumar *et al*., 2021).

According to Panda *et al*. (2022), plants subjected to Cr toxicity have changed xylem and phloem architecture, which impairs the movement of nutrients and water. According to Singh *et al*. (2020), microscopic investigations show that the thicker cell walls in the roots, the enhanced lignification, and the plasmolysis in the mesophyll cells of the leaves all contribute to a decrease in plant development and production.

Impact on Plant Physiology

Important physiological functions like respiration, water interactions, and photosynthesis are all hampered by chromium poisoning. Reduced chlorophyll content is one of the main consequences of Cr stress, which lowers photosynthetic

efficiency. According to a study on *Brassica juncea*, exposure to Cr (VI) seriously degraded the pigments that make up chlorophyll and damaged the thylakoid membranes, which interfered with the photosynthetic machinery (Yadav *et al*., 2022). Oxidative stress brought on by Cr also affects enzyme activity and mitochondrial respiration. Plant physiological processes are further compromised by the production of reactive oxygen species (ROS) during Cr stress, which causes lipid peroxidation, protein oxidation, and DNA damage (Sharma *et al*., 2021). Additionally, Cr exacerbates its toxicity by interfering with the absorption and movement of vital nutrients like Mg, K, and N (Sathya and Jaleel, 2019).

Secondary Metabolite Production Under Chromium Stress

The toxicity of Cr has a major impact on secondary metabolite formation, which is essential for plant defence and therapeutic qualities. Under Cr (VI) stress, studies on *Ocimum basilicum* showed a decrease in essential oil yield and a change in phenolic content (Rajput *et al*., 2021). According to Ahmad *et al*. (2020), exposure to Cr also decreased the content of menthol in Mentha piperita by inhibiting the formation of monoterpenes. It is interesting to note that some plants use increased production of specific secondary metabolites, such flavonoids, alkaloids, and phenolics, as a defensive strategy in response to Cr stress (Pourrut *et al*., 2011). *Withania somnifera*, for instance, produced more withanolide while under mild Cr stress, indicating that secondary metabolite pathways were activated by stress (Jaiswal *et al*., 2023).

Medicinal Potential of Plants Under Chromium Stress

Because chromium poisoning changes the concentration and composition of bioactive chemicals, it has a negative impact on plants for therapeutic use. Due to oxidative damage and impaired metabolite production, medicinal plants like *Centella asiatica* and Aloe vera show decreased therapeutic efficiency when cultivated in Cr-contaminated soils (Kumar *et al*., 2022). But other plants are resilient to Cr stress, and through adaptation processes, they preserve or even improve their therapeutic qualities. Because of elevated antioxidant defence systems, *Artemisia annua*, for example, demonstrated increased production of artemisinin, a powerful antimalarial

chemical, under controlled Cr (VI) stress (Nafis *et al*., 2020). This dual reaction emphasizes the need for more study to maximize the growth of medicinal plants in areas contaminated by chromium.

Bioaccumulation Patterns of Chromium in Plants

The bioaccumulation of chromium varies greatly among plant species and is impacted by plant physiology, chromium speciation, and soil pH. Poor mobility of chromium in the xylem causes it to accumulate in the root tissues of most plants. As demonstrated by research on *Brassica napus* and *Triticum aestivum*, Cr is mostly stored in the root apoplast, where it forms complexes with pectins and lignins, two components of the cell wall (Ali *et al*., 2021). According to studies conducted on *Spinacia oleracea* in hydroponics, Cr (VI) is quickly converted to the less hazardous Cr (III) in root tissues, which prevents it from moving to shoots (Zhang *et al*., 2020). An efficient method for reducing chromium toxicity is compartmentalization. Furthermore, some hyperaccumulator plants, such as *Brassica juncea* and *Sedum alfredii*, show increased Cr translocation, which makes them attractive options for phytoremediation (Feng *et al*., 2022).

Tolerance Mechanisms Adopted by Plants

In order to deal with chromium toxicity, plants use a variety of strategies:

Chelation and Detoxification: To bind chromium and lower its bioavailability, plants create chelating compounds such organic acids (citrate, malate) and amino acids (proline). Citric acid production in rhizosphere soil improved chromium immobilization, according to a study on Helianthus annuus (Gupta *et al*., 2021).

Antioxidant Defence System: The overexpression of cellular antioxidants such as, glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) reduces oxidative stress caused by chromium. Better resistance to Cr (VI) stress in *Brassica napus* was associated with higher activity of these enzymes (Ahmad *et al*., 2022).

Cell Wall Modifications: Because sulphate and Cr (VI) share structural similarities, nonspecific transporters, such as sulphate transporters, mediate chromium uptake. In

order to restrict chromium entry, plants under Cr stress downregulate these transporters (Zhao *et al*., 2019). Metal-binding proteins and ABC transporters also aid in the sequestration and outflow of chromium.

Vacuolar Compartmentalization: To stop chromium from interacting with vital cellular components, plants store it in vacuoles. Certain tonoplast transporters implicated in chromium detoxification were found in studies on *Arabidopsis thaliana* (Singh *et al*., 2021).

Regulation of Transporters: Chromium-responsive genes and proteins implicated in metal homeostasis, stress signalling, and detoxification have been identified by transcriptomic and proteomic investigations. Cr stress increased metal binding and sequestration in *Oryza sativa* by inducing the expression of genes encoding metallothioneins and phytochelatin synthase (Das *et al*., 2022).

Recent Advances in Understanding Chromium Stress Responses

Deeper understanding of how plants react to chromium toxicity has been made possible by recent developments in molecular biology and omics technology. Energy metabolism and amino acid biosynthesis are two important metabolic pathways that are disturbed by Cr stress, according to metabolomic research on *Zea mays* (Cao *et al*., 2021). By focusing on genes involved in metal uptake and detoxification, genomic techniques like CRISPR/Cas9-based gene editing are being investigated to create crops that can withstand chromium (Zhang *et al*., 2022).

COPPER

Copper is an essential micro-element inevitable for plant growth and function. In spite of this fact, excess concentration of Cu results in phytotoxicity and is well documented and reported by many researchers (Wu and Antonovics, 1975; Wu *et al*., 1975; De Vos *et al*.,1991; Maksymiec, 1997; Ali *et al*., 2002). Although Cu is an essential element, human activity has caused significant amounts of Cu to be released into the environment, which has a negative impact on plant growth. Approximately 939,000 tons of Cu were discharged into the environment worldwide over the previous fifty years (Singh *et al*., 2003). As indicated by Malagoli *et al*. (2013), the Cu level in

grapevine soils varies from 200 to 500 mg kg-1, but in arable lands it typically ranges between 5 and 30 mg kg-1. The amount of copper in soil has increased even more as a result of further releases brought on by human activity.

Effect of Copper Toxicity on Plant Morphology and Anatomy

Consequences of Cu toxicity on plant root development was examined by Sheldon A.R. and Menzies N.W. (2005) and announced that, root growth in plants was severely inhibited by Cu contamination. According to authors, the roots were impeded and the cuticle became harden and broken. The cuticular layer appeared as brownish. Large number of impaired lateral roots were formed at low concentrations of Cu. Root hair formation decreases with increasing the Cu concentration. Copper toxicity changes the entire root morphology.

Khatun *et al*. (2008) studied and elucidated the role of antioxidant enzymes due to Cu toxicity in *Withania somnifera*. Those authors recorded an overall reduction in shoot length, fresh weight, and pigment content due to Cu toxicity and also noted an enhanced activity of many antioxidant enzymes. As per reports, the same result was obtained with dry weights also. Inhibition of growth and reduction of biomass production are general responses of higher plants to heavy metal toxicity.

The toxic effects of Cu on crop plants were summarized by Adrees M. (2015), in a review article. As per the research, it is disclosed that Cu is a necessary element for plants in low quantities, but it is severely toxic at morphological, physiological, biochemical and molecular levels when it accumulates in excess. Copper disrupts a number of metabolic functions that are essential to the growth and development of plants. The sensitivity of different plant species and cultivars within those species to Cu toxicity varies greatly. With the right mix of soil types and plant species/cultivars, Cu harmful effects on growth and yield can be minimised.

Chen J *et.al*., (2014) studied root ultra-structure of *Phyllostachys pubescens.* Those authors reported that, most of the plants did not survive beyond the application of 400 µM Cu. Root morphology severely affected than shoot system. Transmission electron microscopy and scanning electron microscopy studies of root revealed that,

under Cu contamination ultra structure of root especially cell wall, cellular organelles, and vascular tissues underwent considerable changes.

The main way that Cu toxicity alters plant shape is by preventing root extension and resulting in reduced growth. Reduced root hair development and impaired nutrition and water absorption result from its disruption of structural integrity of the root system (Rashid *et al*., 2023). Cu builds up in the root tissues, damaging the cell walls and membranes and resulting in cell necrosis, according to histological investigations (Ghosh *et al*., 2022).

Cell wall thickening, a decrease in root surface area, and changes in leaf structure are among the anatomical changes brought on by Cu toxicity. Reduced gas exchange and photosynthetic efficiency due to stomatal closure brought on by Cu-induced alterations further hinder plant growth (Duc *et al*., 2021). According to research, Cu stress can cause aberrant starch granules to accumulate in the mesophyll cells of leaves (Banerjee *et al*., 2023).

Impact on Plant Physiology

Numerous physiological functions are negatively impacted by copper poisoning. Excess accumulation of copper in plants will leads to reduced chlorophyll synthesis and inhibition of photosynthetic activities (Jiang *et al*., 2013), metabolic activities are disordered (Remans *et al*., 2012), and cell division is inhibited (Jiang *et al*., 2000). The transport and absorption of vital nutrients, including calcium, magnesium, and potassium, are hampered by excess copper, leading to deficits that worsen stress (Sharma *et al*., 2022). Moreover, copper causes reactive oxygen species (ROS) to be produced, which results in oxidative stress and harm to DNA, proteins, and lipids within cells (Zhang *et al*., 2022).

Yruela (2013) reviewed the role of copper and other micronutrients in plant photosynthetic functions as well as the mechanisms involved in their homeostasis within chloroplasts. a crucial aspect of copper toxicity is the breakdown of chlorophyll, which lowers photosynthetic efficiency (Yin *et al*., 2023). A third factor contributing to the decline in photosynthetic capability is copper-induced suppression

of photosystem II (PSII) activity (Liu *et al*., 2023). More recently, according to Kaur *et al*. (2023), copper exposure also inhibits ATPase activity, which affects cellular energy metabolism and a number of ATP-dependent processes, such as protein synthesis and nutrition transport.

Secondary Metabolite Production Under Copper Toxicity

Copper toxicity induces alterations in the secondary metabolite profile of plants associated with defence and therapeutic function. According to recent research, copper stress promotes the production of certain alkaloids, flavonoids, and phenolic compounds that chelate copper ions and function as antioxidants, hence reducing the effects of oxidative stress (Hassan *et al*., 2022).

The overall pattern of secondary metabolite production is complicated, though, and some compounds-like terpenoids-may decrease under copper stress while others may rise. This decrease is frequently connected to the downregulation of biosynthetic enzymes that produce particular beneficial substances (Ghosh *et al*., 2022). The therapeutic efficacy of medicinal plants such as *Ocimum basilicum* and *Lavandula angustifolia* has been shown to be impacted by copper-induced suppression of essential oil production (Ahmed *et al*., 2022).

Medicinal Potential of Plants Under Copper Stress

The therapeutic potential of plants is significantly impacted by copper toxicity, which modifies the profiles of bioactive chemicals. Copper stress causes a decrease in the production of active substances like alkaloids and saponins, which are necessary for the therapeutic qualities of plants like *Withania somnifera* and *Centella asiatica* (Yadav *et al*., 2022). Nevertheless, under copper stress, certain plants, like *Artemisia annua*, preserve or even improve particular bioactive components, like as flavonoids and terpenoids, hence maintaining or even increasing their medicinal potential (Rathore *et al*., 2023).

According to Meena *et al*. (2023), *Andrographis paniculata* has elevated amounts of certain phenolic acids when exposed to copper stress, which may amplify its antioxidant and anti-inflammatory characteristics. Therefore, the type of secondary

metabolite production and the particular plant species under copper toxicity determine the therapeutic potential of plants.

Bioaccumulation Patterns of Copper in Plants

Depending on the plant type and environmental factors, copper can move to the aerial portions of plants, such as leaves, stems, and flowers, although being mainly concentrated in the roots due to its toxicity (Ghosh *et al*., 2022). Bioaccumulation of copper is frequently linked to its binding to lignins and pectins, which are components of the cell wall. There, it forms insoluble complexes that decrease the mobility of plant (El-Desoky *et al*., 2021). By containing the metal and lessening its cytotoxic effects, plants can also reduce toxicity by sequestering copper in vacuoles (Zhao *et al*., 2023).

Research has shown that hyperaccumulator plants, such *Salvinia molesta*, have a remarkable ability to absorb and store copper, which makes them applicable to phytoremediation techniques (Zhou *et al*., 2021). Additionally, the ability of plants like *Helianthus annuus* and *Brassica juncea* to bioaccumulate and remediate soils contaminated with copper has been investigated (Jadhav *et al*., 2022).

Tolerance Mechanisms Adopted by Plants

Several strategies are used by plants to deal with copper toxicity:

Chelation and Detoxification: Copper ions are chelated by organic acids such as citrate, malate, and oxalate, which inhibit their entry into plant cells and decrease their bioavailability in the rhizosphere (Rashid *et al*., 2023).

Antioxidant Defence System: Antioxidant enzymes such superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) are activated to scavenge reactive ROS and shield cellular structures from copper-induced oxidative stress (Meena *et al*., 2023).

Cell Wall Modifications: Increased lignin and other phenolic compound deposition in the cell walls aids in immobilizing copper, lessening its harmful effects (Kaur *et al*., 2023).

Vacuolar Compartmentalization: The sequestration of copper ions in vacuoles reduces their toxicity to plant cells (Zhao *et al*., 2023).

Regulation of Transporters: Copper uptake, efflux, and detoxification are regulated by specialized transporter proteins that plants have produced, such as P-type ATPases and COPT (copper transporter) (Ahmed *et al*., 2022).

Recent Advances in Understanding Copper Stress Responses

Deeper understanding of the mechanisms underlying copper tolerance has been made possible by recent developments in molecular biology, genomics, and proteomics. Important molecular pathways involved in copper absorption, transport, and detoxification have been discovered through research on genes and proteins that respond to copper (Sharma *et al*., 2022). By altering the expression of copper transporters, CRISPR-Cas9 technology has been investigated as a means of engineering copper-tolerant crop types (Bai *et al*., 2023).

Plants can adapt at the molecular level to lessen the negative effects of excess copper, according to proteomic studies that have shown alterations in stress-responsive proteins under copper toxicity (Ghosh *et al*., 2022).

IRON

Effect of Iron Toxicity on Plant Morphology and Anatomy

Plant morphology and anatomy are negatively impacted by excessive iron since it damages cellular structures. Leaf bronzing, chlorosis, and necrotic patches are obvious signs of iron toxicity, especially in rice and other crops that get a lot of water (Becker and Asch, 2005). Plants exposed to high iron levels have been shown to undergo anatomical changes, including lignification and thickening of the root cell walls (Stein *et al*., 2019). These changes are adaptations that restrict the translocation of iron to aerial regions.

Recent studies demonstrate that the growth and suberization of root hairs brought on by iron toxicity act as physical barriers that lower iron absorption (Wu *et al*., 2022). According to research on *Oryza sativa*, too much iron builds up in the

apoplast, causing oxidative stress and compromising the integrity of the cell membrane (Fang *et al*., 2021). Chloroplast structural abnormalities and mitochondrial enlargement are visible under a microscope, indicating impaired photosynthetic and respiratory processes (Kobayashi *et al*., 2019).

Impact on Plant Physiology

Iron toxicity has a major impact on physiological functions, mostly through the production of ROS, which harm cells. The Fenton reaction, which is catalysed by excess iron, results in hydroxyl radicals and oxidative stress (Morrissey and Guerinot, 2009). Inhibition of nutrient intake, stomatal dysfunction, and decreased photosynthetic efficiency are important physiological effects.

Iron is an important constituent of several plant proteins and enzymes like leghemoglobin, cytochromes, ferredoxin, catalase, peroxidase, aconitase, and superoxide dismutase (Marschner 1995). However, elevated levels of iron cause production of ROS, that is, free radicals, which alter membrane permeability and damage membrane structure (De Dorlodot *et al*., 2005).

Excess iron damages the photosynthetic machinery by breaking down D1 protein and interfering with PSII function, according to a study on soybeans (Glycine max) (Li *et al*., 2022). Furthermore, deficits in vital elements like phosphorus, potassium, and magnesium result from elevated iron concentrations in the rhizosphere, which disrupt nutritional balance (Sperotto *et al*., 2020). These disturbances change the relationship between plants and water, which restricts growth and output.

Secondary Metabolite Production Under Iron Toxicity

The synthesis of secondary metabolites, which are essential for plant defence and therapeutic qualities, is changed by iron toxicity. Research on *Camellia sinensis* shows that high iron levels affect the antioxidant capacity of plant by lowering its catechin and flavonoid content (Yadav *et al*., 2021). Similarly, it was discovered that iron stress inhibits the manufacture of artemisinin in *Artemisia annua* by

downregulating the expression of important genes involved in the biosynthesis pathway (Nafis *et al*., 2019).

In contrast, several plants produce more phenolic compounds when they are under iron stress, most likely as a protective measure against oxidative damage (Ma *et al*., 2022). *Brassica juncea* has been shown to respond to iron-induced ROS by accumulating more lignin and other phenolics, which suggests that secondary metabolites have a dual function in stress tolerance and therapeutic potential.

Medicinal Potential of Plants Under Iron Stress

Changes in the secondary metabolite profiles of plants under iron stress can impair their therapeutic qualities. Growing in surroundings contaminated with iron, for instance, reduces the therapeutic efficiency of medicinal herbs such as *Ocimum sanctum* and *Withania somnifera* (Sharma *et al*., 2020). These alterations are caused by oxidative damage to bioactive molecules and the downregulation of metabolite production pathways.

Some plants, nevertheless, show resilience by adjusting their metabolic processes to preserve their therapeutic value. According to Jaiswal *et al*. (2023), *Centella asiatica*, for example, maintains its asiaticoside concentration under moderate iron stress by triggering the formation of secondary metabolites and antioxidant enzymes.

Bioaccumulation Patterns of Iron in Plants

Plant species, soil conditions, and environmental iron concentration all affect iron bioaccumulation patterns. Since iron is not very mobile in phloem, it mostly builds up in root tissues (Connorton *et al*., 2017). As a tolerance strategy, rice uses iron plaque deposition on the root surfaces to limit translocation to shoots (Audebert and Fofana, 2009).

Iron was found to be compartmentalized in the vacuoles of *Nicotiana tabacum* and *Arabidopsis thaliana* in recent studies employing X-ray fluorescence imaging, reducing its harmful effects (Kim and Guerinot, 2020). For the phytoremediation of

iron-contaminated soils, plants with a high capacity for bioaccumulation, like *Typha latifolia* and Phragmites australis, are being investigated (Luo *et al*., 2022).

Tolerance Mechanisms Adopted by Plants

Different strategies are used by plants to withstand iron toxicity, such as:

Iron Chelation and Sequestration: To sequester excess iron and decrease its availability in cytosolic compartments, plants create chelators such nicotianamine and organic acids (citrate, malate) (Curie and Briat, 2003). Increased citrate secretion under iron stress has been found to be a crucial detoxifying strategy in maize studies (Kobayashi *et al*., 2021).

Antioxidant Defence System: Mitigating oxidative stress brought on by iron excess requires increased activity of antioxidant enzymes such ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) (Mittler, 2017). These enzymes were found to be upregulated in *Brassica napus* during iron stress, which improved stress tolerance (Zhang *et al*., 2022).

Regulation of Transporters: To avoid excessive absorption, iron transporters such NRAMP (Natural Resistance-Associated Macrophage Protein) and IRT1 (Iron-Regulated Transporter) are strictly controlled (Bashir *et al*., 2021). Function of IRT1 in iron homeostasis was highlighted by knockout studies in Arabidopsis, which showed decreased iron toxicity when IRT1 expression was inhibited.

Subcellular Compartmentalization: According to Kim and Guerinot (2020), vacuolar sequestration of iron lowers its cytoplasmic concentration, safeguarding essential cellular components. OsVIT, or vacuolar iron transporter, has been found to be a crucial component of vacuolar iron storage in *Oryza sativa*, improving tolerance to iron toxicity (Zhang *et al*., 2019).

Recent Advances in Understanding Iron Stress Responses

Technological developments in omics and molecular biology have improved our knowledge of how plants react to iron toxicity. According to Sperotto *et al*. (2020), iron-responsive genes implicated in detoxification, transport, and antioxidant defence

have been found through transcriptomic analyses of maize and rice. The post-translational modifications that mediate stress responses have been uncovered by proteomic investigations, which have shown alterations in stress-related proteins under iron overload (Cao *et al*., 2021).

MERCURY

Mercury exists in multiple chemical forms, namely metallic mercury, inorganic mercury and organic mercury compounds. Excessive level of Hg^{2+} is toxic to plant cells and induces evident injuries and physiological disorders in plants (Chen and Yang 2012).

Morphological and Anatomical Effects

By preventing the growth of roots and shoots, decreasing the size of leaves, and resulting in chlorosis and necrosis, mercury toxicity affects the morphology of plants. Mercury exposure causes aberrant elongation, swelling, and decreased branching in roots (Patra & Sharma, 2020). In terms of anatomy, mercury destroys cellular integrity, interferes with vascular development, and alters the structure of xylem and phloem, especially in the mesophyll and epidermal tissues (Singh *et al*., 2021).

Inhibiting root and shoot growth, decreasing leaf size, and resulting in chlorosis and necrosis are among ways that mercury exposure affects plant morphology. According to Patra and Sharma (2020), roots exposed to mercury show signs of aberrant elongation, swelling, and decreased branching. Mercury harms cellular integrity, impedes vascular development, and alters xylem and phloem structure, especially in the mesophyll and epidermal tissues (Singh *et al*., 2021).

Physiological Effects

Mercury affects mitochondria, leaf stomata, binds to water channel proteins and acts as a barrier of water flow in plants. It negatively acts on photosynthesis, transpiration, water uptake and chlorophyll synthesis (Sandhi *et al*., 2023).

According to Boening (2000), mercury has been linked to iron accumulation, potassium depletion, manganese loss, and magnesium loss. Mercury affects the antioxidant defence system by interfering with both enzymatic antioxidants such ascorbate peroxidase, superoxide dismutase, and glutathione reductase as well as nonenzymatic antioxidants like glutathione and thiols (Israr *et al*., 2006, Roychoudhury and Chakraborty, 2020). Mercury can also damage cell membranes and organelles, inactivate or denaturize proteins, and interfere with the delivery of essential micronutrients.

Mercury impedes stomatal function and chlorophyll biosynthesis, which inhibits photosynthetic processes. By increasing ROS and lipid peroxidation, it causes oxidative stress (Islam *et al*., 2022). Mercury further decreases metabolic efficiency by inhibiting enzymatic activity such as superoxide dismutase, peroxidase, and catalase (Sharma *et al*., 2023).

Secondary Metabolite Production Under Mercury Toxicity

Secondary metabolites including flavonoids, alkaloids, and phenolics are produced in response to mercury stress, frequently as a defence against oxidative damage. *Brassica napus* and *Mentha arvensis* under mercury stress have been shown to produce more phenolic compounds (Rajawat *et al*., 2022).

Medicinal Potential of Plants Under Mercury Stress

Mercury affects the profiles of bioactive compounds in plants, changing their therapeutic value. Mercury exposure alters the composition of essential oils in plants such as *Artemisia annua* and *Withania somnifera*, decreasing the safety and effectiveness of treatment (Saxena *et al*., 2020).

Bioaccumulation Patterns

Mercury accumulates in roots and then moves to aerial regions due to its strong affinity for thiol groups in plant proteins (Das *et al*., 2021). Different accumulation patterns are shown by plants based on species, mercury form, and environmental

factors. For instance, whereas methylmercury moves to leaves, inorganic mercury tends to collect more in roots.

Tolerance Mechanisms

According to Gupta *et al.* (2022), plants use strategies such as interacting with phytochelatins and metallothioneins, activating antioxidant enzymes, and sequestering mercury in vacuoles to reduce toxicity. By increasing nutrient absorption and detoxifying capacity, mycorrhizal connections also significantly contribute to improving mercury tolerance (Shukla *et al*., 2023).

Recent Research Insights

Mercury Stress and Growth: Mercury reduces biomass and alters root development in *Oryza sativa* by interfering with auxin transport (Zhang *et al*., 2022).

Photosynthetic Alterations: In *Triticum aestivum*, mercury obstructs energy transfer processes by inhibiting photosystem II and chlorophyll fluorescence (Chatterjee *et al*., 2023).

Secondary Metabolism and Stress Response: According to Banerjee *et al*. (2022), in *Ocimum basilicum* flavonoid concentration increased due to mercury stress, denoting a stress-relieving function.

Bioremediation Strategies:

There is possibility of Hg phytoremediation through the use of genetically engineered plants with increased phytochelatin production (Kumar *et al*., 2021).

***ARTEMISIA NILAGIRICA* (CLARKE) PAMP**

The medicinal herb *Artemisia nilagirica* (Clarke) Pamp., which belongs to the family Asteraceae, is found in the hilly areas of Southeast Asia and India. This plant is well-known for its diverse pharmacological properties and has long been used as an anti-inflammatory and to treat a number of illnesses, such as fever, gastrointestinal issues, and urinary diseases. Recent studies have confirmed the presence of many bioactive molecules with therapeutic actions, further elucidating its medical potential.

Biochemical and Physiological Responses

Cellular constituents such as proteins, lipids, and DNA underwent oxidative stress due to enhanced ROS generation brought on by heavy metal toxicity. According to Kumar *et al*. (2023), the enhanced antioxidant defence mechanisms by plant reduces the oxidative damage under Cd and Pb stress, which is crucial for avoiding cellular homeostasis. The ability of *A. nilagirica* to control ROS production demonstrates its adaptive response to metal toxicity (Kumar *et al*., 2023).

Crucial physiological functions of *A. nilagirica* are also changed by exposure to heavy metals. Pb interferes with stomatal conductance and water consumption efficiency, resulting in decreased transpiration rates, while Cd exposure reduces chlorophyll concentration, disrupting photosynthetic efficiency (Shao *et al*., 2022). The overall growth and production of the plant are adversely affected by these changes.

Phytochemical Composition

The major constituent of essential oil of *A. niligirica* is a monoterpene, α-Thujone. Germacrene-D is another major component. Thujone is generally considered as a principle active constituent of worm wood oil. Diverse bioactive substances, such as flavonoids, alkaloids, terpenoids, are found in *A. nilagirica*. Its medicinal qualities are attributed to the presence of sesquiterpene lactones and polyphenolic chemicals, both of which have been extensively documented.

Recent studies have discovered substances including camphor, β-caryophyllene, and derivatives of artemisinin that have antibacterial, anti-inflammatory, and antioxidant properties (Sharma *et al*., 2023). Additionally, the potent antioxidant properties of essential oils derived from *A. nilagirica* leaves and flowers indicate that they could be utilized to treat diseases linked to oxidative stress (Kumar *et al*., 2022).

Panneerselvam *et.al*. (2014) and Parameswri *et al*. (2019) announced that, *A. nilagirica* is an important source of phytochemicals with pharmaceutical potential. The leaf extract showed potent activity against various bacteria and fungus indicate

that the plant could "lead" for the isolation of novel agents with good efficacy to treat various diseases and disorders. *A. nilagirica* leaf extracts could be a best natural friendly approach for the vector control initiatives.

Anti-inflammatory and Analgesic Properties

The anti-inflammatory and analgesic qualities of *Artemisia nilagirica* constitute some of its most important medical applications. By adjusting the levels of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β, studies have demonstrated that plant extracts can successfully reduce inflammation (Sharma *et al*., 2023). According to Kumar *et al*. (2023), anti-inflammatory properties of the plant are believed to be mediated by suppressing nuclear factor kappa B (NF-κB) signalling pathways and inhibiting cyclooxygenase (COX) enzymes. Its analgesic properties have also been verified in animal models, where it showed pain alleviation on par with that of traditional analgesics such as ibuprofen (Jeyaraj *et al*., 2021).

Antioxidant and Anticancer Activities

Gul *et al*. (2017) demonstrated the phytochemical and antioxidant activities of *A. nilagirica* employing a series of antioxidant assays. The crude extracts of *A. nilagirica* performed high potential of preventing free radical formation. Eight distinct human cancer cell lines were significantly reduced in their proliferation by the ethyl acetate and alcoholic extracts, which did not harm normal peritoneal macrophages. As a result, *A. nilagirica* may be very important for the prevention and treatment of illnesses when oxidants and free radicals are involved.

Hence, *A. nilagirica* can be used potentially as ready accessible and valuable bioactive source for isolation of antioxidant and anticancer agents. The ethyl acetate and alcoholic extracts strongly inhibited the growth of 8 different human cancer cell lines and were nontoxic to normal peritoneal macrophages. Substantial antioxidant and antiproliferative capacities of *A. nilagirica* extracts may be mainly attributed to the presence of the high level of Phyto constituents. Consequently, *A. nilagirica* may have a great relevance in the prevention and therapy of diseases in which free radicals and oxidants are implicated.

Antioxidant potential of *A. nilagirica*. has been extensively studied. The plant extracts are abundant in phenolic chemicals, which have been shown to scavenge free radicals and stop oxidative stress-induced cellular damage. Chronic diseases like diabetes, cancer, and cardiovascular disorders can be avoided thanks to these antioxidant qualities (Kumar *et al*., 2023). Antioxidant-rich extracts of *A. nilagirica* have been demonstrated in studies to shield cells from lipid peroxidation and DNA damage, two processes that are precursors to cancer (Sharma *et al*., 2023).

Furthermore, in a number of in vitro and in vivo models, *A. nilagirica* has shown anticancer activity. In some tumours, such as breast and colon cancer, it has been shown to decrease metastasis, trigger apoptosis, and stop the growth of cancer cells. Flavonoids and sesquiterpenoids, in particular, have been found to be important components responsible for the anticancer potential of plant (Kumar *et al*., 2023). Chemo preventive qualities of *A. nilagirica* raise the possibility that it could be used as an adjuvant cancer treatment.

Antimicrobial Activity

It is well recorded that *Artemisia nilagirica* possess antimicrobial properties. The plant has demonstrated activity against a wide range of microbes, such as viruses, fungus, and bacteria. It is effective against drug-resistant bacterial strains including Escherichia coli and Staphylococcus aureus, which have grown to be a significant worry in modern medicine, according to recent research (Shao *et al*., 2022). According to Jeyaraj *et al*. (2021), the essential oil of *A. nilagirica* has demonstrated exceptional efficacy in combatting pathogenic fungi that cause a variety of human infections, such as *Aspergillus niger* and Candida albicans.

Hepatoprotective and Antidiabetic Effects

Recent research has demonstrated that *Artemisia nilagirica* possesses hepatoprotective and antidiabetic benefits in addition to its anti-inflammatory and antimicrobial qualities. According to Ali *et al*. (2022), its extract has been shown to shield the liver from oxidative stress and toxin-induced damage. The plant may be

used as a therapeutic agent to treat diabetes since its constituents, especially flavonoids, have been connected to blood glucose management (Kumar *et al*., 2023).

Heavy Metal Stress Effects on Plant Growth and Morphology

Large number of studies have focused into how heavy metals affect growth and morphology of *A. nilagirica*. Shoot length, leaf area, and root length have all been shown to significantly decrease when exposed to heavy metals including Cd, Pb, and As. Pb and Cd significantly reduced the growth of roots and shoots, causing morphological changes, according to research by Sharma *et al*. (2022). According to the author, lead exposure also resulted in leaf chlorosis and reduced biomass accumulation, whereas arsenic produced oxidative damage and leaf necrosis. Since the primary location for metal uptake in plants is the roots, the detrimental impacts of these metals are more obvious there.

Biochemical and Physiological Responses

Cellular constituents such as proteins, lipids, and DNA underwent oxidative stress as a result of increased production of ROS brought on by heavy metal toxicity. Increased levels of the lipid peroxidation marker MDA and increased activity of antioxidant enzymes like POD, CAT, and SOD have been found in *A. nilagirica*. According to Kumar *et al*. (2023), the enhanced antioxidant defence mechanisms by plant reduced oxidative damage under Cd and Pb stress, which is crucial for avoiding cellular homeostasis. The ability of *A. nilagirica* to control ROS production demonstrates its adaptive response to metal toxicity (Kumar *et al*., 2023).

Major physiological functions of *A. nilagirica* are also changed by exposure to heavy metals. Lead interferes with stomatal conductance and water consumption efficiency, resulting in decreased rate of transpiration, while Cd exposure reduces chlorophyll synthesis, disrupting photosynthetic efficiency. The overall growth and development of the plant were adversely affected (Shao *et al*., 2022).

Bioaccumulation and Translocation of Heavy Metals

Artemisia nilagirica is a promising candidate for phytoremediation because of its capacity to accumulate heavy metals in a variety of plant components, especially the roots. According to recent research, *A. nilagirica* has a significant capacity for heavy metal bioaccumulation, particularly for Pb and Cd. Roots of *A. nilagirica* serve as a sink for these metals, and there is little translocation to the shoot system (Sharma *et al*., 2022). *A. nilagirica* may be a viable option for extracting metals from contaminated soils without running the risk of them spreading to other plant parts or the food chain, according to this restricted translocation.

In this regard, a study conducted in 2021 by Jeyaraj *et al*. discovered that the plant exhibited a considerable build-up of Cd in the roots following exposure to elevated levels of the metal, with little transfer to the shoots. The reduced risk of metal toxicity in other plant parts was demonstrated by the low translocation factor (TF) and bioaccumulation factor (BF), which suggested that *A. nilagirica* could efficiently store metals in the roots.

Tolerance Mechanisms to Heavy Metal Toxicity

Artemisia nilagirica uses a variety of tolerance mechanisms to endure severe heavy metal stress. The chelation and sequestration of metals within the plant are the main mechanisms. Metals can be chelated to lessen their toxicity by using proteins like metallothioneins (MTs) and organic acids like citric acid. Additionally, polyphenolic compounds, which have been demonstrated to aid in metal detoxification by binding to metals and halting their detrimental effects on cellular structures, are accumulated by *A. nilagirica* (Kumar *et al*., 2023).

Additionally, the plant uses a technique called vacuolar sequestration to keep metals out of delicate areas of the cell. One well-known process by which plants tolerate elevated metal concentrations is vacuolar compartmentalization of heavy metals. According to recent research by Ali *et al*. (2022), *A. nilagirica* minimizes the harmful effects of excess metals like Pb and Cd by storing them in vacuoles.

Furthermore, several transporters that help keep metals out of the cytoplasm are upregulated in *A. nilagirica*. It is believed that transport proteins such metal ion transporters (MTPs) and ATP-binding cassette (ABC) transporters are essential for halting the buildup of hazardous metal ions in plant tissues (Shao *et al*., 2022). This active transport mechanism increases the resistance to stress by lowering the availability of metal for metabolic functions.

Medicinal Implications of Heavy Metal Exposure

In addition to affecting growth and development of *Artemisia nilagirica*, heavy metal accumulation may also have an effect on therapeutic qualities of the plant. According to recent research, exposure to heavy metals may change phytochemical makeup of a plant, which could impact the ability of plant for treatment. For example, the quantities of secondary metabolites like flavonoids, terpenoids, and phenolic compounds, which are responsible for the therapeutic properties of plant, as well as essential oils, have been demonstrated to change when Cd and Pb buildup occurs (Jeyaraj *et al*., 2021).

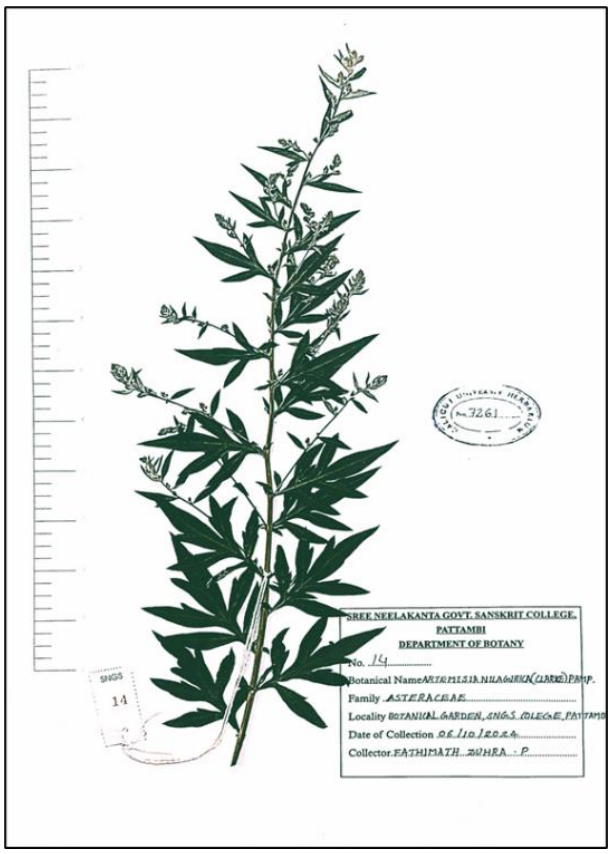
A. nilagirica has been shown to create higher quantities of antioxidant chemicals in response to oxidative stress caused by metals, even though exposure to heavy metals can lower the concentration of some bioactive molecules. Its therapeutic qualities in treating disorders linked to oxidative stress may be improved by this adaptive response. However, if the plant is utilized medicinally without a sufficient assessment of its metal content, the long-term buildup of heavy metals may provide health hazards to humans (Ali *et al*., 2022).

CHAPTER 3
MATERIALS AND METHODS

CHOICE OF PLANT

Artemisia nilagirica growing in the botanical garden, SNGS College, Pattambi was selected for the present investigation. The twigs consisting of 7-8 pairs of fully opened leaves and approximately 20 cm length were collected. A voucher specimen of the collected species has been preserved and stored, and is deposited in the National Repository at the Calicut University Herbarium (CALI) and catalogued under the reference number 7261. Additionally, all relevant documentation, including location, collection date, and environmental conditions, has been recorded.

Fig. 1 Herbarium specimen of *Artemisia nilagirica* (Clarke) Pamp.



CULTIVATION FOR THE EXPERIMENTATION

Healthy plants were maintained for the availability throughout the period of experimentation. Healthy cuttings of length 16 cm consisting of 5-6 nodes and approximately 5-6 pairs of leaves were collected and grown in double distilled water for root initiation. After 12-15 days rooted propagules with 4-5 roots were transferred to Hoagland nutrient medium.

COMPOSITION AND PREPARATION OF NUTRIENT SOLUTION

Hoagland solution (Epstein, 1972) prepared as described by Taiz and Zeiger (2002) was used for hydroponic study (Table 1). The stock solution of each nutrient was prepared separately and appropriate volume of each was mixed together to make up the final volume and concentration of the nutrient solution. pH of the solution was adjusted to 6.8 using 0.1N HCl or NaOH.

Table 1. Composition of nutrient solution (modified Hoagland solution) employed in the present study

Compounds	Molecular weight g/mol	Concentration of stocksolution		Volume of stock/L of final solution
		mM	g/L	
Macronutrients				
KNO_3	101.10	1000	101.0	6.0
$Ca(NO_3)_2.4H_2O$	236.16	1000	236.16	4.0
NH_4Cl	53.49	1000	53.49	2.0
$Mg(NO_3)_2.6H_2O$	256.41	1000	256.41	1.0
Micronutrients				
KCl	74.55	25	1.864	2.0
H_3BO_3	61.83	12.5	0.773	2.0
$ZnSO_4.7H_2O$	287.54	1.0	0.288	2.0
$MnSO_4.H_2O$	169.01	1.0	0.169	2.0
$CuSO_4.5H_2O$	249.68	0.25	0.062	2.0
H_2MoO_4	161.97	0.25	0.04	2.0
NaFeEDTA	558.50	53.7	30.0	0.3

TREATMENT WITH HEAVY METALS

One Molar stock solution of the heavy metal salts for treatment prepared and required dilutions were made. The optimal concentrations were determined by trial-and-error method. Rooted propagules were grown in different concentrations. Concentrations of these metals to impart about 50% growth retardation symptoms maintaining the survival of the plant was determined and these concentrations were selected for further experiments. Aluminium chloride ($AlCl_3$) - 80 µM, potassium dichromate ($K_2Cr_2O_7$) - 70 µM, copper sulphate ($CuSO_4$) - 50 µM, ferric sodium EDTA (NaFeEDTA) - 70 µM, mercuric chloride ($HgCl_2$) - 10 µM.

EXPERIMENTAL SETUP

Fifty milliliters of each of the solution was taken in glass culture tube consisting of 25x150 mm size. Rooted cuttings (1 number) were planted in one culture tube containing 50 mL of Hoagland solution to which the heavy metal solutions were added to obtain the final standardized concentrations. The hydroponic system was maintained under greenhouse conditions. Plants cultivated in Hoagland solution without any heavy metal salt served as the control.

SAMPLING

Cultured plants of treatments and control were collected at comparable interval of 4 days up to 20 days of growth. At each interval (0-20) plants were harvested from each treatment and control washed thoroughly in distilled water and blotted to dryness. A minimum of 5 plants of each treatment were cut to separate root, stem, leaf and each were cut into pieces, randomized and sampled in triplicates for each analysis.

MORPHOLOGICAL PARAMETERS

Growth of plants were assessed in terms of root length, stem length and leaf area.

ROOT LENGTH, STEM LENGTH AND LEAF AREA

The sampled propagules were washed in distilled water, blotted and length of root and stem were measured manually using a graduated scale. Leaf area was measured using Image J Software. Leaf area measurements were made on newly opened leaves because no significant differences were found due to the treatments with heavy metals in the already matured leaves.

TOLERANCE INDEX PERCENTAGE

Tolerance index percentage was calculated according to the method of Turner (1994).

$$\text{TI} = \frac{\text{Observed value of root length in solution with metal}}{\text{Observed value of root length in solution without metal}} X\ 100$$

STOMATAL INDEX

Stomatal density on abaxial and adaxial sides of the leaf was counted under a light microscope, by using nail polish impressions of leaf surface. Stomatal index was calculated according to the method of Meidner and Mansfield (1968)

$$\text{Stomatal Index} = \frac{\text{Number of } stomata\ per\ unit\ area}{\text{No. of stomata} + \text{No. of epidermal cells per unit area}} X\ 100$$

ANATOMICAL STUDIES

Uniformly cut pieces of root, stem and leaf of control and treatments were collected on 20th day of experiment and fixed in FAA. Free hand sections were stained with Toluidine blue according to the procedure of Khasim (2002). Sections were observed using Leica DM 1000 microscope and photographs were taken using DFC 295 Camera.

SEM-EDX ANALYSIS

For ultra structural detailing scanning electron microscopy was done by leaf segments as well as cross sections of root, stem and leaf were fixed in 2.5%

glutaraldehyde made on 0.1 M phosphate buffer (pH 6.9) for overnight. After fixation specimens were rinsed twice with distilled water and dehydrated gradually by passing through alcohol series (50%, 60%, and 70% ethanol was used) for 10 minutes each. Dried specimens were mounted onto grooves cut on aluminium stubs using double side adhesive conducting carbon tapes to expose the sections. After it the specimens were gold coated and later photomicrographs were taken using the photographic attachment of the Scanning Electron Microscope. Energy dispersive X-Ray Analysis (EDX) technique used to identify the elements presented in the samples. SEM-EDAX Model: Jeol 639Ola/ OXFORD XMX N with Accelerating Voltage: 0.5 to 30KV. Filament: Tungsten, Magnification X300000, Elemental Mapping, EDX resolution 136 eV, EDAX detector area 30 mm^2 was used for study.

For experiments control and treated plant samples were collected and washed thoroughly in distilled water and blotted to dryness. Minimum 5 samples of each control and treatment were used for studies.

LEAF MICROMORPHOLOGICAL CHARACTERS

Micromorphology of stomata and trichomes of *A. nilagirica* leaves were evaluated using scanning electron microscope (SEM) on 20 d of exposure to heavy metals. The leaf cuttings of different treatments and control were fixed in 2.5% glutaraldehyde, prepared in 0.1 M phosphate buffer (pH 6.9). The specimens were dehydrated by passing through ascending concentration of acetone series. Then the samples were dehydrated using Critical Point Dryer (Quorum K850, Houston, Texas). Dried specimens were mounted on aluminium stubs using double side adhesive conducting carbon tapes and further subjected to gold-palladium coating for 60 sec using Sputter Coater (Quorum SC7626, Houston, Texas). Photomicrographs were taken using field emission scanning electron microscope (FESEM Carl-Zeiss Gemini 300, Jena, Germany).

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES

DRY WEIGHT

Suitable quantities of root, stem and leaves collected and processed as

described earlier were weighed in pre-weighed containers using electronic balance. Fresh weights obtained were recorded and the weighed samples were then placed in hot air oven at 100 ^{0}C for one hour followed by a 60 ^{0}C for overnight. Dry weight of each sample was taken on the next day and drying and weighing were repeated until values become constant.

Dry weight Percentage = (Dry weight / Fresh weight) ×100

ESTIMATION OF PIGMENT COMPOSITION OF THE LEAF

Estimation of chlorophyll and carotenoids were done according to Arnon (1949). Fresh leaves of control as well as experimental were washed with water and blotted between sheets of filter paper. To estimate chlorophyll and carotenoids, 80% acetone was used as the extracting medium. One hundred milligram fresh leaf sample was weighed in an electronic balance and crushed using mortar and pestle in 20 mL of 80%acetone (v/v). Then the homogenate was centrifuged at 5000 rpm for 10 minutes and the supernatant was collected. The residue was re extracted with 80% acetone and centrifuged. The process was repeated till the pellet become colourless. The final volume of the pooled supernatant was noted. The absorbance was read at 663 nm, 646 nm, 750 nm and 470 nm against the solvent blank (80% acetone). Then quantity of chlorophyll and carotenoids present in the extract was calculated as µg chlorophyll and carotenoids per gram dry weight using the following formula.

Chlorophyll *a* µg/g fresh weight = 12.7(A663) - 2.69(A645) V/1000 x W

Chlorophyll *b* µg/g fresh weight = 22.9(A663) – 4.68(A663) V/1000 x W

Chlorophyll *a*+*b* µg/g fresh weight = 20.2(A645) + 8.2(A663) V/1000 W

$$\text{Carotenoid µg/g fresh weight} = \frac{1000(A470) + 3.27(\text{Chl a} - \text{Chl b})\ xV}{\text{Fresh weight of the sample x } 229}$$

ESTIMATION OF TOTAL SOLUBLE SUGAR CONTENT

Total soluble sugar content was estimated using phenol-sulphuric acid method (Dubois *et al*., 1956).

Extraction: five hundred mg plant samples were homogenized in 5 mL of 80% (v/v) ethanol using pre-chilled glass mortar and pestle. The homogenate was refluxed for 2-3 hours on a water bath after cooling, centrifuged at 10000 rpm for 10 min, and the supernatant was collected. The pellet was re-extracted using 80% ethanol and after centrifugation, the supernatant was collected. The combined supernatant was evaporated to dryness over a boiling water bath and eluted in 4 mL double distilled water and cleared by centrifugation. The supernatant was used for the estimation of total soluble sugars.

Estimation: Estimation of sugar was done according to Montgomery (1957). Suitable aliquots were taken and the volume was made up to 1 mL using double distilled water. To this 0.l mL 80% (w/v) phenol was added and shaken well. Five mL of concentrated sulphuric acid was added quickly from a burette and allowed to cool. After cooling, the optical density of the solution was measured at 490 nm using a spectrophotometer (Shimadzu Corp A12425780917). D-glucose was used as the standard.

ESTIMATION OF STARCH CONTENT

Extraction: The method of Pucher *et al*. (1948) described by Whelan (1955) was used to determine the starch content of samples. Two hundred milligram of tissue in duplicate was weighed and homogenized in a glass mortar and pestle by adding 30% prechilled perchloric acid. The homogenate was centrifuged for five minutes and the supernatant was collected. The residue was re-extracted six times with 30% perchloric acid to ensure the complete extraction of starch. The supernatants were pooled and the volume of the combined extract was noted.

A known volume of the extract was pipetted and an equal volume of the freshly prepared iodine potassium iodide reagent was added and thoroughly mixed. After 10 minutes, it was centrifuged for 10 minutes and the supernatant was decanted. The precipitate was washed with alcoholic sodium chloride solution to remove the excess iodine potassium iodide reagent. After centrifugation, the blue precipitate obtained was treated with alcoholic sodium hydroxide solution till blue color was completely discharged. It was then centrifuged and washed again with alcoholic sodium chloride solution to remove liberated iodine. The precipitate was dissolved in a known quantity of 10% (v/v) sulphuric acid by heating in a boiling water bath, cooled and centrifuged for 10 minutes.

Estimation: Estimation of starch was done according to Montgomery (1957). Suitable aliquot was taken and the volume was made up to 1 mL using double distilled water. To this 0.l mL 80% (w/v) phenol was added and shaken well. Five milliliters of concentrated sulphuric acid was added quickly from a burette and allowed to cool. The optical density of the solution was measured at 540 nm using a spectrophotometer (Shimadzu Corp A12425780917). Soluble starch was used as standard.

ESTIMATION OF TOTAL PROTEIN

Total protein of *A. nilagirica* was estimated according to Lowry *et al*., (1957). Bovine Serum Albumin fraction 5 protein (66 KDa) was used as a standard.

Extraction

Two hundred milligram of root, stem and leaves from the randomized samples of each treatment and control were weighed separately. The weighed tissues were ground using mortar and pestle in chilled distilled water. The homogenate was transferred to centrifuge tubes and equal volume of 10% trichloroacetic acid (TCA) was added, mixed well and kept undisturbed in a refrigerator for flocculation.

The precipitated homogenate was centrifuged for 10 minutes; supernatant was decanted off and 2% TCA was added to the residue and again centrifuged and supernatant was decanted off. The precipitate was washed with 80% acetone to remove the pigments. Two washes were carried out in 80% acetone and final washing in anhydrous acetone. Five mL of 0.1 N NaOH was added to the pellet in each centrifuge tube and boiled for 5 minutes in water bath, cooled and centrifuged. The supernatant was then transferred to test tubes and used to protein estimation.

Estimation

A - 2% sodium carbonate in 0.1 N sodium hydroxide.

B - 0.5% $CuSO_4$ in 1% potassium sodium tartarate

C - Alkaline copper sulphate solution. Mixed 50 mL A and 1mL of B prior to use.

D - Folin-ciocalteau reagent.

Suitable aliquots were taken in duplicates from each preparation. Volume was made up to 1 mL with double distilled water. Then 5 mL of reagent C was added to each tube mixed well and kept at room temperature for 10 minutes and 0.5 mL 1n folin ciocalteau reagent was added with immediate mixing. The tubes were kept for 30 minutes for colour development. Absorbance was read at 700 nm using UV-Visible spectrophotometer.

ESTIMATION OF PROLINE CONTENT

Proline content in the plant parts was estimated according to the method of Bates *et al*., (1973).

Extraction

One-gram fresh tissue of sample such as root, stem and leaves each of the experimental and control plants was homogenized in 10 mL of 5% aqueous sulfocylic acid using a clean glass mortar and pestle. The homogenate was transferred to centrifuge tubes and centrifuged for 10 minutes at 10,000 rpm and the supernatant was collected and estimation of proline was done using acid ninhydrin.

Preparation of acid ninhydrin

Acid ninhydrin was prepared by dissolving 1.25 g of ninhydrin in a mixture of 30 mL of glacial acetic acid and 20 mL of 6 M orthophosphoric acid.

Estimation

From the supernatant 2 mL was taken in test tubes in triplicate and equal volume of glacial acetic acid and ninhydrin were added to it. The tubes were then heated in a boiling water bath for one hour and then the reaction was terminated by placing the tubes in ice bath. For colour development, 4 mL of toluene was added to the reaction mixture and stirred well for 20-30 seconds. Then the chromophoric toluene layer was separated carefully and brought at room temperature. The colour intensity of the solution was measured at a wave length of 520 nm using spectrophotometer. L-proline was used as the standard.

ESTIMATION OF TOTAL PHENOLICS

Total phenolics were estimated using Folin-Denis reagent (Folin and Denis, 1915). Tannic acid was used as the standard.

Extraction

One hundred milligram of fresh tissue was weighed using an electronic balance and homogenized in 80% ethanol in a clean mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 20 minutes and the supernatant was collected. The residue was re extracted with 80% ethanol. The homogenate was again centrifuged and supernatant was pooled. The supernatant was evaporated to dryness at 60 ^{0}C. The residue was dissolved in 5 mL of distilled water.

Estimation

Aliquots of 50 µL in triplicate were pipetted out and made up to 2 mL. Equal volume of Folin-Denis reagent was added to it. The contents were thoroughly mixed and after 3 minutes, 2 mL of 1N sodium carbonate was added. This mixture was kept for one hour after thorough mixing for colour development. The optical density of the resultant solution was measured at 700 nm using a spectrophotometer.

ESTIMATION OF MALONDIALDEHYDE (MDA) CONTENT

The MDA content estimation was done according to Heath and Packer (1968).

Extraction

Two hundred milligram of plant tissue was weighed in duplicate and homogenized in 5 mL of 5% trichloroacetic acid. The homogenate was centrifuged at 12,000 rpm for 15 minutes. The supernatant was collected and used for the estimation of MDA.

Estimation

Two milliliters of the supernatant was mixed with an equal aliquot of 0.5% of Thio barbituric acid (TBA) in 20% trichloroacetic acid (TCA). The solution was heated at 95 ^{0}C for 24 minutes, cooled and then centrifuged at 3000 rpm for 2 minutes. The absorbance of the supernatant was measured at 532 nm and 600 nm against reagent blank using UV- visible spectrophotometer. The absorbance value at 532 nm was corrected for non-specific turbidity by subtracting absorbance value at 600 nm and then the MDA content was calculated using an extinction coefficient of 155.

QUANTITATIVE ESTIMATION OF SELECTED HEAVY METALS

Selected heavy metals namely aluminium, chromium, copper, iron and mercury in root, stem and leaf tissues were analyzed using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). ICP-OES instrument PERKIN ELMER-AVIO 200 at Kerala Research Institute (KFRI), Peechi, Thrissur was used for the estimation of heavy metals present in the digested samples.

Extraction

The digestion of plant samples to analyze the metal content was carried out according to the method of Allan (1969).

The samples of root, stem and leaves from the control and treatments sampled on 4th,12th and 20th days were dried in an oven at 60 °C until a constant weight was achieved. Known weight of each dried sample was transferred into Kjeldahl flasks, and then a mixture of nitric and perchloric acids prepared in 10:4 ratio was added. The samples were refluxed for digestion in the heating mantle at 60 °C until the solution became colorless. Subsequently, the digest was filtered using Whatman's filter paper, transferred to a standard flask and volume was made up to 50 mL with distilled water.

Estimation

Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) was used to estimate metal present in the digested samples. The content of metals in the plant samples was expressed in micrograms per gram dry weight. The Bioaccumulation factor (BCF) and Translocation factor (TF) of metals in the plant parts was calculated by using the following equations.

$$\text{Bioaccumulation factor} = \frac{\text{Concentration of metal in the roots}}{\text{Concentration of metal in the solution}}$$

$$\text{Translocation factor } (\text{TF}) = \frac{\text{Concentration of metal in the shoots}}{\text{Concentration of metal in the root}}$$

ENZYMATIC ANTIOXIDANTS

SUPEROXIDE DISMUTASE (SOD)

Assay of SOD activity in the fresh plant samples was done as per the modified protocol of Giannopolitis and Ries (1977).

Extraction: The enzyme extraction was carried out according to the method of Polle *et al*. (1994). Pre-weighed fresh plant samples were homogenized in 5 mL of 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, using pre-chilled glass mortar and pestle. The homogenate was filtered through four-layered muslin cloth, and the filtrate was centrifuged at 14000 rpm for 15 min at 4 °C, and the supernatant was used for the enzyme assay.

Enzyme Assay: The reaction mixture consisted of 0.1 mL of 1.5 M sodium carbonate, 0.3 mL of 130 mM methionine, 0.3 mL of 13 µM riboflavin, 0.3 mL of 10 µM EDTA, 0.3 mL of 0.63 mM nitrobluetetrazolium (NBT) and 0.1 mL enzyme extract, and the reaction mixture was made up to 3 mL using 100 mM potassium phosphate buffer (pH 7.0). Different assay systems were set, viz. test samples, dark-control and light-control. Test tubes containing all the assay mixtures including enzyme extract, illuminated under fluorescent lamp for 30 min served as the test sample and the set of tubes placed in dark served as the dark control. The assay mixture without enzyme extract illuminated under fluorescent lamp for 30 min served as light control. The mixture without NBT and enzyme extract served as the blank. The blue formazan accumulation in different tubes was quantified using spectrophotometer by recording the absorbance at 560 nm against the blank. One unit of SOD activity was defined as the amount of enzyme required to inhibit the photochemical reduction of NBT to blue formazan by 50%.

Specific activity of each enzyme was calculated after determining soluble protein concentration in the enzyme extract.

$$\text{Specific activity} = \frac{\text{Enzyme activity units}}{\text{mg protein / mL enzyme extract}}$$

CATALASE (CAT)

Catalase (CAT) activity in the fresh plant samples was assayed by the method of Chance and Maehly (1955) with some modifications.

Extraction: The enzyme extraction was carried out according to Polle *et al*. (1994). Pre-weighed fresh plant samples were homogenized in 5 mL of 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, using pre-chilled mortar and pestle. The homogenate was filtered through four-layered muslin cloth, and the filtrate was centrifuged at 14000 rpm for 15 min at 4°C, and the supernatant was used for the enzyme assay.

Enzyme assay: The reaction mixture contained 1 mL of 100 mM potassium phosphate buffer (pH 7.0), 1 mL of 30% (w/v) H_2O_2 and 0.1 mL of enzyme extract. The activity of CAT was recorded by observing the decrease in absorbance at 240 nm for 90 s at 15 s interval using UV-VIS spectrophotometer. One unit of CAT activity was defined as the amount of enzyme required for the decomposition of 1 μM of H_2O_2 per min.

GC-MS ANALYSIS

Fresh leaves on 20th day were collected and shade dried. Dried leaves were ground into fine powder and 5 g of which was subjected to extraction using 100 mL of methanol in Soxhlet apparatus. After running several cycles, concentrated extract was obtained. The extract was used for GC-MS analysis.

Gas chromatography Mass Spectrometry was performed by using Shimadzu GC-MS, model number QP2010S at Kerala Forest Research Institute, Thrissur, Kerala. ELITE-5MS column with 30-meter length, 0.25 mm internal diameter and

0.25 µm thickness was used. Helium gas was used as the carrier gas at constant flow rate of 1 mL/min and an injection volume of 1 µL was employed. The injection port temperature was set at 260 ºC and ion source temperature at 220 ºC. The oven temperature was programmed from 60 ºC to 270 ºC. The total GC running time was 63 min. Data available from the NIST 11 & WILEY 8 Libraries were used for identification of the components. The software used was GCMS Solutions.

FTIR ANALYSIS

For Fourier Transform Infrared analysis, healthy leaves collected on 20th day from both control and treated plant samples then shade dried and made into fine powder with clean mortar and pestle. Perkin Elmer Spectrum Two FT-IR Spectrometer (Fourier Transform Infrared Spectrometer) was used for the analysis of functional groups present in phytochemicals in the range of 400 - 4000 cm^{-1}.

CYTOTOXICITY STUDY

The plant samples were studied for short term in vitro cytotoxicity using Dalton's Lymphoma Ascites cells (DLA).

Extraction

Fresh leaves on 20th day were collected and shade dried. Dried leaves were ground into fine powder and 5 g of which was subjected to extraction using 100 mL of methanol in Soxhlet apparatus. After running several cycles, concentrated extract was obtained. The extract was air dried till complete removal of methanol. The dried powder was used for cytotoxicity study.

Estimation

The tumor cells aspirated from the peritoneal cavity of tumor bearing mice were washed thrice with PBS or normal cell line. Cell viability was determined by trypan blue exclusion method. Viable cells suspension (1X10^6 cells in 0.1 mL) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1mL using phosphate buffered cell line (PBS). Control tube contained only cell suspension. These assay mixtures were incubated for 3 hours at

37ºC. Further cell suspension was mixed with 0.1 mL of 1% trypan blue and kept for 2-3 minutes and loaded on a hemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The numbers of stained and unstained cells were counted separately.

$$\%\ Cytotoxicity\ = \frac{\text{No. of dead cells}}{\text{No. of live cells} + \text{No. of dead cells}}\ X\ 100$$

STATISTICAL ANALYSIS

The study results were statistically analyzed using one-way ANOVA. Dunnett's test was used to assess significant treatment effects ($p < 0.05$). The data represent average recordings from three different studies, each with five replicates ($n = 15$). The data represents the mean ± standard error.

CHAPTER 4

RESULTS

STANDARDIZATION

Table 2. Standardization of aluminium for *Artemisia nilagirica* (Clarke) Pamp. using different concentrations of $AlCl_3$.

Growth Parameter	Interval	Control	Treatment ($AlCl_3$)				
			20 µM	40 µM	60 µM	80 µM	100 µM
Root Length	0th day	4.5±0.22	4.5±0.13	4.5±0.01	4.5±0.20	4.5±0.32	4.5±1.21
	4th day	8.70±0.02	8.94±0.05	8.89±0.4	8.86±1.3	8.82±0.14	5.44±1.5
	8th day	11.14±0.22	12.67±0.11	12.43±0.01	11.35±0.02	9.64±0.16	5.81±0.32
	12th day	13.03±0.2	15.35±1.5	13.14±0.04	13.01±0.02	10.68±0.14	6.42±1.23
	16th day	15.23±0.3	16.74±0.5	15.13±1.4	15.01±0.26	11.75±0.1	6.92±0.12
	20th day	17.62±0.22	18.45±0.20	18.32±1.01	17.91±0.02	12.90±0.16	8.05±1.01
Stem Length	0th day	16±0.03	16±0.01	16±0.20	16±0.14	16±1.40	16±1.30
	4th day	19.20±0.01	19.15±0.05	19.06±0.02	18.34±0.1	17.84±1.4	16.46±0.02
	8th day	21.98±1.2	21.95±0.01	21.42±0.02	21.25±1.3	20.12±2.4	17.35±1.5
	12th day	24.80±1.1	24.92±0.03	24.36±0.05	24.54±0.01	23.84±1.2	17.55±2.02
	16th day	27.54±2.5	27.48±1.2	27.43±2.2	27.12±0.06	25.84±1.2	18.04±1.43
	20th day	29.42±1.4	29.56±2.02	29.33±1.1	29.25±0.03	29.02±1.6	19.62±2.12
Leaf Area	0th day	4.35±0.22	4.36±2.30	4.33±1.5	4.42±2.06	4.26±0.18	4.46±1.24
	4th day	4.75±0.12	4.81±2.01	4.75±0.52	4.70±0.06	4.88±0.14	4.49±0.01
	8th day	5.86±0.04	5.85±1.15	5.64±1.20	5.83±1.04	5.62±0.07	4.86±1.23
	12th day	6.74±0.02	6.82±0.06	6.71±1.25	6.69±1.32	6.52±0.04	5.32±1.05
	16th day	8.36±0.04	8.28±1.35	8.26±1.26	8.25±1.04	7.56±0.02	5.84±2.14
	20th day	10.75±0.06	10.34±0.05	10.36±1.02	10.22±1.45	9.98±0.1	6.22±0.21

Values given are mean of 5 replicates ± S. E

Among the different concentrations of $AlCl_3$ (20 µM, 40 µM, 60 µM, 80 µM, 100 µM) studied, 80 µM $AlCl_3$ showed moderate growth retardation and at the same time the plant survived. At concentrations below 80 µM, all samples were noted with almost equal growth rate of control. Whereas, the concentration above 80 µM resulted extreme growth retardation. Hence 80 µM Al considered as the standardized concentration for *A. nilagirica* (Table.2).

Table 3. Standardization of chromium for *Artemisia nilagirica* (Clarke) Pamp. using different concentrations of $K_2Cr_2O_7$.

Growth Parameter	Interval	Control	Treatment ($K_2Cr_2O_7$)				
			10 µM	30 µM	50 µM	70 µM	90 µM
Root Length	**0th day**	4.5±0.22	4.5±0.11	4.5±0.02	4.5±0.05	4.5±0.20	4.5±0.14
	4th day	8.70±0.02	9.08±0.04	8.95±1.14	8.98±0.13	9.07±0.03	5.44±1.5
	8th day	11.14±0.22	11.17±1.12	11.03±1.34	10.95±1.05	10.12±0.4	6.65±0.04
	12th day	13.03±0.2	13.35±0.15	13.12±0.02	11.05±1.52	10.36±0.02	8.91±1.36
	16th day	15.23±0.3	15.24±0.03	15.13±1.54	13.25±0.26	11.20±0.24	9.14±1.42
	20th day	17.62±0.22	17.45±0.04	17.32±1.06	17.21±1.22	12.02±0.05	9.65±1.06
Stem Length	**0th day**	16±0.03	16±1.52	16±0.18	16±1.02	16±1.16	16±0.13
	4th day	19.20±0.01	19.15±1.12	19.06±2.4	18.35±0.05	17.66±1.6	16.36±2.04
	8th day	21.98±1.2	21.84±2.02	21.42±0.33	21.25±1.05	19.42±1.3	17.25±1.25
	12th day	24.80±1.1	24.82±0.15	24.56±0.06	23.34±1.04	21.34±0.23	18.65±0.14
	16th day	27.54±2.5	27.36±0.15	27.33±2.15	27.12±1.65	22.52±1.3	22.04±1.11
	20th day	29.42±1.4	28.36±1.45	28.23±2.03	29.25±1.12	23.2±1.4	22.15±0.05
Leaf Area	**0th day**	4.35±0.22	4.26±1.03	4.33±2.05	4.42±1.25	4.25±0.2	4.26±1.42
	4th day	4.75±0.12	4.51±1.54	4.46±1.25	4.45±0.05	4.37±0.14	4.27±0.52
	8th day	5.86±0.04	5.64±1.54	4.84±2.04	4.93±2.52	4.58±0.02	4.30±2.14
	12th day	6.74±0.02	6.35±1.23	5.41±1.04	5.32±1.22	4.72±0.11	5.04±0.05
	16th day	8.36±0.04	7.28±0.26	7.16±0.01	6.36±0.42	5.76±0.09	5.14±1.26
	20th day	10.75±0.06	9.35±1.04	8.44±2.42	8.22±0.25	7.14±0.14	5.26±1.04

Values given are mean of 5 replicates ± S. E

Among the different concentrations of $K_2Cr_2O_7$ (10 µM, 30 µM, 50 µM, 70 µM, 90 µM) studied, 70 µM $K_2Cr_2O_7$ showed moderate growth retardation and at the same time the plant survived. At concentrations below 70 µM, all samples were noted

with almost equal growth rate of control. Whereas, the concentration above 70 µM caused extreme growth retardation in tested plants. Hence 70 µM Cr considered as the standardized concentration for *A. nilagirica* (Table. 3).

Table 4. Standardization of copper for *Artemisia nilagirica* (Clarke) Pamp. using different concentrations of $CuSO_4$.

Growth Parameter	Interval	Control	Treatment ($CuSO_4$)				
			20 µM	**30 µM**	**40 µM**	**50 µM**	**60 µM**
Root Length	**0th day**	4.5±0.22	4.5±0.14	4.5±0.25	4.5±0.06	4.5±0.24	4.5±0.02
	4th day	8.70±0.02	8.74±1.24	8.69±0.06	8.46±1.38	7.86±0.19	5.50±1.02
	8th day	11.14±0.22	11.2±0.22	11.03±0.14	10.55±0.03	8.92±0.32	6.42±1.25
	12th day	13.03±0.21	13.44±0.05	12.25±1.23	11.85±0.25	10.13±0.18	6.56±1.32
	16th day	15.23±0.35	14.28±1.21	14.13±0.04	12.01±1.05	11±0.13	7.52±2.01
	20th day	17.62±0.22	16.44±0.35	15.62±1.62	15.31±0.04	12.10±0.15	10.05±1.5
Stem Length	**0th day**	16±0.03	16±0.01	16±1.24	16±1.22	16±1.40	16±0.23
	4th day	19.20±0.01	19.05±0.02	18.46±1.34	18.11±0.22	17.24±1.4	16.56±0.04
	8th day	21.98±1.2	20.66±1.2	20.12±0.04	19.75±0.02	18.30±2.9	17.05±1.34
	12th day	24.80±1.1	22.34±1.2	21.86±0.26	21.54±2.01	20.0±1.80	19.33±1.52
	16th day	27.54±2.5	24.56±0.02	23.43±1.52	22.85±0.01	21.00±1.13	20.05±1.46
	20th day	29.42±1.4	26.24±0.12	24.63±0.06	23.44±1.35	21.84±2.1	20.32±1.05
Leaf Area	**0th day**	4.35±0.22	4.26±0.04	4.33±1.02	4.42±2.14	4.55±0.23	4.46±0.52
	4th day	4.75±0.12	4.31±0.03	4.45±1.02	4.50±0.11	4.62±0.04	4.48±0.05
	8th day	5.86±0.04	4.85±1.03	5.15±0.22	4.93±2.12	4.88±0.12	4.56±0.14
	12th day	6.74±0.02	5.62±1.52	5.34±0.04	5.09±1.45	5.26±0.14	4.92±2.02
	16th day	8.36±0.04	6.45±0.02	6.23±1.45	5.88±1.22	5.64±0.1	4.97±0.04
	20th day	10.75±0.06	9.62±0.02	8.84±1.23	8.22±0.25	5.75±0.18	5.12±0.16

Values given are mean of 5 replicates ± S. E

Among the different concentrations of $CuSO_4$ (20 µM, 30 µM, 40 µM, 50 µM, 60 µM) studied, 50 µM $CuSO_4$ showed moderate growth retardation and at the same time the plant survived. At concentrations below 50 µM, all samples were noted with almost equal growth rate of control. Whereas, at concentration above 50 µM plant showed extreme growth retardation. Hence 50 µM Cu considered as the standardized concentration for *A. nilagirica* (Table. 4).

Table 5. Standardization of iron for *Artemisia nilagirica* (Clarke) Pamp. using different concentrations of NaFeEDTA.

Growth Parameter	Interval	Control	Treatment (NaFeEDTA)				
			10 µM	**30 µM**	**50 µM**	**70 µM**	**90 µM**
Root Length	**0th day**	4.5±0.22	4.5±0.13	4.5±1.05	4.5±0.23	4.5±0.13	4.5±1.12
	4th day	8.70±0.02	8.74±1.2	8.69±0.04	8.12±1.5	8.26±0.22	6.44±0.02
	8th day	11.14±0.22	11.07±1.5	10.9±0.11	10.35±0.42	9.22±0.25	8.01±1.06
	12th day	13.03±0.2	12.6±1.02	11.14±0.32	11.01±0.05	10.21±0.22	8.42±1.01
	16th day	15.23±0.3	13.44±1.02	12.23±0.15	12.01±0.04	10.76±0.03	8.94±0.03
	20th day	17.62±0.22	14.36±0.12	13.51±1.5	13.22±1.05	11.89±0.03	9.05±0.24
Stem Length	**0th day**	16±0.03	16±0.05	16±0.02	16±1.04	16±0.03	16±0.06
	4th day	19.20±.01	17.8±1.23	17.56±1.04	17.34±0.05	17.42±1.9	16.4±1.02
	8th day	21.98±1.2	20.95±1.1	19.42±1.52	19.25±0.02	18.90±2.9	16.85±2.25
	12th day	24.80±1.1	23.8±0.02	21.5±0.05	21.64±1.5	20.24±.08	18.4±0.14
	16th day	27.54±2.5	26.45±1.2	25.43±0.03	23.12±0.05	21.72±2.3	19.04±1.65
	20th day	29.42±1.4	28.06±1.21	26.3±0.04	25.35±0.15	22.48±1.1	20.42±1.32
Leaf Area	**0th day**	4.35±0.22	4.26±2.11	4.33±1.04	4.42±0.06	4.62±0.13	4.46±0.12
	4th day	4.75±0.12	4.71±0.04	4.52±1.02	4.48±1.16	4.95±0.06	4.49±0.05
	8th day	5.86±0.04	5.75±1.5	5.53±0.01	5.25±1.03	5.12±0.05	4.81±1.2
	12th day	6.74±0.02	6.62±2.04	6.13±1.03	6.69±1.52	5.42±0.08	5.02±0.06
	16th day	8.36±0.04	8.16±1.2	8.05±1.04	7.88±0.06	6.1±0.14	5.45±2.01
	20th day	10.75±0.06	10.45±0.08	10.11±1.24	9.76±0.02	6.42±0.16	5.84±1.15

Values given are mean of 5 replicates ± S. E

Among the different concentrations of NaFeEDTA (10 µM, 30 µM, 50 µM, 70 µM, 90 µM) studied, 70 µM NaFeEDTA showed moderate growth retardation and at the same time the plant survived. At concentrations below 70 µM, all samples were noted with almost equal growth rate of control. Whereas, the concentration above 70 µM caused extreme growth retardation in tested plants. Hence 70 µM Fe considered as the standardized concentration for *A. nilagirica* (Table. 5).

Table 6. Standardization of mercury for *Artemisia nilagirica* (Clarke) Pamp. using different concentrations of $HgCl_2$.

Growth Parameter	Interval	Control	Treatment ($HgCl_2$)				
			5 µM	**10 µM**	**15 µM**	**20 µM**	**25 µM**
Root Length	**0th day**	4.5±0.22	4.5±0.13	4.5±0.24	4.5±0.23	4.5±0.13	4.5±1.12
	4th day	8.70±0.02	8.34±1.2	7.13±0.3	5.16±0.05	4.86±0.22	4.12±1.5
	8th day	11.14±0.22	10.4±0.02	8.30±0.14	5.35±0.01	5.22±0.25	5.01±0.02
	12th day	13.03±0.2	12.25±0.1	9.14±0.35	6.45±1.24	5.52±0.22	5.12±0.01
	16th day	15.23±0.3	14.1±0.06	9.98±0.25	6.81±1.35	5.76±0.03	5.33±2.02
	20th day	17.62±0.22	15.4±0.14	10.72±0.6	7.21±0.21	5.88±0.03	5.35±1.5
Stem Length	**0th day**	16±0.01	16±0.13	16±0.26	16±1.12	16±0.05	16±1.3
	4th day	19.20±.01	18.15±1.1	16.58±1.5	16.3±0.04	16.12±1.2	16.05±0.1
	8th day	21.98±1.2	19.23±1.2	17.56±2.5	17.25±1.1	16.80±2.3	16.2±0.08
	12th day	24.80±1.1	22.92±0.01	18.44±1.5	18.04±1.3	17.34±.08	16.45±0.02
	16th day	27.54±2.5	23.44±1.4	19.30±1.3	18.12±1.6	17.74±0.3	16.84±1.25
	20th day	29.42±1.4	25.6±0.02	20.3±1.4	18.25±1.2	17.88±1.1	16.86±0.03
Leaf Area	**0th day**	4.35±0.22	4.26±2.11	4.5±0.22	4.70±1.22	4.42±1.04	4.49±0.16
	4th day	4.75±0.12	4.48±2.4	4.74±0.15	4.83±0.02	4.60±0.01	4.51±0.04
	8th day	5.86±0.04	5.85±1.6	4.94±0.16	4.90±1.6	4.72±0.08	4.84±1.22
	12th day	6.74±0.02	6.14±1.1	5.35±0.06	5.15±0.25	4.81±0.14	4.92±0.02
	16th day	8.36±0.04	8.24±0.2	6.12±0.04	5.32±0.02	5.10±0.16	5.02±1.04
	20th day	10.75±0.06	10.2±1.54	6.4±0.08	5.74±1.42	5.25±1.42	5.11±1.26

Values given are mean of 5 replicates ± S. E

Table. 6 states that, among the different concentrations of $HgCl_2$ (5 µM, 10 µM, 15 µM, 20 µM, 25 µM) tested, 10 µM $HgCl_2$ showed moderate growth retardation and at the same time the plant survived. At concentrations below 10 µM, all samples were noted with almost equal growth rate of control. Whereas, at concentrations above 10 µM plants showed extreme growth retardation. Hence 10 µM Hg considered as the standardized concentration for *A. nilagirica*.

Fig. 2 Growth performance of *Artemisia nilagirica* plants treated with metals like aluminium, chromium, copper, iron and mercury after 20 days of growth. A-control; B-aluminium; C-chromium; D-copper; E-iron F- mercury

Fig. 2 shows the images on 20th day, of control and plantlets treated with standardized concentration of each metal.

ROOT LENGTH

The findings in Table. 7 indisputably show how heavy metal toxicity inhibits *Artemisia nilagirica* root growth. The presence of Al, Cr, Cu, Fe and Hg at particular concentrations had a considerable impact on root elongation, a crucial indicator of plant health and development. Varying levels of inhibition by metals demonstrated variations in their phytotoxicity. The root length in the control group, which stands for ideal development conditions free from metal exposure, rose gradually over time, peaking at 17.62 cm on day 20. This highlights the ability of *Artemisia nilagirica* for natural growth in the absence of environmental disturbances. On the other hand, exposure to heavy metals caused growth to be stunted to varied degrees, with the amount of the reduction depending on the metal in concern.

The toxicity of Al, Cr, Cu, Fe and Hg caused *Artemisia nilagirica* to exhibit observable growth retardation in root length when compared to control. For all heavy metals, the rate of growth retardation was almost same. Starting on the 8th day and continuing for the duration of the development period, all metals showed obvious retardation. When compared to the control, plants treated with metals gradually decreased root growth, plants treated with Hg showed the greatest retardation in root growth. Between treatments, root growth was retarded in the following order: Hg <Fe< Cr < Cu < Al (Fig. 3).

Root development was moderately reduced by Al (80 µM). Initially, its impacts were insignificant in comparison to the control; nevertheless, the growth rate slowed considerably as the exposure period lengthened. The mean root length decreased to 12.90 cm after 20 days, suggesting that extended exposure to Al interferes with the processes necessary for root elongation, most likely by interfering with the formation of the cell wall or nutrient intake.

A similar pattern of decreased root elongation was observed with Cr (70 µM), although its effects were noticeable sooner than those of Al. With a root length of 12.02 cm at the end of the trial, Cr may potentially hinder cellular functions like ion and water uptake. In addition to causing oxidative stress, Cr poisoning can further impede root growth.

The roots treated with Cu (50 µM) had decreased growth from the beginning of the experiment, indicating a more noticeable toxic effect. The root length was only 12.10 cm after 20 days, indicating the significant phytotoxicity of Cu. Its capacity to produce reactive oxygen species, which oxidatively damages cellular structures and prevents root cell elongation, is probably the cause of copper toxicity.

Despite being a necessary micronutrient, Fe (70 µM) showed negative effects on root growth when absorbed in excess. A metabolic imbalance brought on by too much iron can result in less cell division and elongation. In comparison to the other metals examined, Fe was somewhat hazardous, as evidenced by the root length under exposure of 11.89 cm after 20 days. Out of all the metals examined, Hg (10 µM) was the most hazardous. Because mercury significantly inhibited root growth from the

beginning of the experiment, the mean root length was only 10.72 cm after 20 days. Because Hg poisoning binds to sulfhydryl groups in proteins, it is known to significantly impair cellular integrity and enzymatic activity, leading to marked growth retardation.

The findings demonstrate the detrimental effects of heavy metal poisoning on *Artemisia nilagirica* root growth. Any disturbance to the process of root elongation, which is essential for the absorption of nutrients and water, has a substantial impact on the general well-being and survival of the plant. Because of their great affinity for producing reactive oxygen species and interfering with vital cellular functions, metals such as Cu, Fe and Hg exhibited the strongest inhibitory effects, while Al and Cr had moderate impacts.

The gradual reduction in root length as exposure time increases over time, which further impede plant growth. This discovery highlights vulnerability of *A. nilagirica* to heavy metal stress and the possible obstacles to its growth in contaminated environments.

Table 7. Effect of aluminium, chromium, copper, iron and mercury on root length (**cm**) in *Artemisia nilagirica* (Clarke) Pamp.

Metals	**Interval - Days**					
	0	**4**	**8**	**12**	**16**	**20**
Control	4.5±0.22	8.70±0.02	11.14±0.22	13.03±0.2	15.23±0.3	17.62±0.22
Aluminium (80 µM)	4.5±0.31	8.82±0.14	9.64±0.16	10.68±0.14	11.75±0.1	12.90±0.16
Chromium (70 µM)	4.5±0.20	9.07±0.03	10.12±0.4	10.36±0.02	11.20±0.24	12.02±0.05
Copper (50 µM)	4.5±0.24	7.86±0.19	8.92±0.32	10.13±0.18	11±0.13	12.10±0.15
Iron (70 µM)	4.5±0.13	8.26±0.22	9.22±0.25	10.21±0.22	10.76±0.03	11.89±0.03
Mercury (10 µM)	4.5±0.24	7.13±0.3	8.30±0.14	9.14±0.35	9.98±0.25	10.72±0.6

Values given are mean of 5 replicates ± S. E

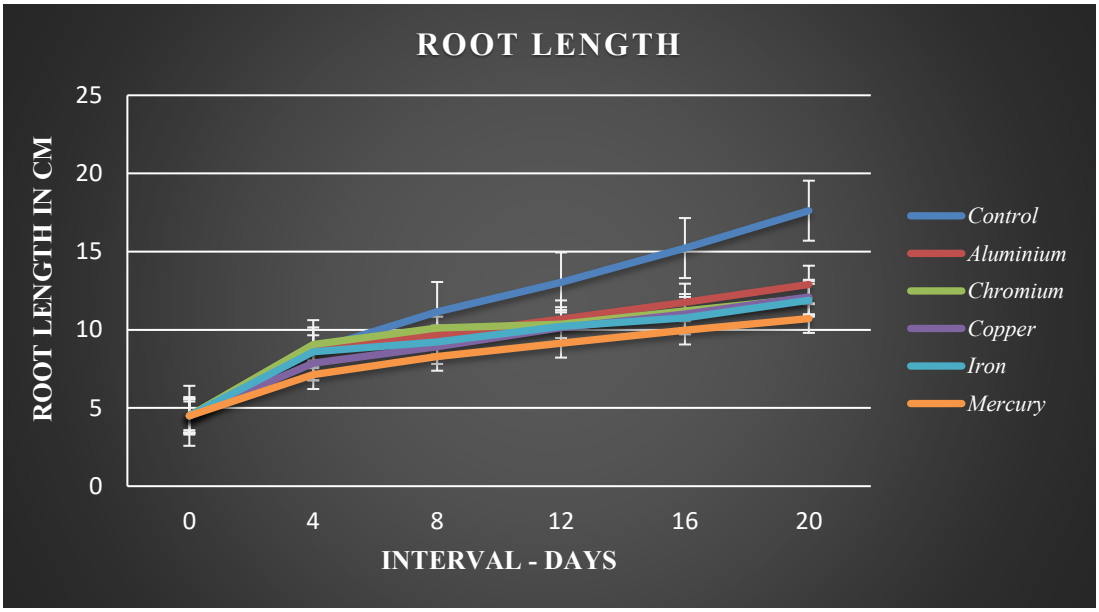


Fig. 3 Effect of aluminium, chromium, copper, iron and mercury on root length (**cm**) in *Artemisia nilagirica* (Clarke) Pamp.

SHOOT LENGTH

Table. 8 & Fig. 4 provides a description of how *A. nilagirica* shoot development is harmed by heavy metals. The effect on shoot length indicates that exposure to heavy metal poisoning considerably reduces plant growth. There is a clear inhibitory tendency in the data as metal concentrations rise. Over the course of 20 days, the shoot growth was evaluated for each metal treatment. Shoot length of the control group increased from 16 cm to 29.42 cm by 20 days, demonstrating steady and robust growth. This acts as a baseline, signifying ideal growth circumstances free from stress brought on by heavy metals.

Aluminium had a comparatively minor effect on shoot elongation; after 20 days, the shoot length was 29.02 cm, which was somewhat less than the control. Although the growth was still around the same as that of the untreated group, Al most certainly had a sublethal effect on cell division and elongation. The slow reduction in growth rate indicates that Al, though not as significantly as other metals, hampered the expansion of the cell wall and the absorption of nutrients.

Exposure to Cr decreased stem growth, resulting in a final length of 23.2 cm. When compared to the control, this indicates a significant decrease. A cumulative toxic effect over time is indicated by the gradual suppression of shoot development.

The toxicity of Cu was more noticeable; by day 20, the shoot length had reached at 21.84 cm. Copper disrupts structural proteins, limits cell growth, which prevents the flow of nutrients and water required for prolonged elongation, is reflected in the growth rate drop that has been seen. The shoot length decreased significantly due to excess Fe, reaching a final value of 22.48 cm. Iron is a necessary nutrient, but too much of it can cause metabolic imbalance and reduced growth.

The most severe toxicity was shown by Hg, which by day 20 had inhibited shoot development to 20.3 cm. Out of all the treatments, Hg treated plant had the slowest growth. The sharp drop in shoot length highlights strong harmful effects of Hg, even at very low amounts.

The distinct toxicity mechanisms of heavy metals are reflected in the varying impacts they have on *A. nilagirica* shoot elongation. The most severe stunting was caused by Cu and Hg, followed by Fe and Cr, while the least inhibiting effect was caused by Al. The accumulated toxicity of these metals over time significantly reduces the ability of plant to maintain growth, underscoring their negative effects on the general health of plants.

Table 8. Effect of aluminium, chromium, copper, iron and mercury on shoot length (**c m**) in *Artemisia nilagirica* (Clarke) Pamp.

Metals	**Interval - Days**					
	0	**4**	**8**	**12**	**16**	**20**
Control	16±0.03	19.20±.01	21.98±1.2	24.80±1.1	27.54±2.5	29.42±1.4
Aluminium (80 µM)	16±1.4	17.84±1.4	20.12±2.4	23.84±1.2	25.84±1.2	29.02±1.6
Chromium (70 µM)	16±1.1	17.66±1.6	19.42±1.3	21.34±0.2	22.52±1.3	23.2±1.4
Copper (50 µM)	16±1.4	17.24±1.4	18.30±2.9	20.0±1.8	21±1.13	21.84±2.1
Iron (70 µM)	16±.03	17.42±1.9	18.90±2.9	20.24±.08	21.72±2.3	22.48±1.1
Mercury (10 µM)	16±1.2	16.58±1.5	17.56±2.5	18.44±1.5	19.30±1.3	20.3±2.6

Values given are mean of 5 replicates ±S. E

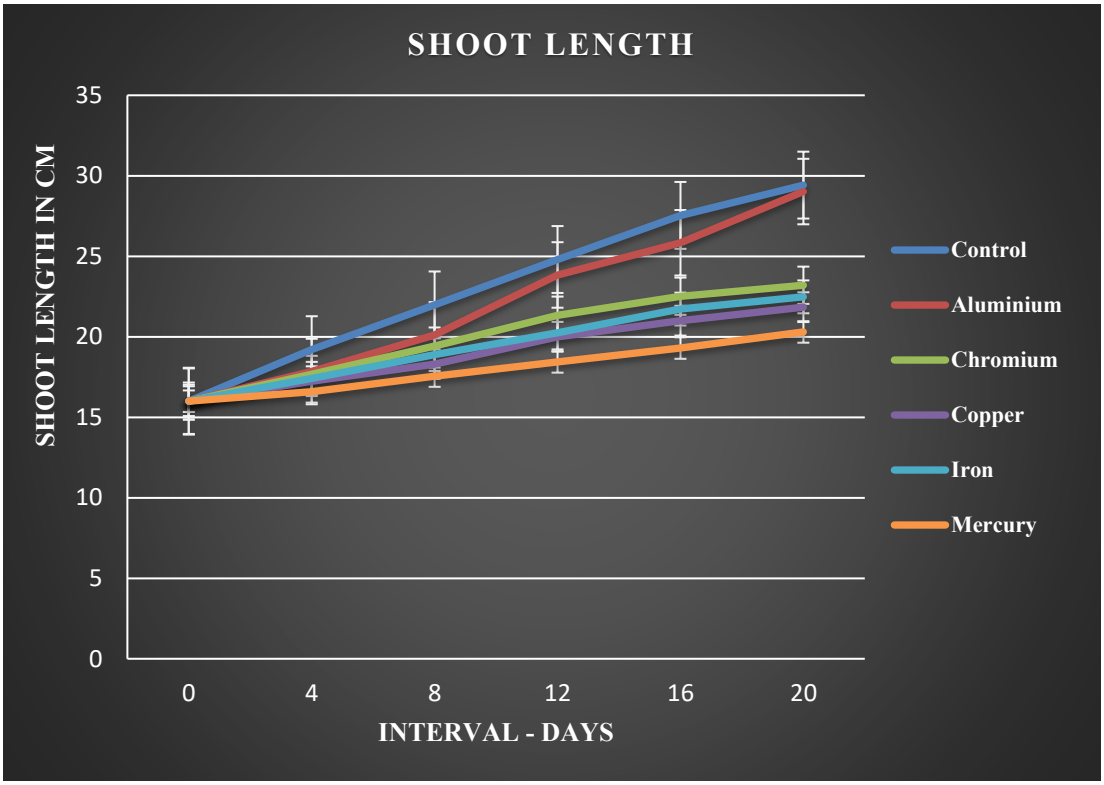


Fig. 4 Effect of aluminium, chromium, copper, iron and mercury on shoot length (**cm**) in *Artemisia nilagirica* (Clarke) Pamp.

Leaf Area

The data (Table. 9) illustrates how heavy metals inhibited leaf area development of *Artemisia nilagirica* over a 20 - day period. Leaf area of the control group increased steadily and significantly, from 4.35 cm^2 to 10.75 cm^2, indicating ideal growth. However, exposure to Al, Cr, Cu, Fe and Hg resulted in varying decreases in the development of leaf area.

The increase in leaf area in the control group illustrates the innate capacity of plant for photosynthetic development in the right circumstances. Active cellular division and expansion in leaf tissues, which are essential for biomass formation, are indicated by the notable rise. Leaf area was moderately impacted by Al, with the final figure coming in at 9.98 cm^2. Al was comparatively less harmful than other metals.

Exposure to Cr considerably reduced the leaf area, resulting in 7.14 cm^2 at 20^{th} day. This compromises energy production for growth by directly affecting the total

surface area available for light collection. Whereas, the leaf area was stunted at 5.75 cm^2 because to the strong inhibitory action of Cu. The leaf area only increased to 6.42 cm^2, indicating moderate toxicity from Fe. The leaf area decreased significantly as a result of Hg as well, to just 6.4 cm^2. The decreased leaf area points to disrupted formation and elongation of mesophyll cells and also the oxidative stress brought on by heavy metals impairing photosynthetic ability.

In *Artemisia nilagirica*, the inhibitory effects of heavy metals on leaf area follow a decreasing toxicity pattern: Al < Cr < Fe ≈ Hg < Cu (Fig. 5). Cell division, growth, and photosynthetic efficiency are among the physiological and biochemical processes that are hampered by the smaller leaf area. These results demonstrate how vulnerable leaf tissues are to stress caused by heavy metals, which may impair the general growth and ecological adaptability of plants.

Table 9. Effect of aluminium, chromium, copper, iron and mercury on leaf area (**cm^2**) in *Artemisia nilagirica* (Clarke) Pamp.

Metals	**Interval - Days**					
	0	**4**	**8**	**12**	**16**	**20**
Control	4.35±0.22	4.75±0.12	5.86±0.04	6.74±0.02	8.36±0.04	10.75±0.06
Aluminium (80 µM)	4.26±0.18	4.88±0.14	5.62±0.07	6.52±0.04	7.56±0.02	9.98±0.1
Chromium (70 µM)	4.25±0.2	4.37±0.1	4.58±0.02	4.72±0.11	5.76±0.09	7.14±0.14
Copper (50 µM)	4.55±0.23	4.62±0.04	4.88±0.12	5.26±0.14	5.64±0.1	5.75±0.18
Iron (70 µM)	4.62±0.13	4.95±0.06	5.12±0.05	5.42±0.08	6.1±0.14	6.42±0.16
Mercury (10 µM)	4.5±0.22	4.74±0.15	4.94±0.16	5.35±0.06	6±0.04	6.4±0.08

Values given are mean of 5 replicates ±S. E

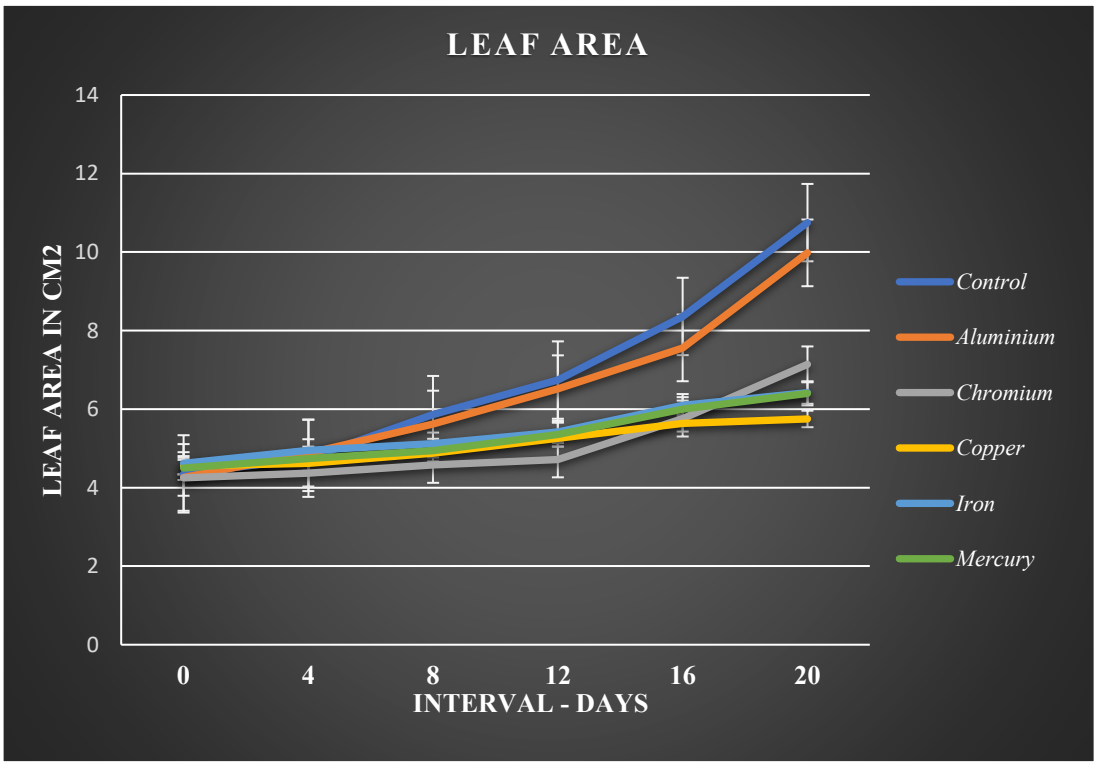


Figure. 5 Effect of aluminium, chromium, copper, iron and mercury on leaf area (**cm^2**) in *Artemisia nilagirica* (Clarke) Pamp.

TOLERANCE INDEX

Plants tested with all metal treatments exhibited a consistent decrease in tolerance index values over time, as determined by comparing the root lengths of the control and treatment groups (Table 10, Fig.6). Under heavy metal stress, tolerance index (TI) of *Artemisia nilagirica* substantially decreased, demonstrating the detrimental effect on the capability of plant to sustain growth and physiological processes. The control plants showed ideal growing conditions by maintaining a TI of 100 at all intervals. But with time, exposure to metals including Al, Cr, Cu, Fe and Hg gradually decreased the TI; by day 20, decrease caused by Hg was the most noticeable.

This decrease emphasizes how heavy metal exposure impairs metabolic and cellular resistance. The greatest decrease in TI was brought on by Hg exposure, Similarly, TI was greatly affected by Cu and Cr also. Iron and aluminium caused decreased TI, though not as much, indicating that the plant may have some tolerance mechanisms.

The accumulated stress brought on by disturbed cellular homeostasis, poor nutrient translocation, and suppressed development processes is indicated by the decrease in TI across all metals. These results highlight susceptibility of *A. nilagirica* to extended exposure to heavy metals, which is indicative of the larger ecological hazards associated with heavy metal contamination.

Table 10. Effect of aluminium, chromium, copper, iron and mercury on tolerance index (**%**) of *Artemisia nilagirica* (Clarke) Pamp. during growth.

Metals	**Interval - Days**					
	0	**4**	**8**	**12**	**16**	**20**
Control	100	100	100	100	100	100
Aluminium (80 µM)	100	95.1±0.13	86.5±0.17	82.13±0.04	75.53±0.12	71.33±0.16
Chromium (70 µM)	100	95.33±0.13	87.83±0.02	84±0.02	76.24±0.09	67.33±0.18
Copper (50 µM)	100	93.66±0.09	84.75±0.15	83.07±0.18	74.1±0.13	66.15±0.21
Iron (70 µM)	100	94.86±0.09	86.33±0.15	84.99±0.18	75.55±0.13	68.3±0.21
Mercury (10 µM)	100	90.89±0.05	78.47±0.13	74.91±0.05	69.66±0.03	62.84±0.06

Values given are mean of 5 replicates ±S. E

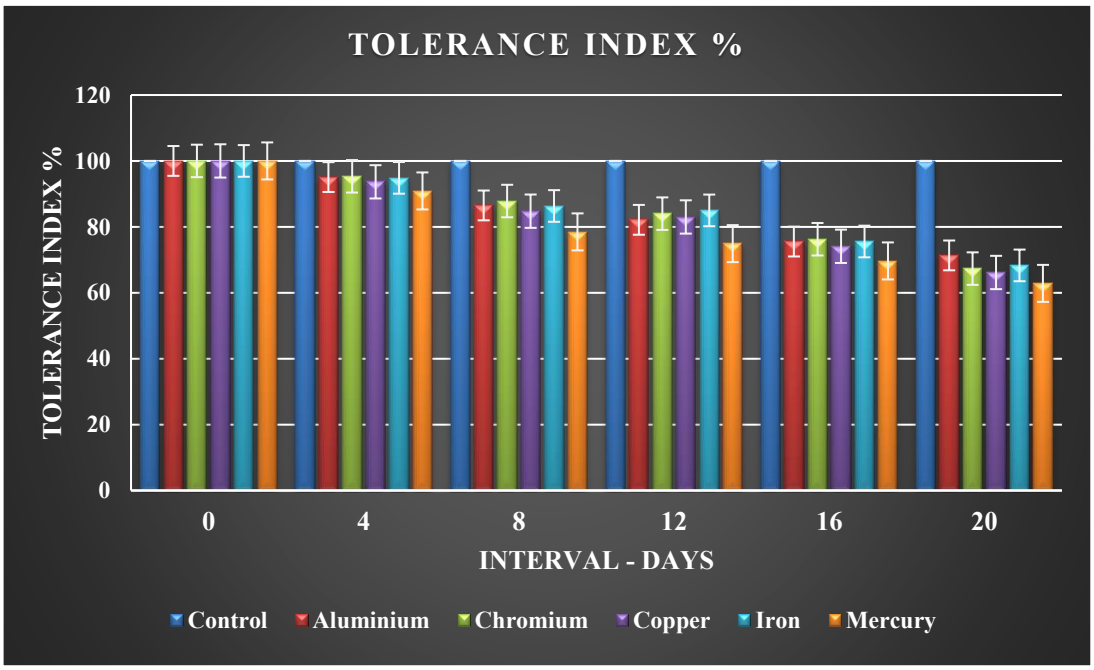


Figure. 6 Effect of aluminium, chromium, copper, iron and mercury on tolerance index (**%**) percentage pertaining to root length in *Artemisia nilagirica* (Clarke) Pamp. during growth

STOMATAL INDEX

The information (Table 11, Figs. 7 & 8) shows how the stomatal index (SI) for the upper and lower epidermis (UE and LE) of *Artemisia nilagirica* changed over the course of 20 days while receiving heavy metal treatments.

Control: Normal growth without stress was indicated by SI values for UE and LE that stayed constant over time, slightly increasing.

Aluminium: Both UE and LE exhibited a minor change in SI, indicating a slight impact on stomatal development.

Chromium: By day 20, SI had significantly increased, particularly in the LE, suggesting a definite reaction to chromium exposure.

Copper: SI increased moderately, especially in LE, indicating vulnerability to copper stress.

Iron: SI increased somewhat, with LE exhibiting greater variance than UE.

Mercury: Treatment with mercury resulted in the largest rise in SI, especially in LE, indicating that this metal has a substantial effect on stomatal growth.

The data showed that different metals have different effects on stomatal indices, with Cu, Hg, and Cr having more noticeable effects than Fe or Al. Throughout each treatment, the lower epidermis continuously showed more SI alterations than the upper epidermis.

Table 11. Effect of aluminium, chromium, copper, iron and mercury on Stomatal index of *Artemisia nilagirica* (Clarke) Pamp. during growth.

Treatments		**Interval - Days**					
		0	**4**	**8**	**12**	**16**	**20**
Control	UE	10.01±0.02	10.12±0.12	10.25±0.14	10.38±0.11	10.43±0.23	10.55±0.03
	LE	10.12±0.13	10.33±0.31	10.49±0.02	10.64±0.19	10.79±0.11	10.85±0.16
Aluminium (80 µM)	UE	10.10±0.22	10.13±0.33	10.16±0.34	10.18±0.14	10.23±0.21	10.24±0.08
	LE	10.12±0.03	10.14±0.02	10.15±0.2	10.16±0.32	10.17±0.17	10.19±0.30
Chromium (70 µM)	UE	10.03±0.12	11.05±0.13	11.65±0.13	12.06±0.15	13.08±0.05	15.09±0.16
	LE	10.15±0.04	12.66±0.18	13.16±0.21	14.06±0.09	15.09±0.23	16.06±0.46
Copper (50 µM)	UE	10.22±0.12	11.16±0.24	13.16±0.23	14.01±0.32	14.32±0.24	15.11±0.25
	LE	10.53±0.03	12.12±0.15	14.06±1.8	15.24±2.23	17.26±2.31	17.63±0.23
Iron (70 µM)	UE	10.32±2.43	11.6±2.34	12.16±0.7	13.21±2.14	13.94±2.36	14.01±1.32
	LE	10.52±2.16	11.68±2.15	13.09±1.6	15.33±3.01	15.97±2.06	16.34±1.26
Mercury (10 µM)	UE	10.14±2.32	12.43±2.34	13.03±2.6	14.33±2.12	15.23±2.34	17.34±4.16
	LE	10.23±1.08	13.31±0.21	15.34±0.26	18.32±1.25	22.04±3.2	23.46±1.24

Values given are mean of 5 replicates ±S. E

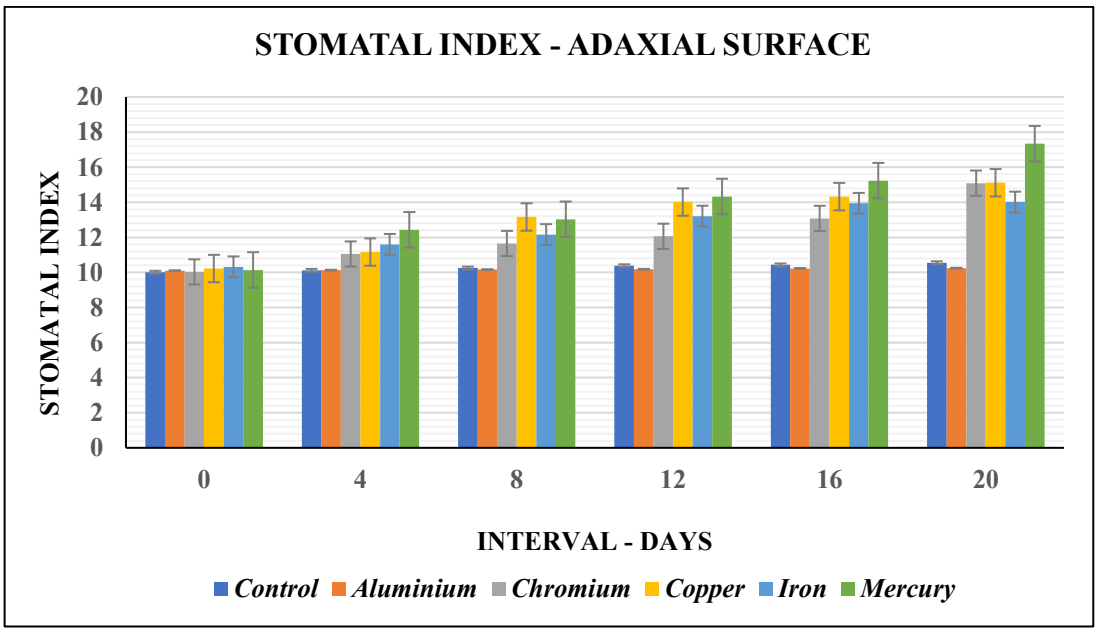


Figure. 7 Effect of aluminium, chromium, copper, iron and mercury on stomatal index of *Artemisia nilagirica* (Clarke) Pamp.

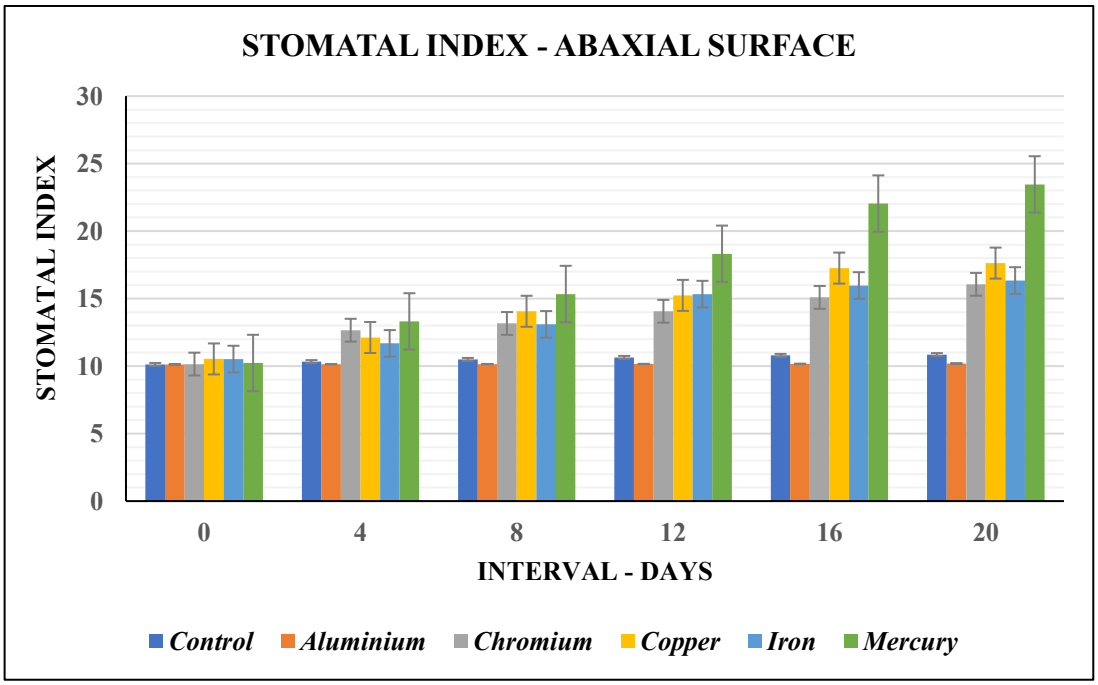


Figure. 8 Effect of aluminium, chromium, copper, iron and mercury on Stomatal index of *Artemisia nilagirica* (Clarke) Pamp.

Scanning Electron Micrographs - Stomata

Significant changes in stomatal morphology and structure under the influence of heavy metal treatments were found in the Scanning Electron Microscopy analysis of stomata on the adaxial side of *Artemisia nilagirica*. These findings demonstrate the effect of metal toxicity on stomatal aperture, structural integrity, and overall stomatal function.

The control sample (Fig. 9-A) showed open aperture and clearly defined guard cells, suggesting that the stomatal structure was intact. This typical form is indicative in unstressed conditions, as stomata efficiently regulate transpiration and gas exchange, which is a feature of optimal physiological functioning. The healthy, regular shape of the stomata serves as an indicator for comparing the treated samples to the control group.

The guard cells in the Al-treated leaves (Fig. 9-B) showed constriction in the stomata, which showed a nearly closed aperture. Al-induced stress may be the cause of this decrease in stomatal opening, which could compromise gas exchange and lower transpiration rates. Under metal stress, the physiological adaptation of plant to reduce water loss is reflected in the constriction of stomatal aperture. Plants treated with Cr showed nearly identical stomatal closure (Fig. 9-C).

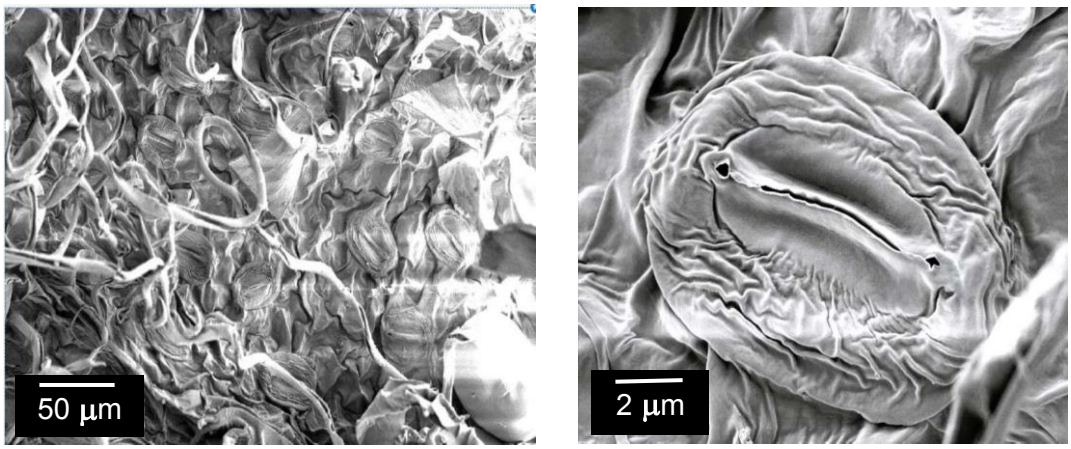
In these situations, the closure is probably a stress-induced reaction meant to reduce oxidative damage and water loss, which is a typical defence strategy in plants under heavy metal stress.

Remarkably, the metals differed in the extent of stomatal opening. Toxic levels of Cu and Fe (Fig. 10-D & E) caused the stomatal aperture to slightly open in comparison to the control. The stomatal regulating system may be moderately disrupted, as evidenced by this partial opening. This could be because these metals interfere with cellular signalling pathways that are linked to guard cell function. Although less strictly controlled, the stomata continued to be largely functioning in spite of the impacts of stress.

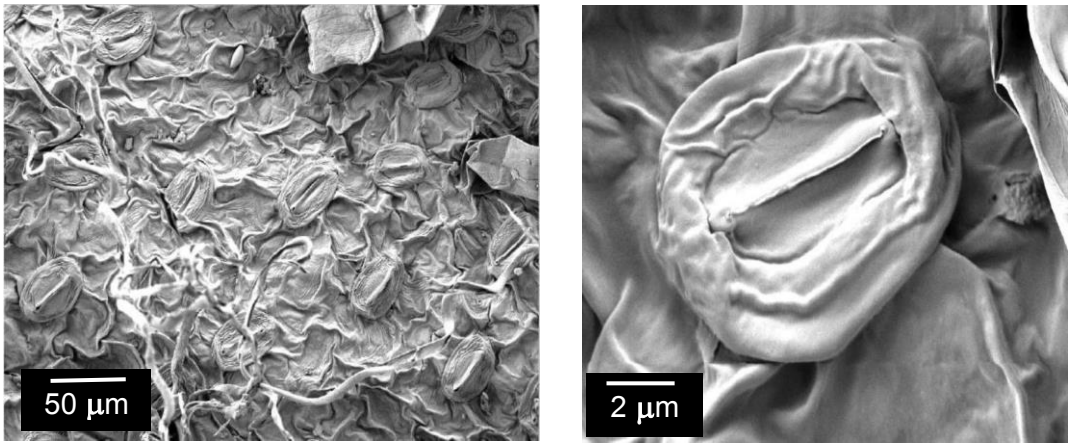
On the other hand, plants treated with Hg (Fig. 10-F) showed greatly distorted guard cells and stomata which appeared open. One of the unique responses to Hg exposure is the substantial opening of the stomata, which suggests a different physiological adaptation. The process of phytovolatilization, in which Hg is transformed into a volatile form and discharged through the stomata, may be the cause of this. The large aperture makes it easier for volatile Hg to be released, which helps the plant detoxify. The significant effect of Hg toxicity on stomatal function is highlighted by the possibility that this adaptation may result in increased water loss and a diminished capacity for regulating gas exchange.

In *A. nilagirica*, the SEM study shows the different effects of heavy metals on stomatal shape and function. Certain metals cause stomatal closure as a defence mechanism, whereas others cause an abnormal opening that may be connected to their phytovolatilization detoxifying process. In contrast to Hg, Cu and Fe have intermediate effects with a slight stomatal opening, which is indicative of their comparatively mild levels of toxicity. These results highlight how the plant uses a variety of strategies to adapt to various heavy metal stresses.

A. Control l



B. Al treated leaf



C. Cr treated leaf

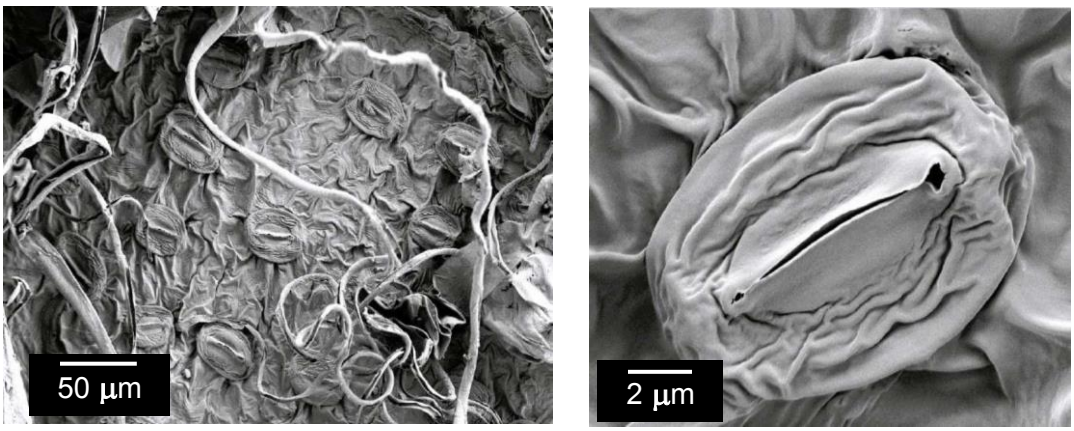
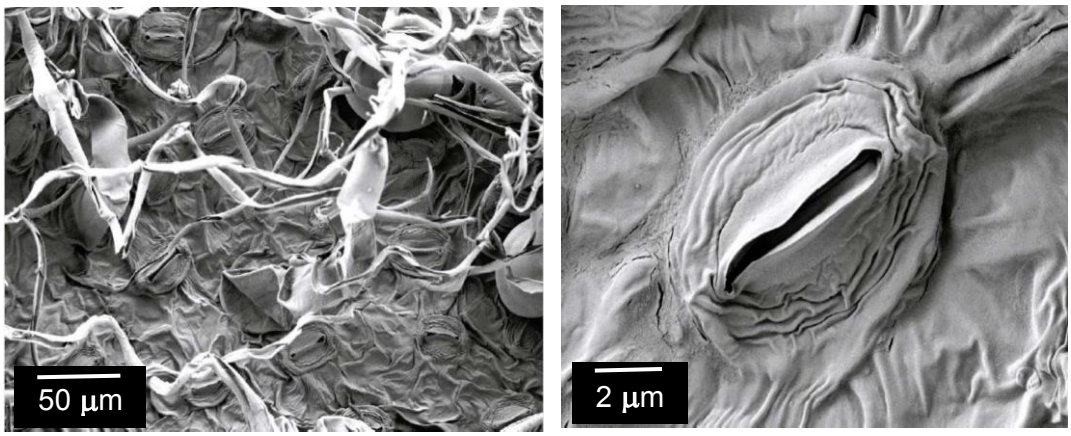
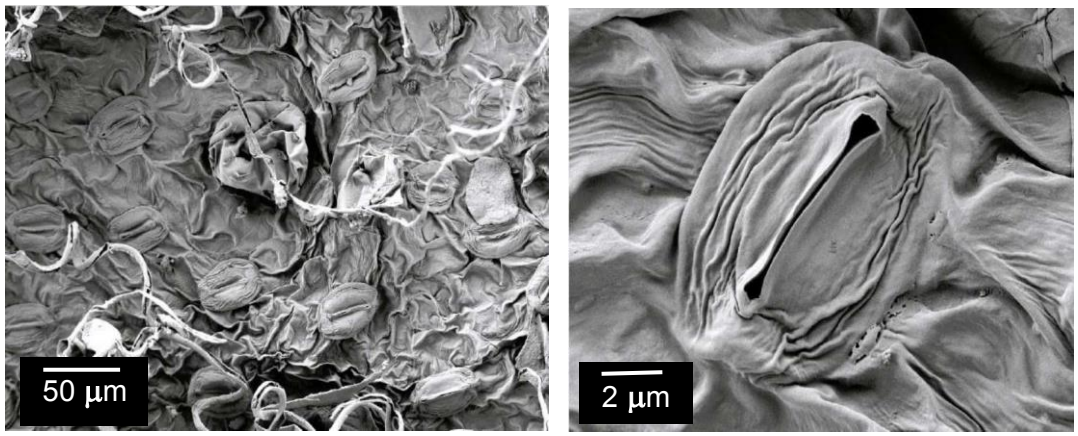


Fig. 9 Scanning Electron Microscopic images of leaf (adaxial side) - stomata of *Artemisia nilagirica* (Clarke) Pamp.

D. Cu treated leaf



E. Fe treated leaf



F. Hg treated leaf

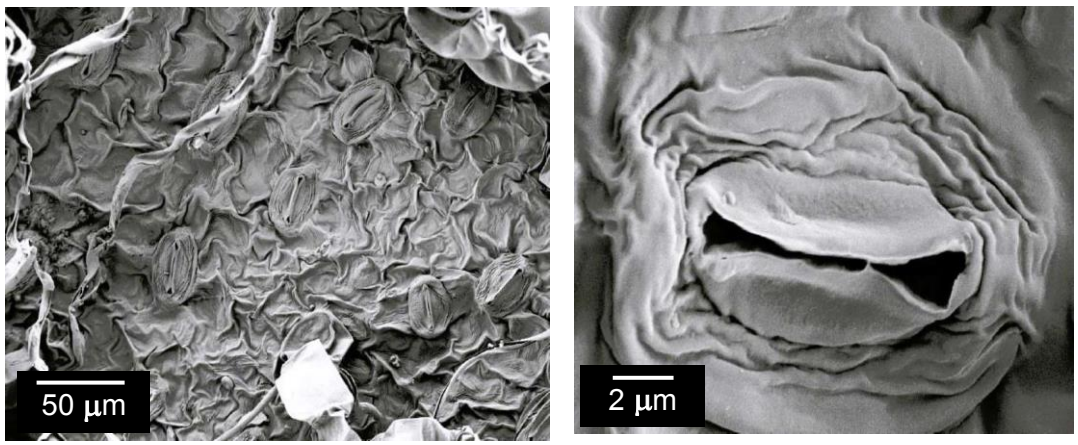


Fig. 10 Scanning Electron Microscopic images of leaf (adaxial side) - stomata of *Artemisia nilagirica*

ANATOMICAL STUDIES

Control

Root

The transverse section of the root of *A. nilagirica* revealed a well-organized structure consisting of a distinct rhizodermis, broad cortex, endodermis, and centrally located vascular cylinder (Fig.11-A). The piliferous layer had an undulating border abundant in root hairs, The cortex was composed of thin-walled parenchymatous cells with conspicuous intercellular spaces. A distinct endodermal layer enclosed the stele. The enlarged view clearly showed that, the vascular region consisted of prominent xylem vessels and thin-walled phloem tissues, while the pith was comparatively narrow and composed of compact parenchymatous cells.

Scanning electron microscopic observations confirmed the anatomical organization of the root, revealing a continuous outer layer with numerous root hairs, compact cortical cells, and a centrally placed vascular cylinder (Fig. 17-A). The vascular tissues appeared well differentiated with numerous xylem vessels was visible in the magnified image. (Fig. 17-B).

Stem

The stem exhibited a typical dicotyledonous organization with a single-layered epidermis bearing numerous epidermal hairs (Fig.11-C). Beneath the epidermis, the cortex consisted of compactly arranged parenchymatous cells. Vascular bundles were arranged in a continuous ring surrounding a broad central pith. Each vascular bundle was characterized by prominent thick-walled xylem vessels and comparatively smaller phloem elements. (Fig.11-D). The pith occupied a major portion of the stem and consisted of large, thin-walled parenchymatous cells.

SEM images corroborated these observations, showing a clearly defined epidermal layer with trichomes, compact cortical tissues and regularly distributed vascular bundles (Fig.17-C), vessel lumina were clearly visible in magnified view (Fig.17-D).

Leaf

The transverse section of the leaf showed a distinct upper and lower epidermis enclosing the mesophyll tissue. (Fig.11-E). Epidermal hairs (trichomes) were present on both upper and lower epidermis. The midrib region was prominent and contained a well-developed vascular bundle composed of xylem and phloem tissues. The mesophyll consisted of compactly arranged cells with conspicuous intercellular spaces, and the vascular bundle was characterized by numerous thick-walled xylem vessels surrounded by phloem tissues. (Fig. 11-F).

SEM images of leaf showed upper and lower epidermis, vasculature and mesophyll tissues. Epidermal cells were covered with cuticle (Fig.17-E). Xylem vessels were clearly visible in the magnified view (Fig.17-F).

Treatment with Aluminium

Root

Compared to control, root hairs reduced in *A. nilagirica* roots due to Al contamination (Fig.12-A). Piliferous cells were slightly damaged and densely stained. Identical to the control, endodermis was very distinct but showed specific cell wall thickening. Cortical cells and vascular tissue were almost similar to the control. Vascular bundles were noticed by increased diameter of xylem vessels.

SEM examination revealed that the quantity of root hairs was significantly reduced as a result of Al toxicity. (Fig.18-A). In comparison to the control, other structural features did not alter. Dense masses were observed in the cortical and stelar regions of the Al-treated root (Fig. 18-B).

Stem

The stem of *A. nilagirica* subjected to Al treatment exhibited a structure nearly identical to that of the control (Fig. 12-C). The stelar area, notably the xylem, becomes more pronounced, comprising some thick deposits (Fig. 12-D). Trichomes were found filled with yellow-coloured occlusions.

SEM images of the Al-treated stem revealed a structure that was essentially the same as the control (Fig. 18-C). Compared to the control, there were less epidermal hairs. Every cell type, including pith, cortex, and vasculature, was clearly visible. The cortical cells and vasculature remarked by the presence of dense masses deposited (Fig. 18-D).

Leaf

Al-treated *A. nilagirica* leaves displayed the usual dicot leaf structure and looked nearly identical to the control (Fig. 12-E). In contrast to the control, yellow-coloured masses were observed in the mesophyll cells.

SEM pictures of an Al-treated *A. nilagirica* leaf reveal scarred epidermis with epidermal hairs like those of the control leaf. Xylem vessels were prominent in the vasculature (Fig. 18-E). Dense masses were clearly visible inside the mesophyll cells in the enlarged view (Fig. 18-F).

Treatment with chromium

Root

One significant effect of the Cr contamination was a decrease in the quantity of root hair and the piliferous layer seemed to be injured. The presence of yellow-coloured occlusions in the cortical region and the prominent stelar region composed of darkly stained vascular tissues were noticeable anatomical features of the root in the chromium-treated plant. Reduced pith was seen (Fig. 13-A&B).

Root of *A. nilagirica* treated with Cr showed a ruptured piliferous layer and fewer root hairs in the SEM analysis (Fig. 19-A). Noticeable stelar region with increased xylem vessel diameter was observed. Dense masses were found accumulated inside the xylem vessels (Fig. 19-B).

Stem

The stem tissues of *A. nilagirica* plants treated with Cr showed structural changes that were nearly identical to the changes exhibited by root cells, such as fewer stem hairs and a weakened rhizodermal layer (Fig. 13-C). It was also evident that the xylem vessels were enlarged. Dense yellow-coloured masses were discovered inside the trichomes and xylem vessels (Fig. 13-D).

The hand part is consistent with the SEM photos. The magnified image (Fig. 19-C & 19-D) showed depositions in the stelar region and increased diameter of xylem vessels.

Leaf

Plants treated with Cr displayed a characteristic dicot leaf structure. It was observed that both epidermis had trichomes (Fig.13-E). Yellow coloured masses were found deposited in mesophyll tissues (Fig.13-F).

When compared to the control, the SEM picture of an *A. nilagirica* leaf treated with Cr revealed very slight anatomical alterations. There were epidermal hairs and a transparent epidermis (Fig. 19-E). Compared to control, the vascular area was noticeable. Although mesophyll cells had robust walls, they seemed to be damaged due to cell wall cracking (Fig. 19-F), and some of the cells were irregularly distributed and contained deposits.

Treatment with Copper

Root

Plants treated with Cu resulted with reduced root diameter and a smaller number of root hairs. Piliferous layer cells were darkly stained (Fig.14-A). Dense masses were found embedded in cortical and vascular tissues (Fig.14-B). vessel diameter increased compared to the control.

SEM images of *A. nilagirica* roots treated with Cu shows thick piliferous layer. Xylem vessels with increased diameter and filled with dense deposits (Fig. 20-A & B).

Stem

Plants treated with Cu showed reduced number of epidermal hairs and other anatomical features were almost similar to the control (Fig.14-C). Vasculature noted with enlarged xylem vessels and dark dense deposits inside the vessels (Fig.14-D).

In comparison to the control, the SEM images of stem of the plant- *A. nilagirica* treated with Cu reveal anatomical alterations (Fig. 20-C). The epidermal layer was complete and the trichomes were nearly intact, cortex was normal but the vasculature consisted of xylem vessels with larger diameters. At the stelar region, cells with uneven deposits of dense masses were seen (Fig. 20-D). The pith region was broad and cells were aligned uniformly.

Leaf

Leaf structure of *A. nilagirica* treated with Cu was noted with reduced size of the lamina and midrib (Fig.14-E). Significant structural change due to Cu toxicity was the presence of large number of glandular and non-glandular trichomes on both upper and lower epidermis (Fig.14-F).

SEM image of *A. nilagirica* leaf treated with Cu showed the same structural changes as that found in hand section (Fig.20-E). Reduced size of lamina was noted. Reduced xylem vessel diameter was observed. Trichomes were prominent in the magnified view. (Fig.20-F).

Treatment with Iron

Root

Plants treated with Fe resulted in reduced number of root hairs. Piliferous layer cells were darkly stained (Fig.15-A). Yellow coloured dense masses were distributed in cortex and vascular region (Fig.15-B).

SEM images of root of *A. nilagirica* treated with Fe reveal a piliferous layer that is discontinuous and darkly stained; the number of root hairs was significantly

reduced; dense masses were visible in the cortex (Fig.21-A). Numerous xylem vessels with increased vessel diameter were found throughout the medullary rays (Fig.21-B).

Stem

The plant treated with Fe, displayed trichome loss and epidermal layer damage. Numerous vascular bundles arranged in concentric ring leaving a large parenchymatous pith, just like in the control stem (Fig. 15-C). The appearance of yellowish dense intercellular deposits in the stem tissues, particularly in the xylem vessels, was the most intriguing finding following the iron contamination (Fig.15-D).

The SEM images (Fig. 21-C) exhibit a structure comparable to that of light microscopic images. There was a regular arrangement of cortex cells. Pith cells had uneven deposits in the enlarged view (Fig. 21-D). The pith region was broad, and the cells were arranged closely together without much intercellular spaces.

Leaf

A. nilagirica treated with Fe had a leaf structure that was nearly identical to the control, with the exception of the lamina and midrib being smaller (Fig. 15-E). There were trichomes on the upper and lower epidermis. (Fig. 15-F) show extensive, yellow-colored deposits in the mesophyll tissues.

SEM study on leaf of *A. nilagirica* contaminated with Fe revealed slightly distorted epidermal layers. There were thick deposits and undamaged mesophyll cells (Fig. 21-E). Mesophyll cells and vascular tissues showed the presence of dense metal deposits in the enlarged view (Fig. 21-F).

Treatment with Mercury

Root

In comparison to the control, the roots of plants treated with mercury had reduced diameter and thicker rhizodermal layer (Fig. 16-A). The piliferous layer was discontinuous with very few root hairs. In the cortical cells, cell wall thickening and dark-coloured deposits were seen. The endodermis was found densely stained. Dark

deposits were also present in the vascular region, in contrast to the control (Fig. 16 B).

Similar structural features, such as an impaired epidermal layer with fewer root hairs, were seen in SEM images (Fig. 22-A). Dense masses of metal complexes filled the xylem vessels were visible in the magnified image (Fig. 22-B).

Stem

Stem of *A. nilagirica* plants treated with Hg showed anatomy similar to control plant such as, epidermal cells followed by few layers of cortical cells. Numerous vascular bundles were arranged in concentric rings towards the periphery, leaving large parenchymatous pith inside. Cortex, xylem vessels and pith cells were found filled with dark deposits. (Fig. 16-C&D).

The SEM image of *A. nilagirica* stem treated with Hg displayed a damaged epidermal layer and fewer trihomes in the SEM image. In contrast to the control, cells with unequal deposits of dense masses were observed in the stelar and pith regions (Fig. 22-C&D).

Leaf

Leaves of *A. nilagirica* treated with mercury displayed a high number of trichomes (Fig. 16-E&F). The cells had shrunk in size and the vascular bundles seemed heavily stained. The most remarkable findings were glandular trichomes with yellowish occlusions (Fig.16-E&F).

The light micrographs and the SEM image of leaf of *A. nilagirica* treated with mercury are consistent. It was evident that there were particular adaptations to mercury toxicity. There was a noticeable decrease in lamina size and a significant increase in trichomes. Mesophyll cells with thick walls were seen (Fig. 22-E&F).

ANATOMY OF CONTROL PLANTS

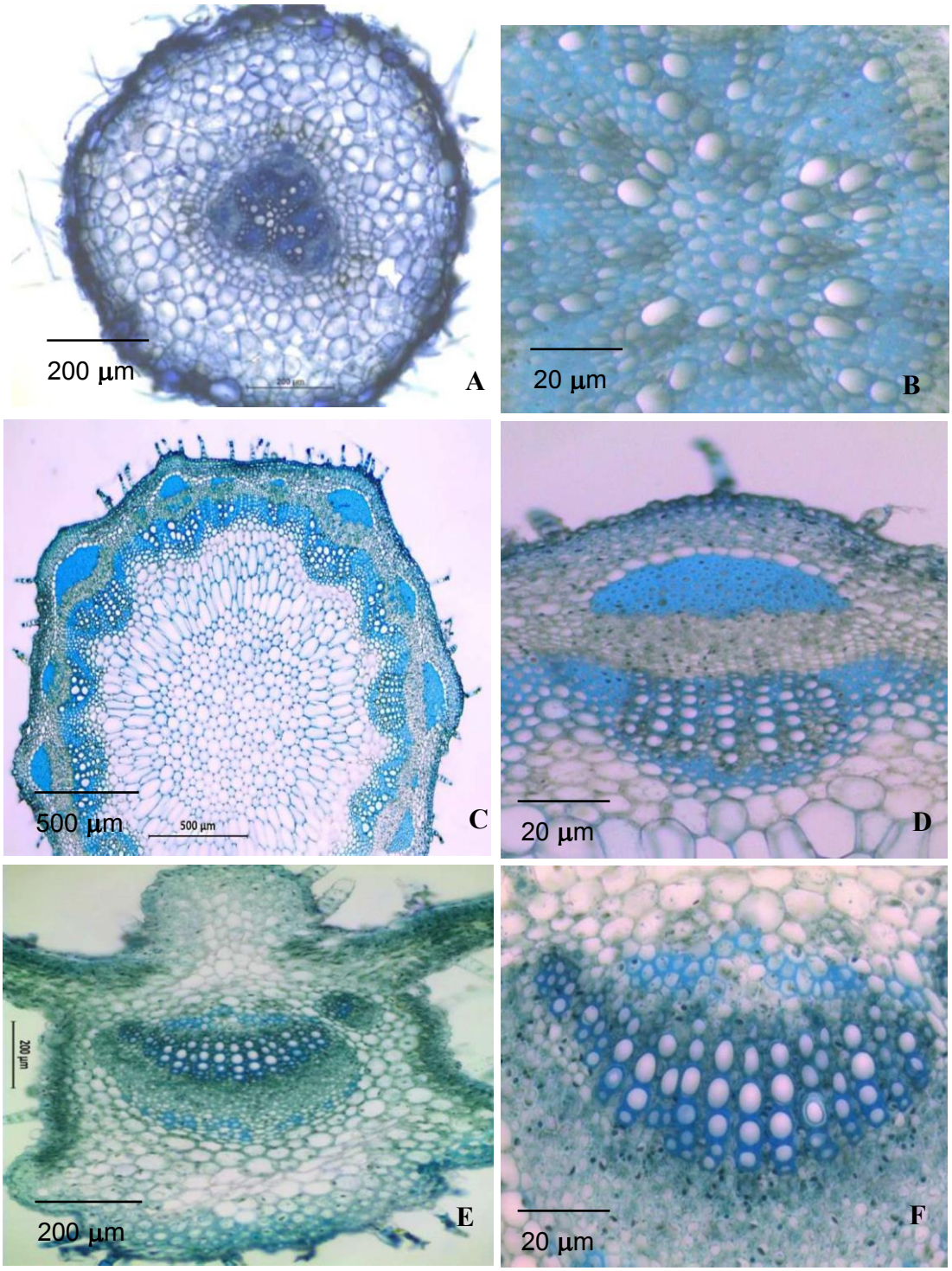


Figure. 11- A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F.** Portion Enlarged (Leaf).

ANATOMY OF PLANT TREATED WITH ALUMINIUM

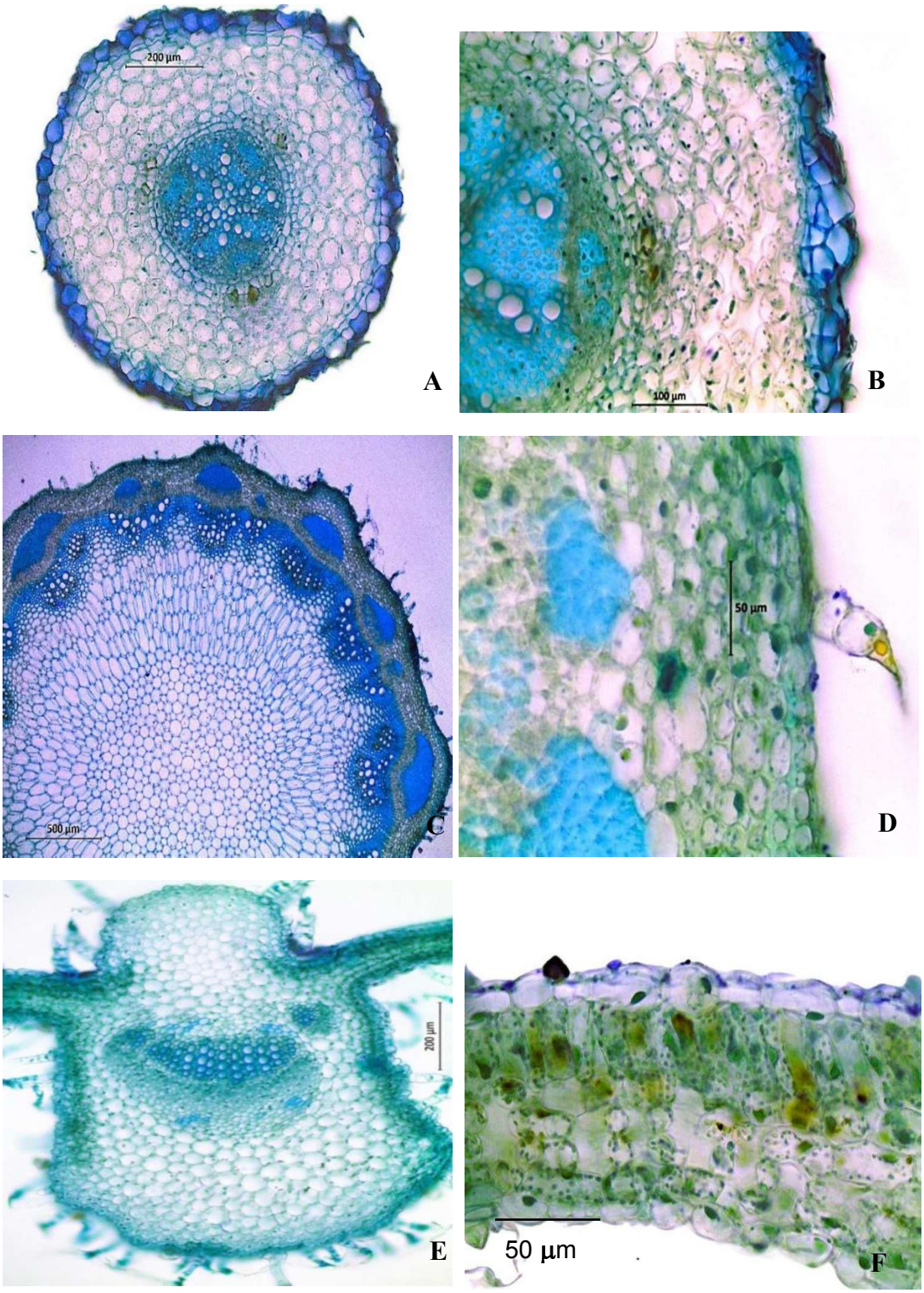


Figure. 12- A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F.** Portion Enlarged (Leaf).

ANATOMY OF PLANT TREATED WITH CHROMIUM

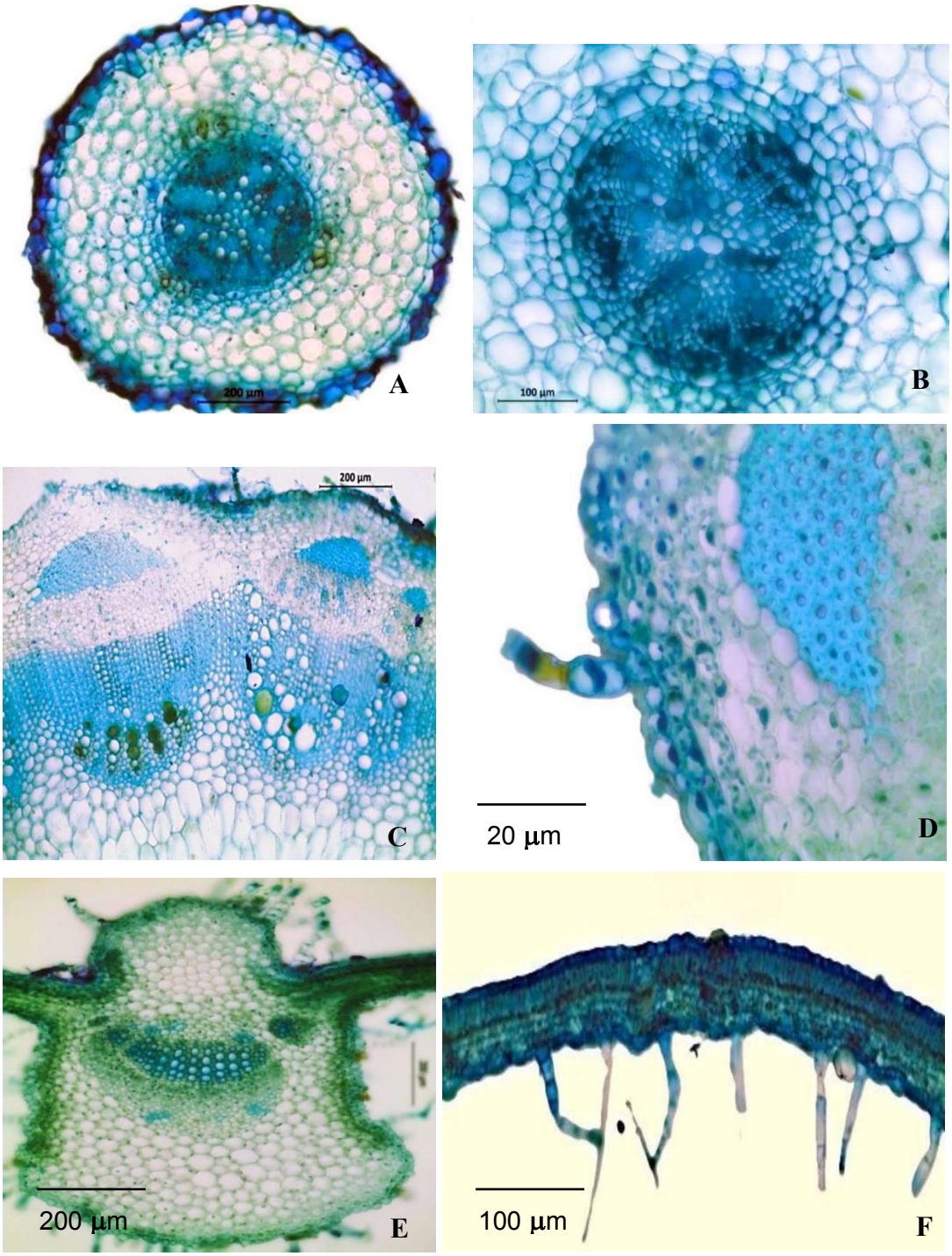


Figure. 13- A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F.** Portion Enlarged (Leaf).

ANATOMY OF PLANT TREATED WITH COPPER

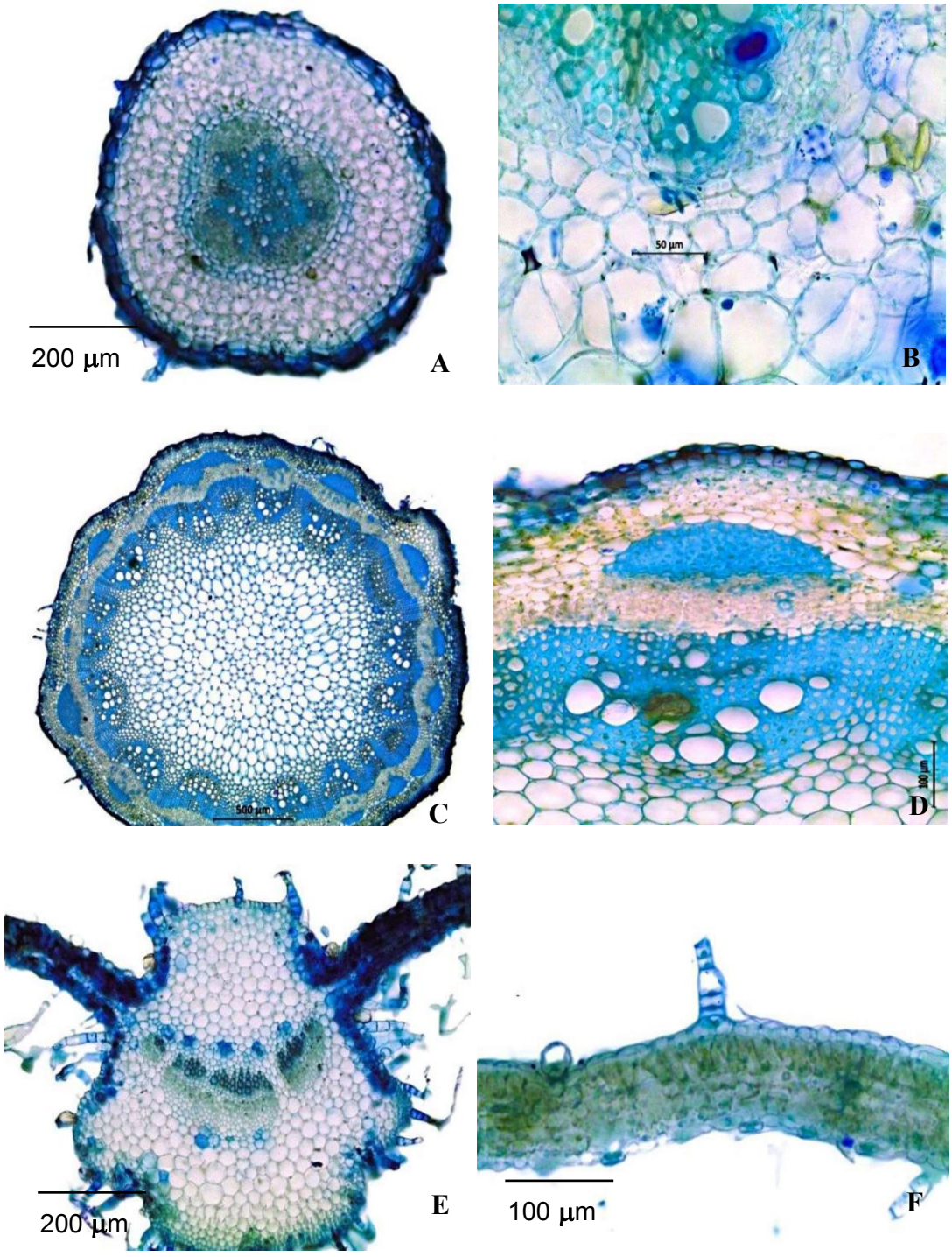


Figure. 14- A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

ANATOMY OF PLANT TREATED WITH IRON

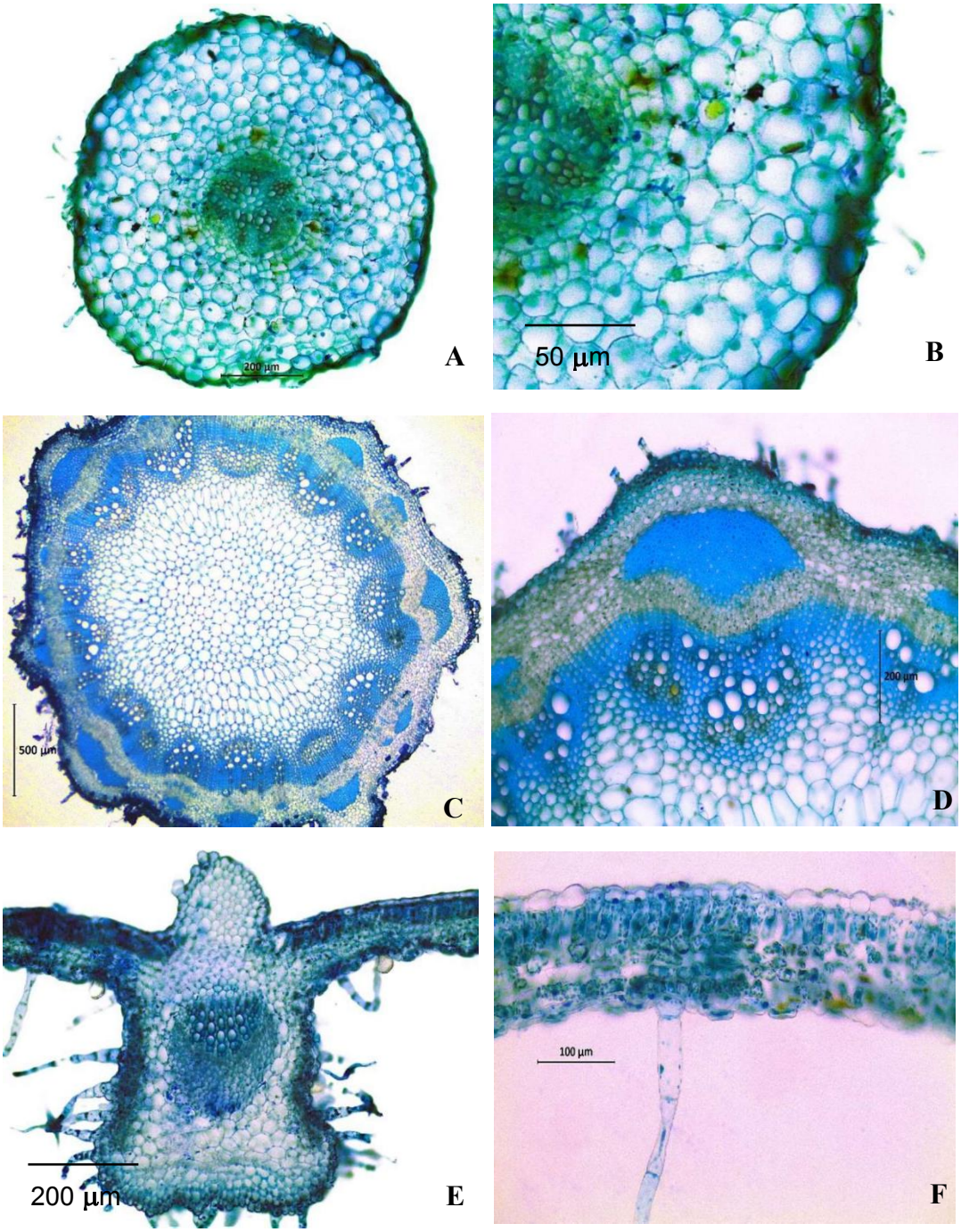


Figure. 15- A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

ANATOMY OF PLANT TREATED WITH MERCURY

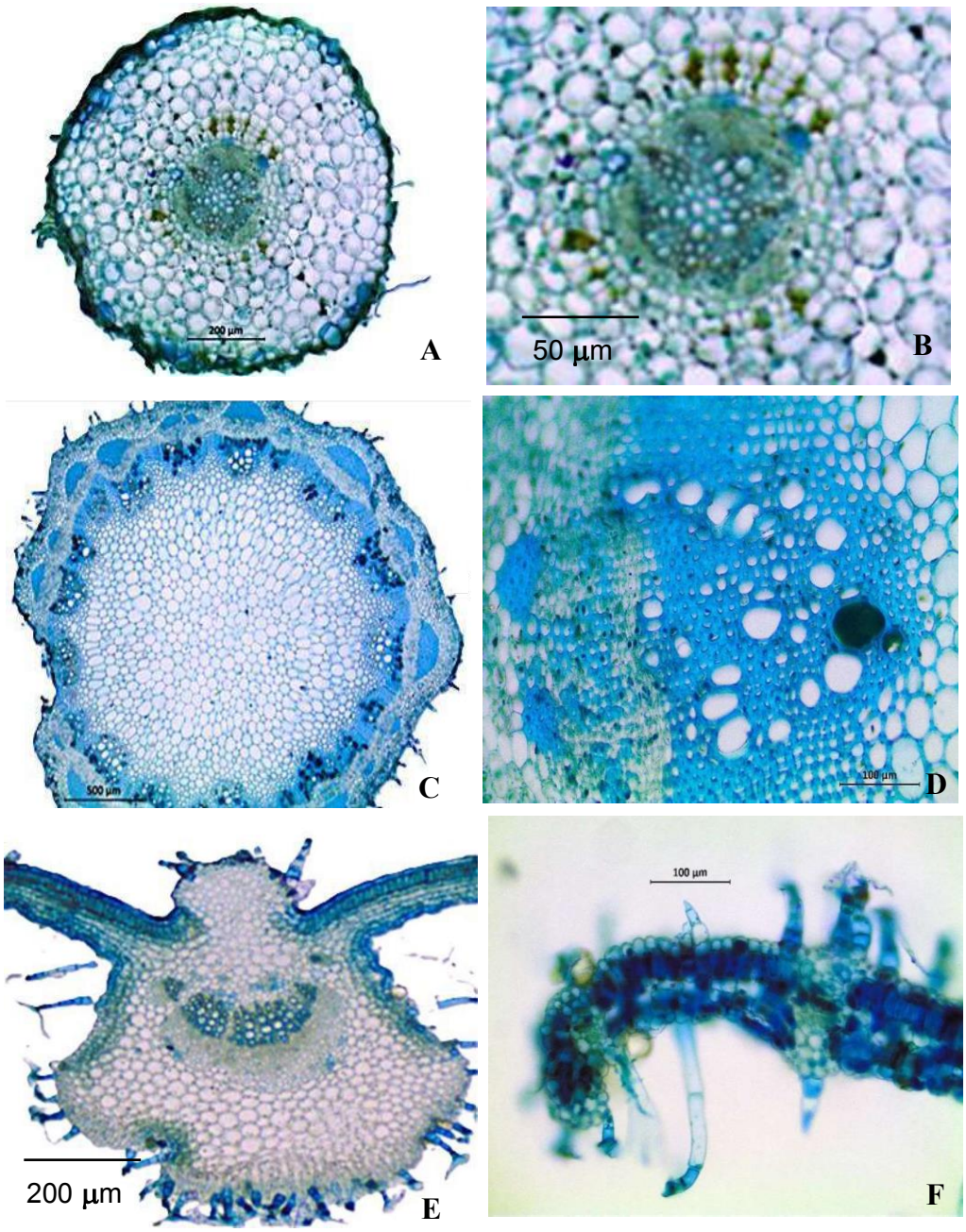


Figure. 16- A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

SCANNING ELECTRON MICROGRAPHS OF *ARTEMISIA NILAGIRICA (CLARKE) PAMP.*

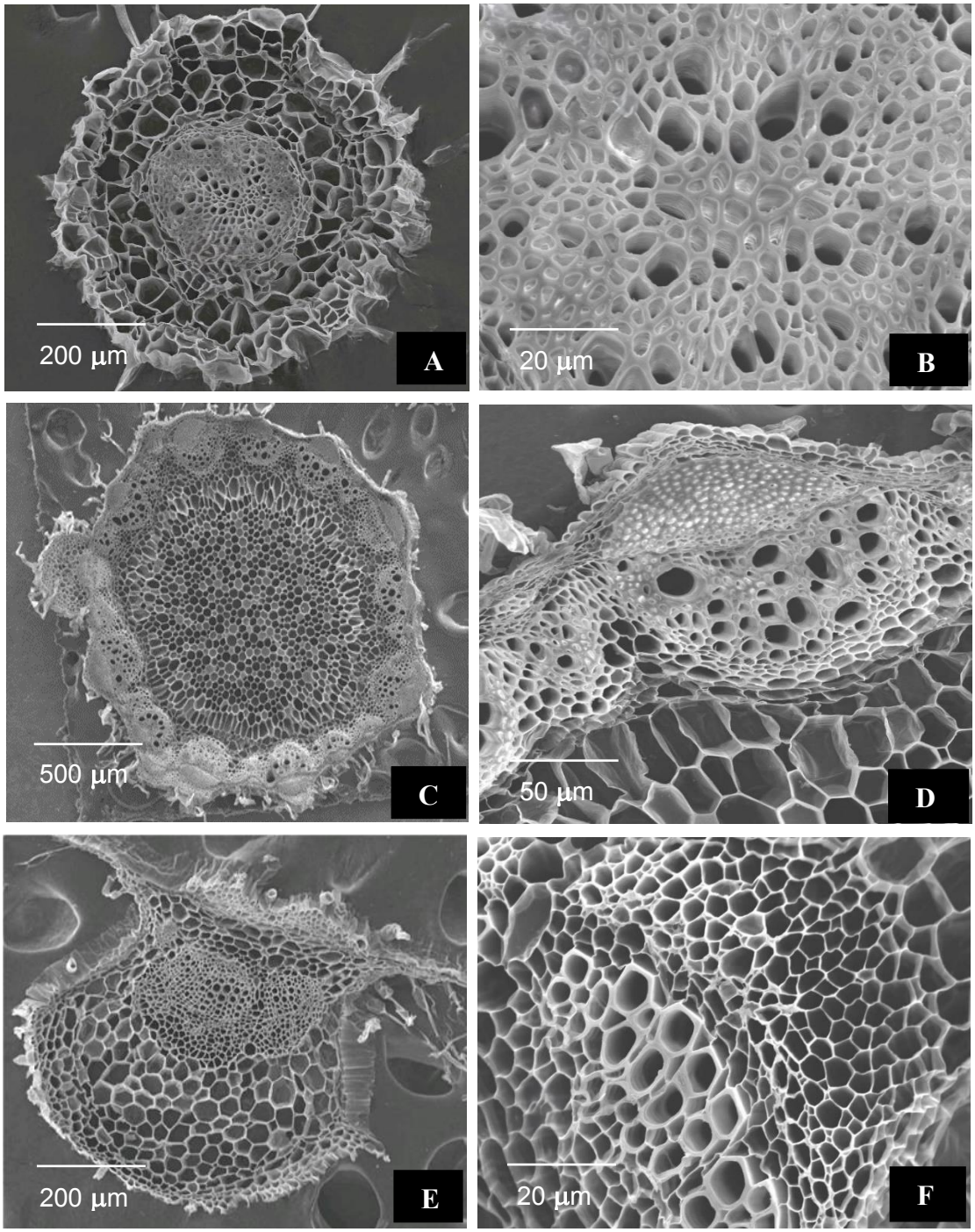


Figure. 17- A. Root T.S; **B**. Portion Enlarged (Root); **C.** Stem T.S, **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

SEM OF PLANT TREATED WITH ALUMINIUM

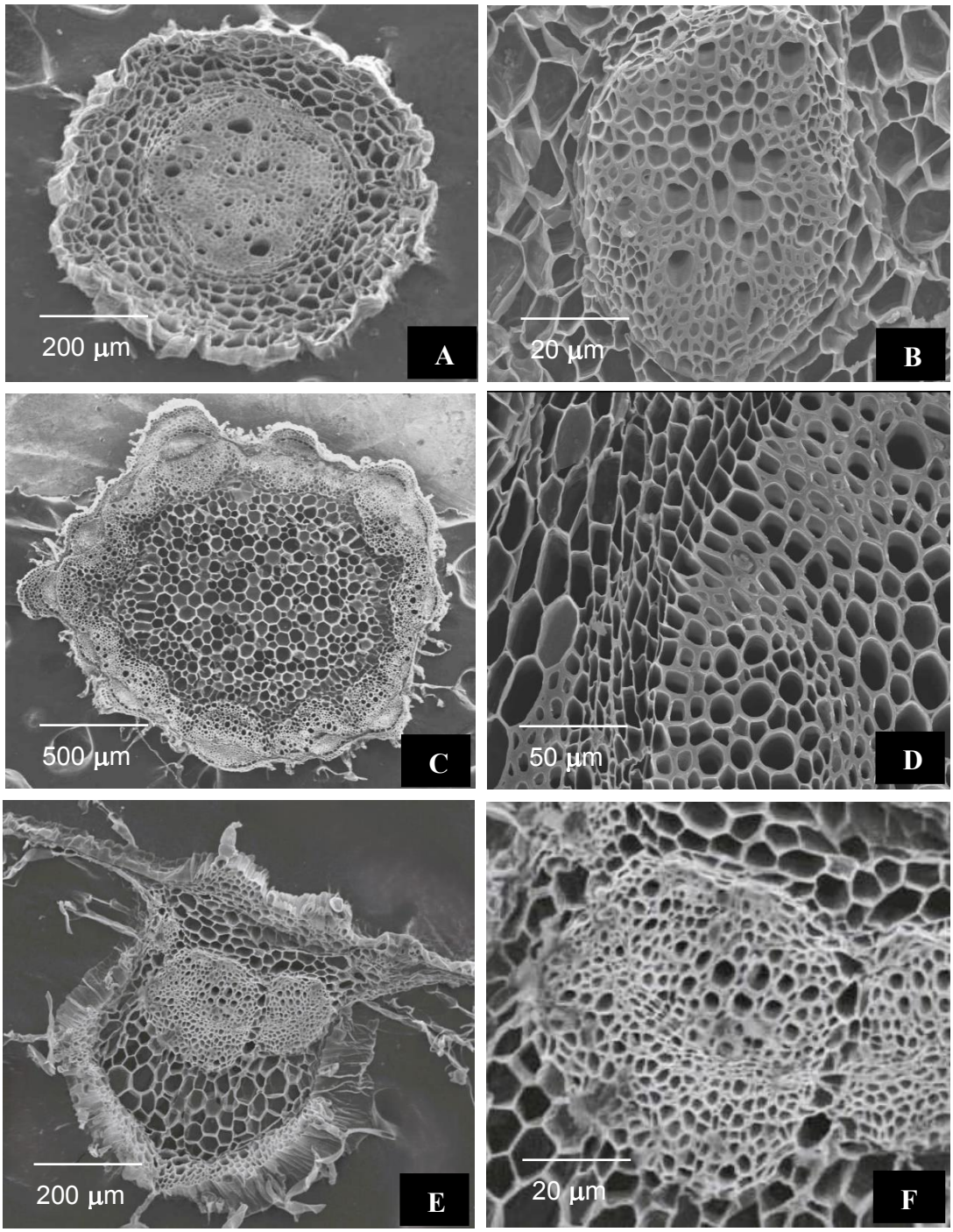


Figure. 18- A. Root T.S; **B**. Portion Enlarged (Root); **C.** Stem T.S, **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

SEM OF PLANT TREATED WITH CHROMIUM

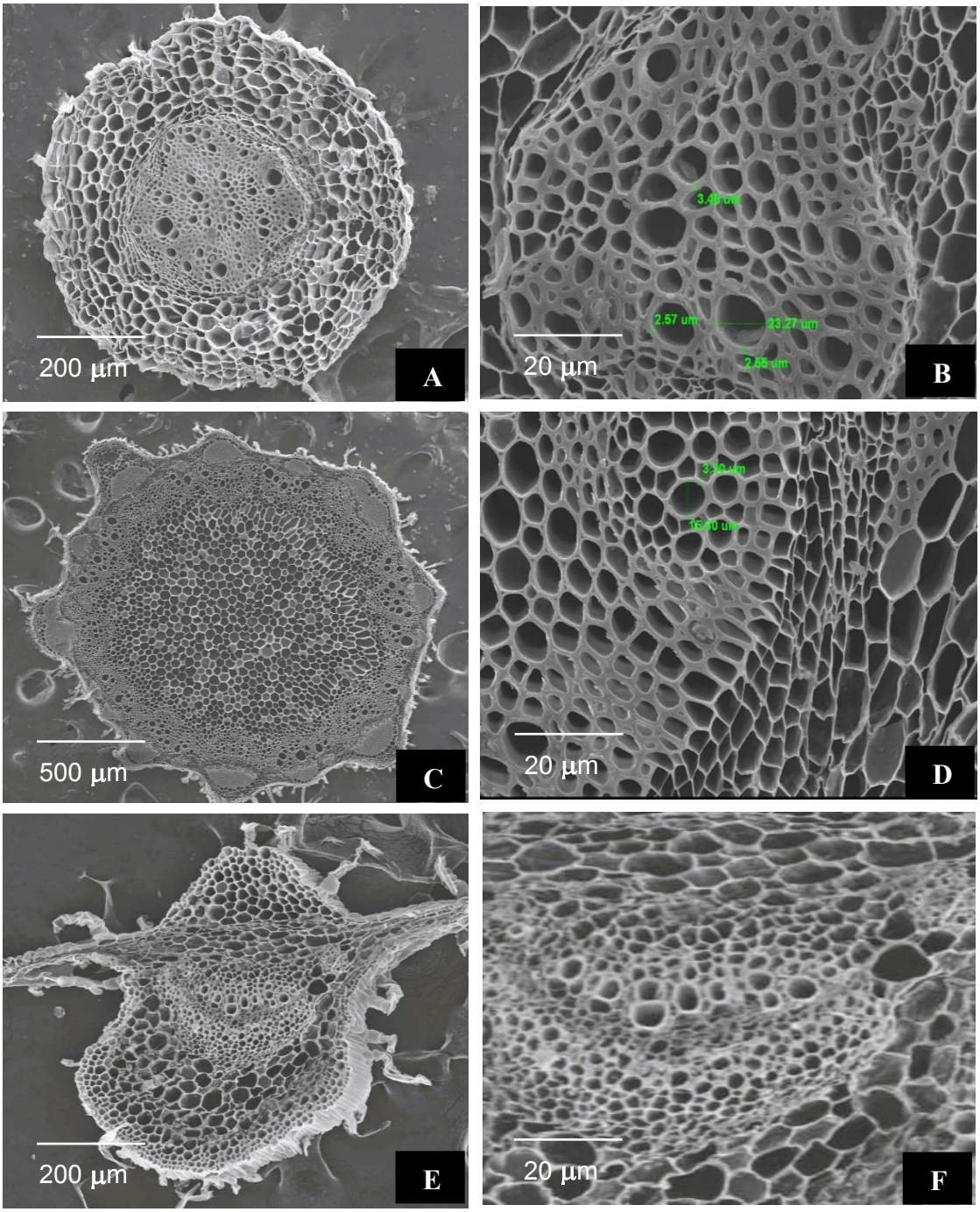


Figure. 19- A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

SEM OF PLANT TREATED WITH COPPER

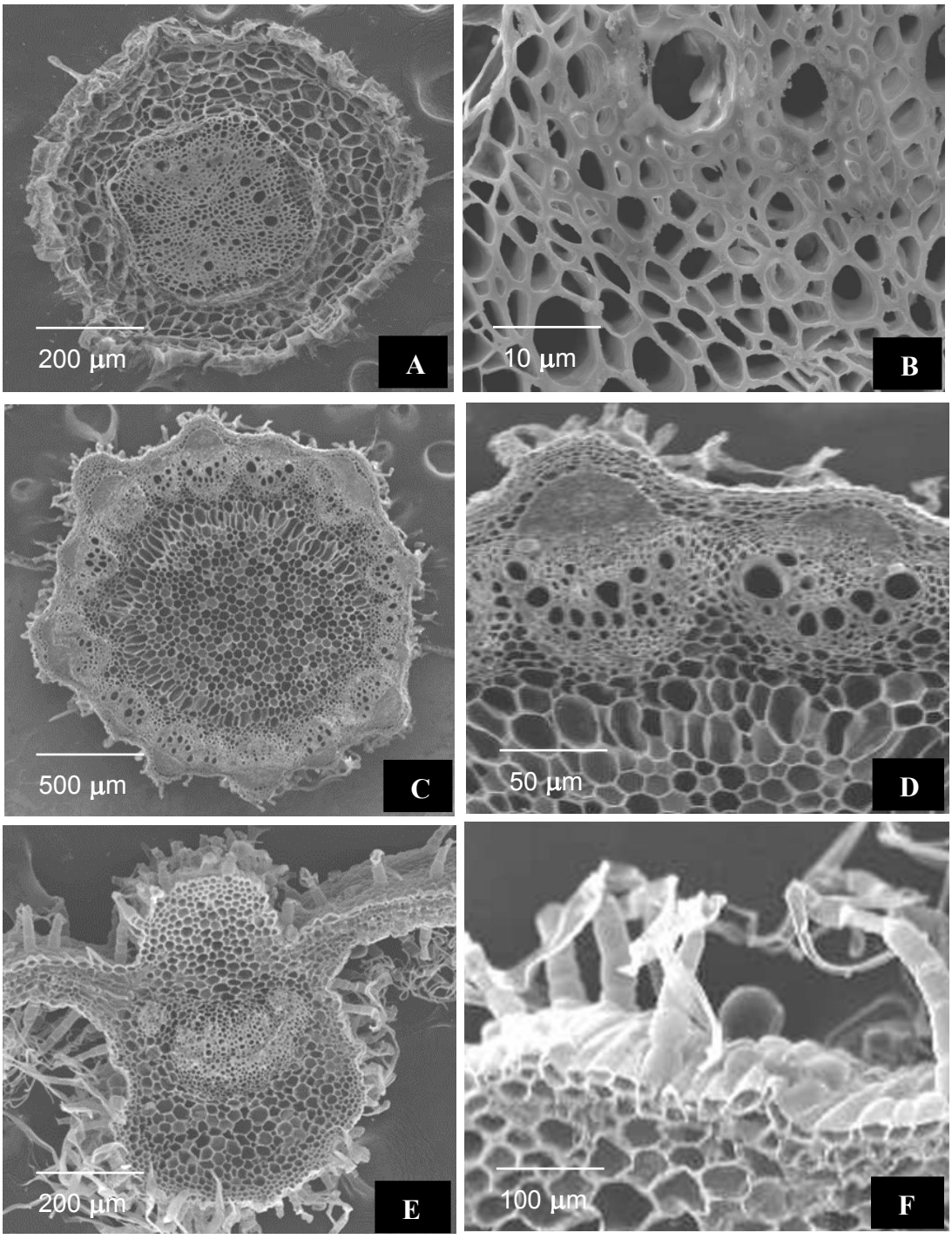


Figure. 20- A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; D. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

SEM OF PLANT TREATED WITH IRON

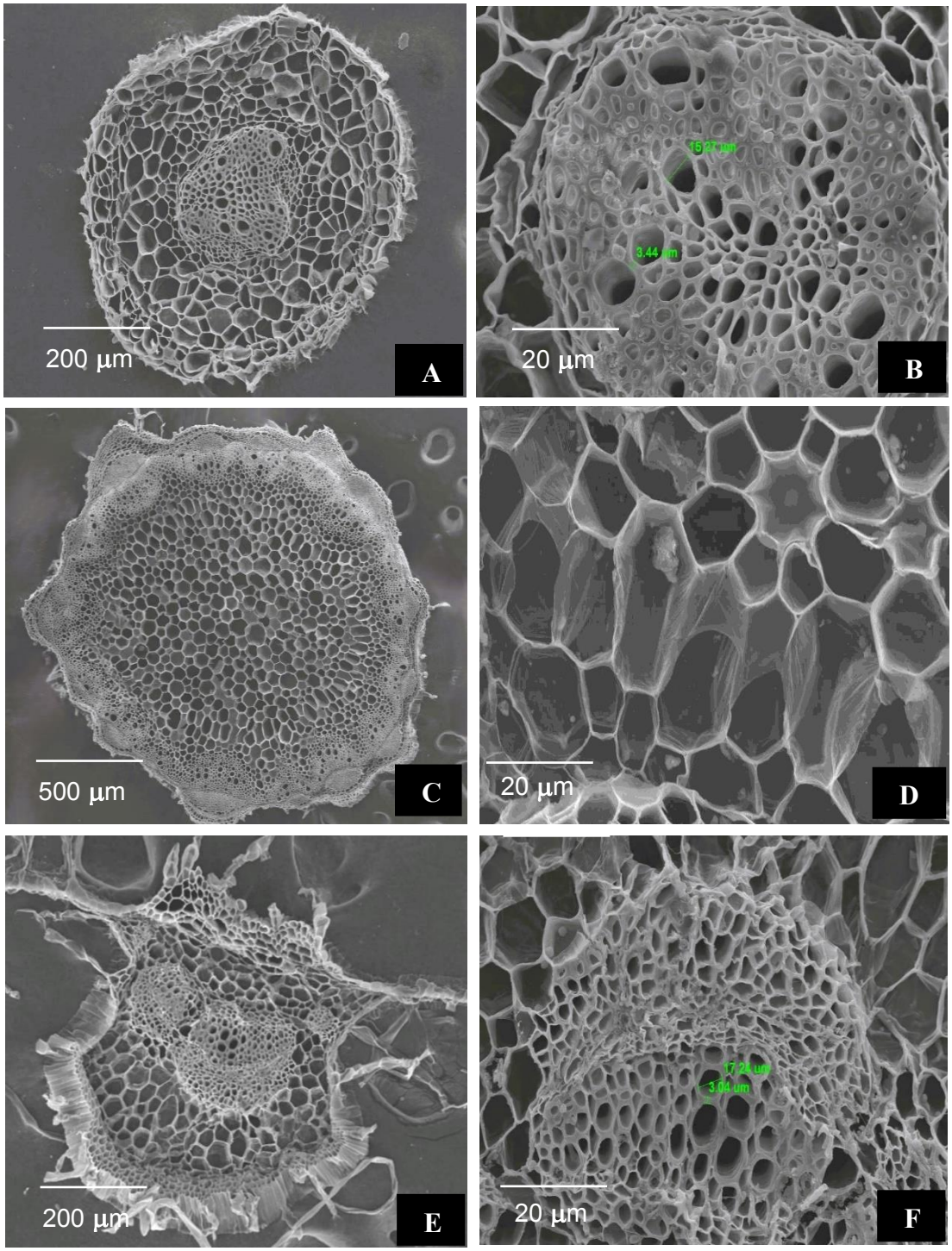


Figure.21- A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

SEM OF PLANT TREATED WITH MERCURY

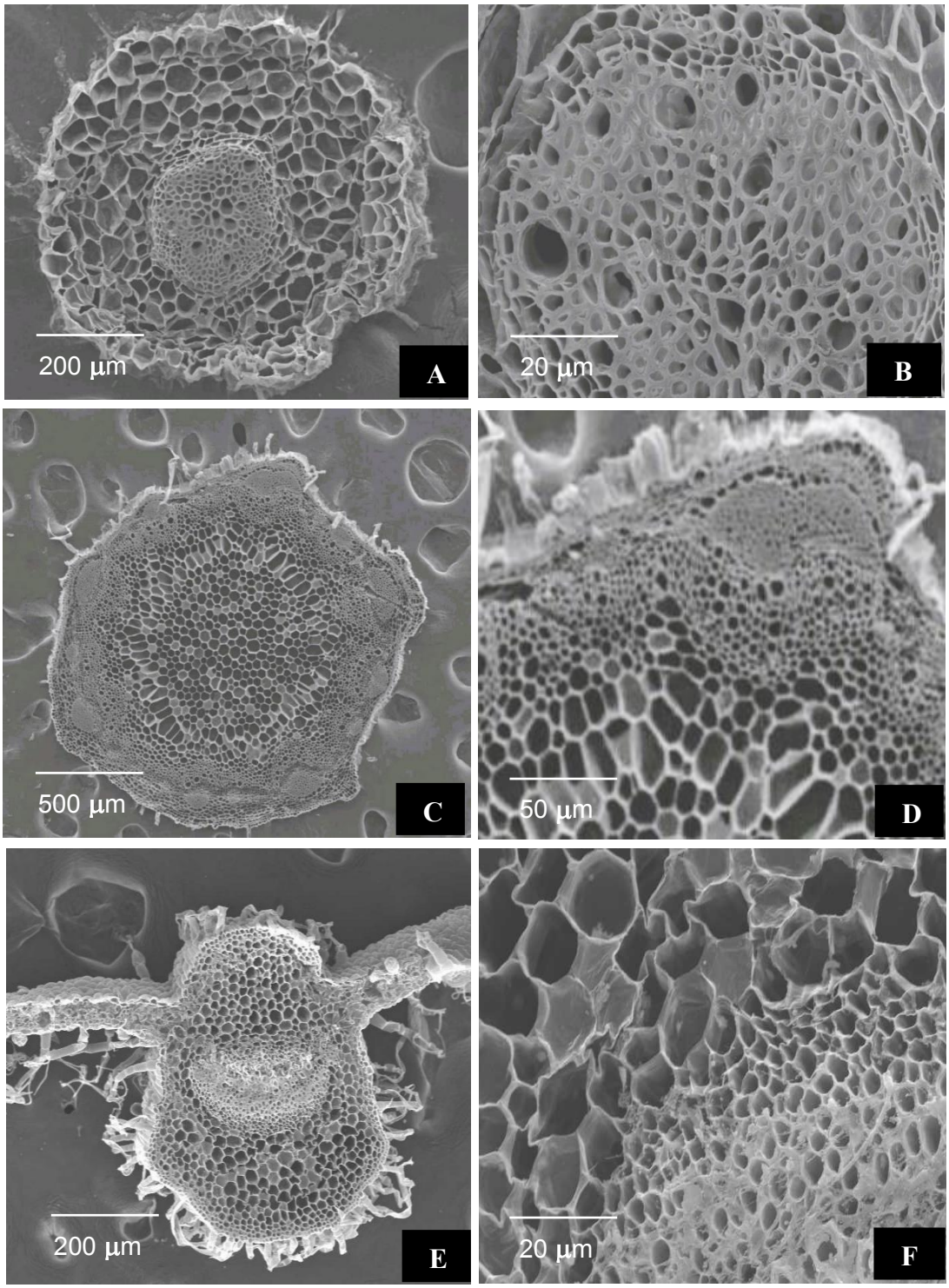


Figure. 22- **A**. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Stem Enlarged; **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

SCANNING ELECTRON MICROGRAPHS AND ENERGY DISPERSIVE X - RAY ANALYSIS OF *ARTEMISIA NILAGIRICA*

CONTROL

The effect of heavy metal stress on the distribution of distinct macro and micro components in different plant sections was investigated using SEM-EDX analysis. EDX data were used to identify the elements and their quantities in the localized masses. *A. nilagirica,* treated with heavy metals remarked notable changes in SEM-EDX peaks, which show distinct macro and micro-elemental distribution patterns in the tissues; the modifications differed based on the metal treated. Carbon, oxygen, magnesium, aluminium, phosphorus, sulphur, chlorine, potassium, calcium, iron, manganese, copper, and zinc were among the essential elements detected in the tissues based on energy dispersive X-ray (EDX) analysis data of a particular region of the *A. nilagirica* root segment (Fig. 23-A & Table.12).

The stem and leaf tissues contained the same basic macro and microelements as the root (Figs. 23- B&C, Table.13&14). The bulk of the significant elements discovered in all plant tissues are composed of C and O.

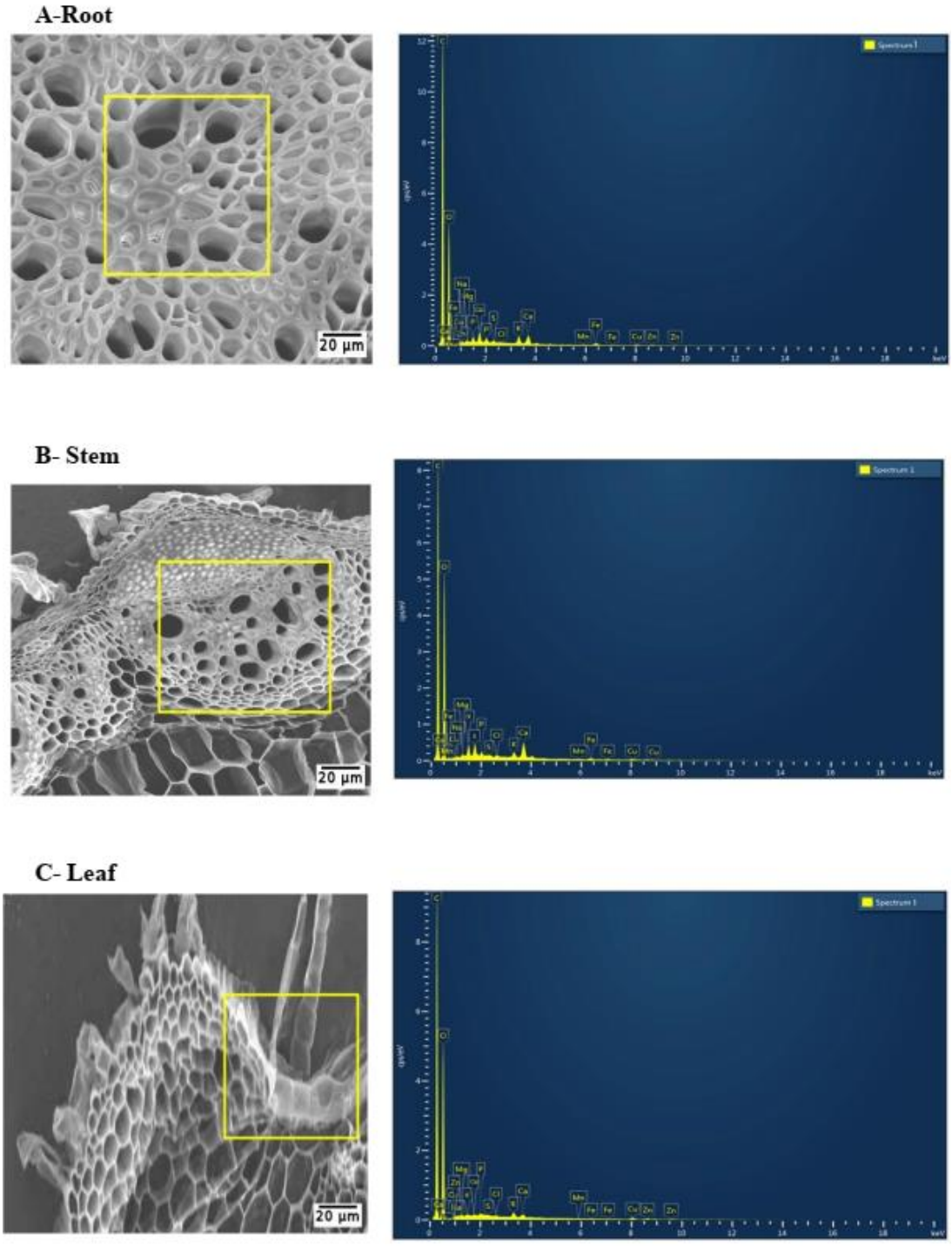


Fig. 23 Scanning Electron Micrographs and EDX spectrum of *Artemisia nilagirica.*

Table 12. SEM-EDX data showing the distribution of various macro and micro-elements (weight %) in the root tissues of *Artemisia nilagirica* on 20th d of treatments.

Essential Elements		Distribution of Elements (Weight %)					
		Control	**Aluminium**	**Chromium**	**Copper**	**Iron**	**Mercury**
Macro-Elements	C	64.27	60.52	50.79	57.35	51.36	52.06
	O	30.82	35.15	41.67	38.74	41.58	42.78
	P	0.61	0.28	0.43	0.24	0.46	0.72
	K	0.41	0.68	2.80	0.37	0.35	1.19
	S	0.57	0.14	0.13	0.36	0.21	0.51
	Ca	1.40	0.93	0.58	0.19	1.27	1.14
	Mg	0.13	0.36	0.14	0.27	0.34	0.25
Micro-Elements	Fe	0.43	0.46	0.38	0.41	2.83	0.51
	Mn	0.04	0.06	0.04	0.01	0.10	0.18
	Cu	0.58	0.47	0.57	1.09	0.26	0.12
	Zn	0.30	0.31	0.35	0.66	0.80	0.15
	Na	0.14	0.16	0.23	0.13	0.26	0.20
	Cl	0.30	0.11	0.24	0.18	0.18	0.04
Treated Metals	Al	0.00	0.37	0.00	0.00	0.00	0.00
	Cr	0.00	0.00	1.65	0.00	0.00	0.00
	Hg	0.00	0.00	0.00	0.00	0.00	0.15
Total		100	100	100	100	100	100

Table 13. SEM-EDX data showing the distribution of various macro and micro-elements (weight %) in the stem tissues of *Artemisia nilagirica* on 20th d of treatments.

Essential Elements		**Distribution of Elements (Weight %)**					
		Control	**Aluminium**	**Chromium**	**Copper**	**Iron**	**Mercury**
Macro-Elements	C	55.51	56.49	56.38	56.27	55.32	54.68
	O	40.15	40.07	39.03	40.70	39.82	40.45
	P	0.39	0.12	0.23	0.15	0.20	0.17
	K	0.68	0.37	0.72	0.36	0.32	0.75
	S	0.64	0.32	0.16	0.08	0.21	0.19
	Ca	0.93	0.26	1.20	0.26	0.37	1.19
	Mg	0.06	0.18	0.12	0.11	0.46	0.18
Micro-Elements	Fe	0.45	0.39	0.26	0.19	1.83	0.29
	Mn	0.05	0.07	0.05	0.07	0.04	0.06
	Cu	0.47	0.64	0.74	0.84	0.60	0.38
	Zn	0.31	0.47	0.06	0.67	0.16	0.24
	Na	0.16	0.25	0.10	0.22	0.48	0.20
	Cl	0.20	0.16	0.12	0.08	0.19	1.03
Treated Metals	Al	0.00	0.18	0.00	0.00	0.00	0.00
	Cr	0.00	0.00	0.83	0.00	0.00	0.00
	Hg	0.00	0.00	0.00	0.00	0.00	0.19
Total		100	100	100	100	100	100

Table 14. SEM-EDX data showing the distribution of various macro and micro-elements (weight %) in the leaf tissues of *Artemisia nilagirica* on 20th d of treatments.

Essential Elements		**Distribution of Elements (Weight %)**					
		Control	**Aluminium**	**Chromium**	**Copper**	**Iron**	**Mercury**
Macro-Elements	C	60.94	56.94	57.08	59.26	56.81	50.17
	O	35.51	39.81	35.91	33.18	34.68	41.41
	P	0.24	0.18	1.32	0.26	1.02	0.65
	K	0.54	0.19	0.47	1.07	1.46	2.85
	S	0.12	0.06	0.17	0.18	0.05	0.32
	Ca	1.37	1.38	1.29	2.54	1.83	2.60
	Mg	0.23	0.18	0.14	1.25	0.18	0.26
Micro-Elements	Fe	0.06	0.04	0.22	0.51	0.98	0.44
	Mn	0.07	0.13	0.16	0.14	0.15	0.12
	Cu	0.40	0.26	0.52	0.94	0.41	0.15
	Zn	0.32	0.24	0.64	0.48	0.86	0.31
	Na	0.11	0.25	1.23	0.16	1.41	0.52
	Cl	0.09	0.19	0.11	0.03	0.16	0.09
Treated Metals	Al	0.00	0.15	0.00	0.00	0.00	0.00
	Cr	0.00	0.00	0.74	0.00	0.00	0.00
	Hg	0.00	0.00	0.00	0.00	0.00	0.21
Total		100	100	100	100	100	100

EFFECT OF ALUMINIUM

Comparative study using energy dispersive X-ray analysis on the effect of Al treatment on *A. nilagirica* showed maximum occurrence of aluminium compared to control (Fig. 23).

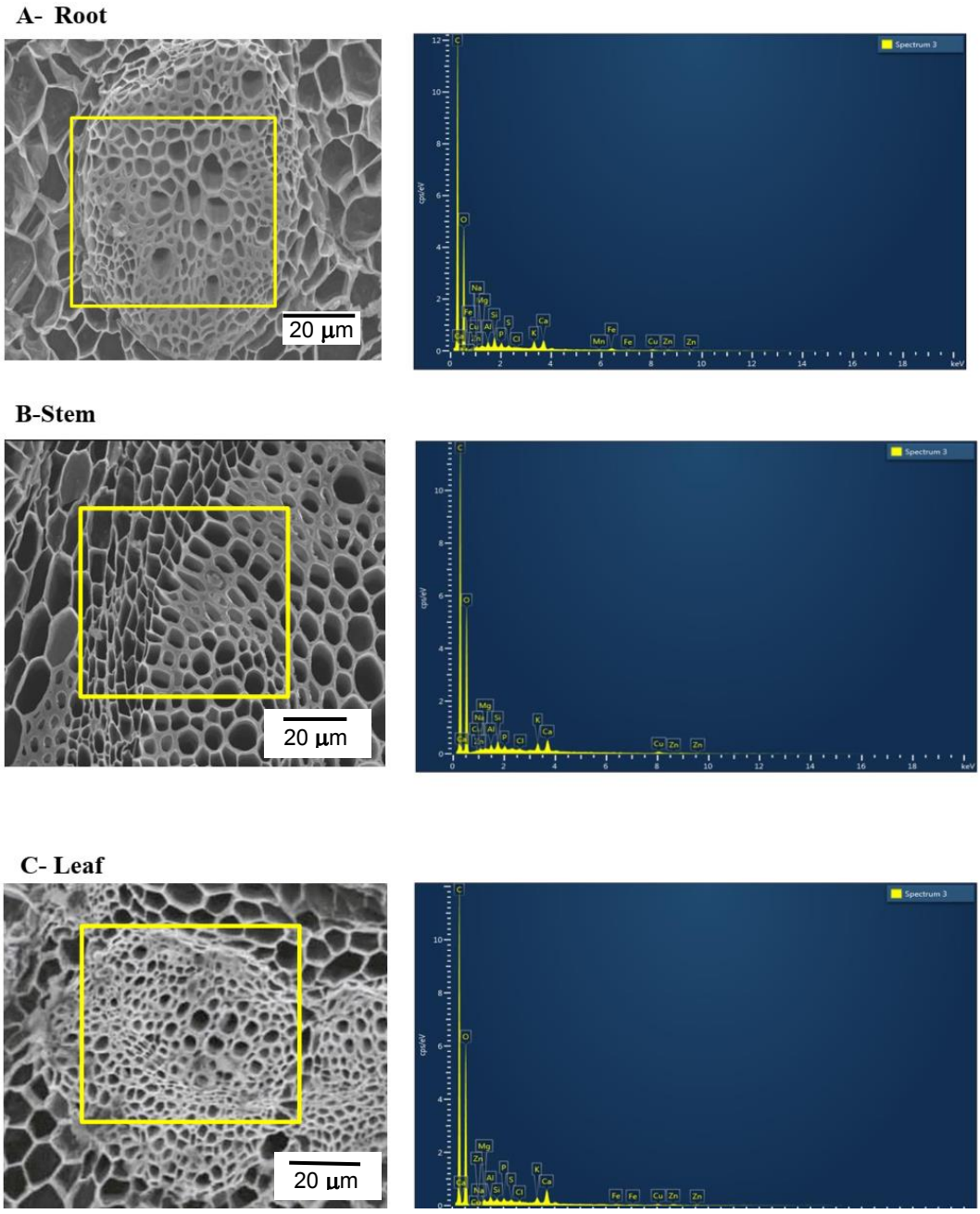


Fig. 24 Scanning Electron Micrographs and EDX spectrum of *Artemisia nilagirica* treated with aluminium

The root tissue of plant treated with Al showed a marginal increase of potassium, magnesium, oxygen and sodium (Fig. 24-A, Table.12). A basal level increase was found in the quantity of iron, manganese and zinc. All other elements decreased. In addition to the essential and non-essential elements aluminium was noted in the root tissues. Root of *A. nilagirica* treated with aluminium, remarked maximum localization of Al in the root than other parts. Decrease of calcium, potassium, sulphur, iron and increase of magnesium, copper, zinc and chlorine was observed in the stem tissues. Presence of aluminium was detected (Fig. 24-B, Table.13). Leaves of plants treated with Al showed lesser aluminium content compared to other tissues (Fig. 24C, Table.14). Phosphorus, potassium, sulphur, magnesium, copper and zinc contents were decreased in the leaf tissues due to Al treatment. Whereas sodium and chlorine increased as compared to the control.

EFFECT OF CHROMIUM

Root tissues of plants treated with chromium showed maximum chromium atoms compared to other tissues (Table.12). Chromium treatment resulted in remarkable variation in the distribution of atoms in the root tissues of *A. nilagirica* (Fig. 25-A). In the root tissues, due to chromium treatment there is increase in the distribution of elements such as oxygen, and magnesium whereas carbon, phosphorus, sulphur, and iron decreased significantly. The other elements remained the same as control. In stem tissues, Cr treatment resulted in increase of calcium, potassium and copper compared to control (Fig.25-B&Table.13). Distribution of magnesium atoms has decreased in the leaf tissues (Table.14). Leaf tissues treated with Cr showed high chromium content (Fig. 25-C). Phosphorous, sulfur and zinc increased in leaf tissues whereas calcium and copper decreased.

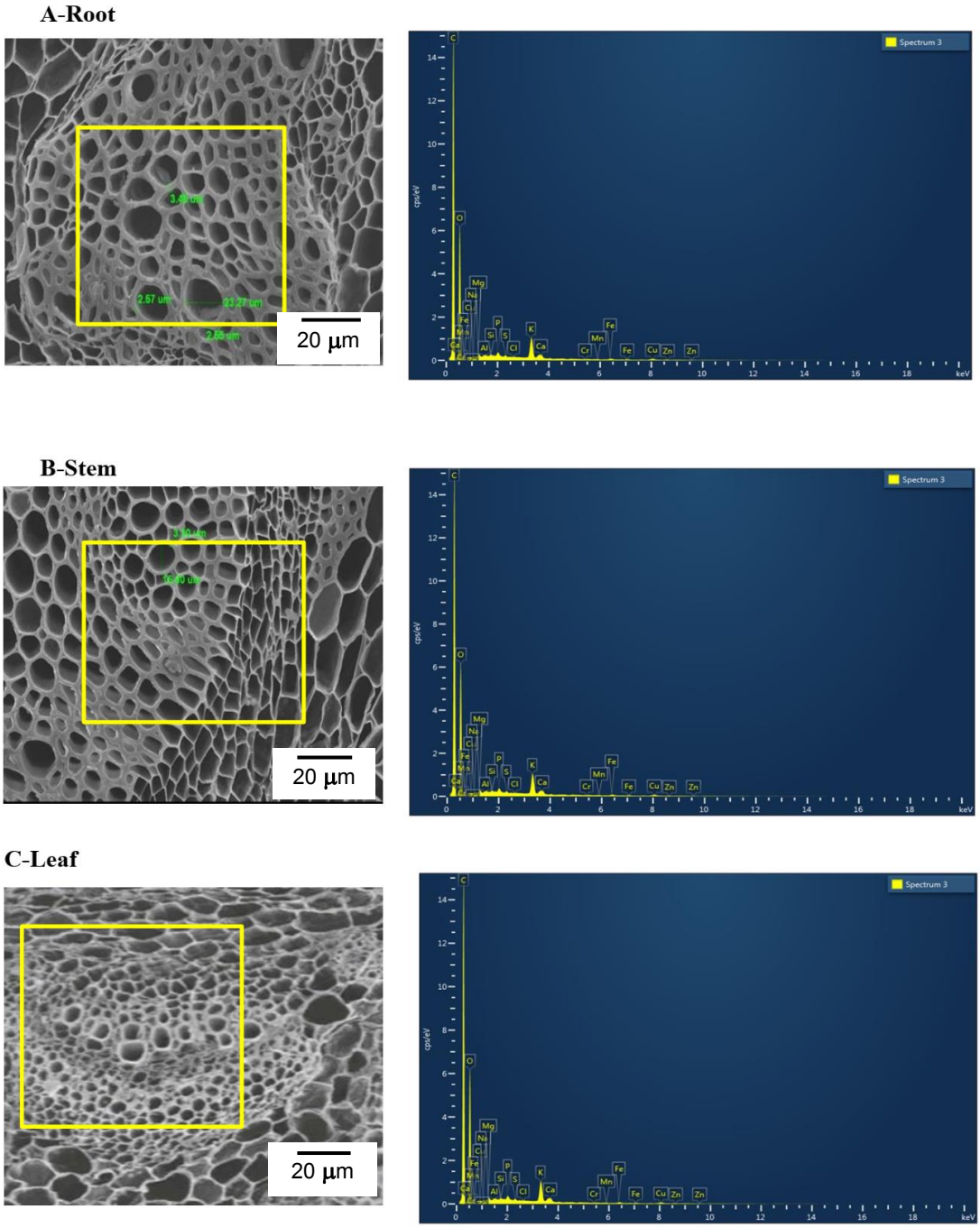


Fig. 25 Scanning Electron Micrographs and EDX spectrum of *Artemisia nilagirica* treated with chromium

EFFECT OF COPPER

Compared to the control, the root of plant treated with copper had more copper atoms. Cu treatment reduced the number of phosphorous, calcium, potassium, sulphur, sodium and iron atoms in the *A. nilagirica* root tissue (Fig.26-A &Table.12).

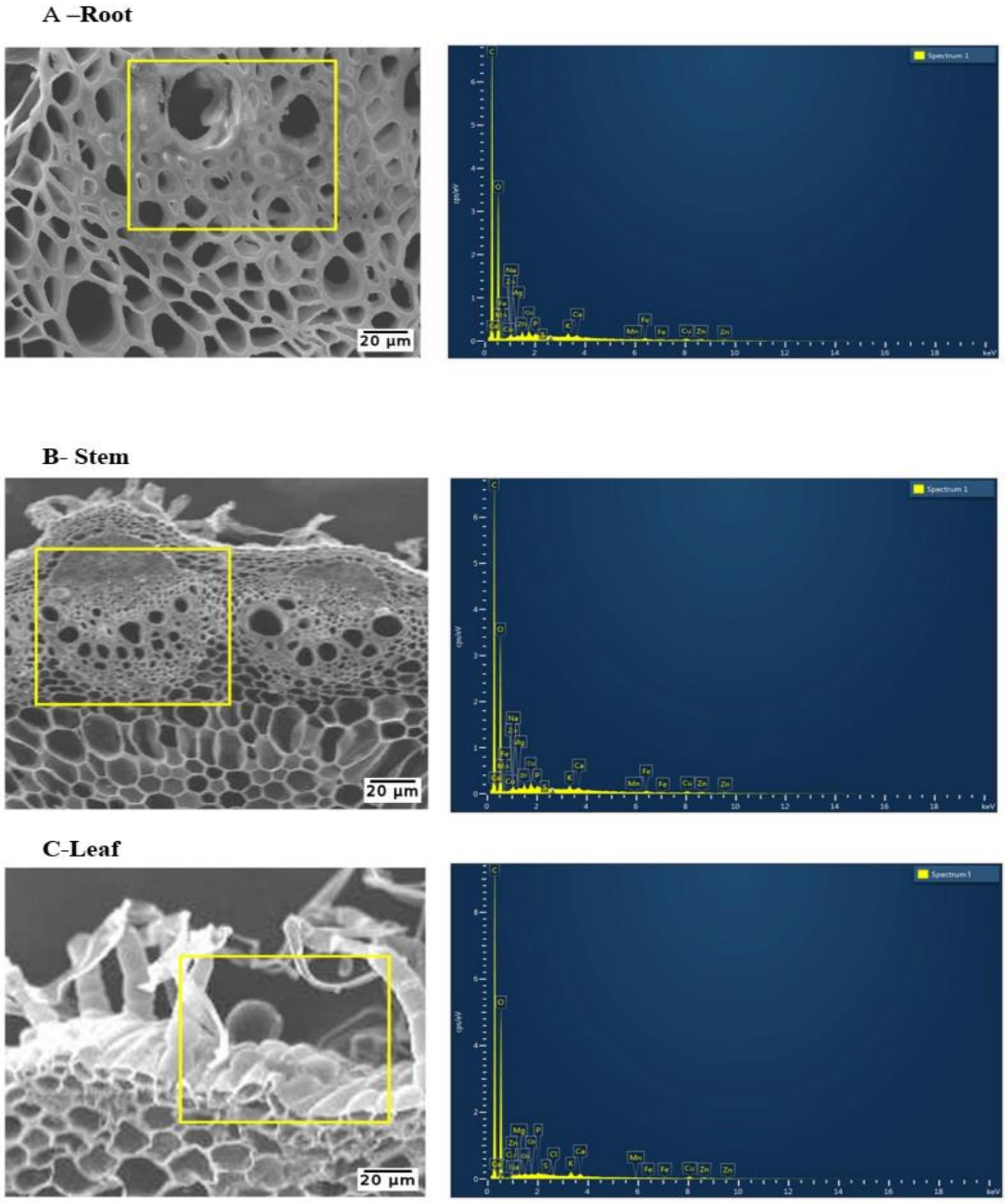


Fig. 26 Scanning Electron Micrographs and EDX spectrum of *Artemisia nilagirica treated* with copper

Magnesium and zinc level slightly elevated. Additionally, compared to the control, stem tissues had more copper atoms (Fig.26-B&Table.13). Magnesium and zinc levels in the stem tissues were elevated. Phosphorus, potassium, sulphur, calcium and iron levels dropped in stem tissues. Copper levels were high in leaf tissues when compared to the control (Fig.26-C&Table.14). leaf of Cu treated plant showed elevated levels of all other elements.

EFFECT OF IRON

In comparison to the control, iron treatment increased the number of Fe atoms in *A. nilagirica*. Localization of Fe is more in root than stem and leaf. Fe treatment resulted in an increase of magnesium, and zinc atoms in the root tissues (Fig.27-A &Table.12).

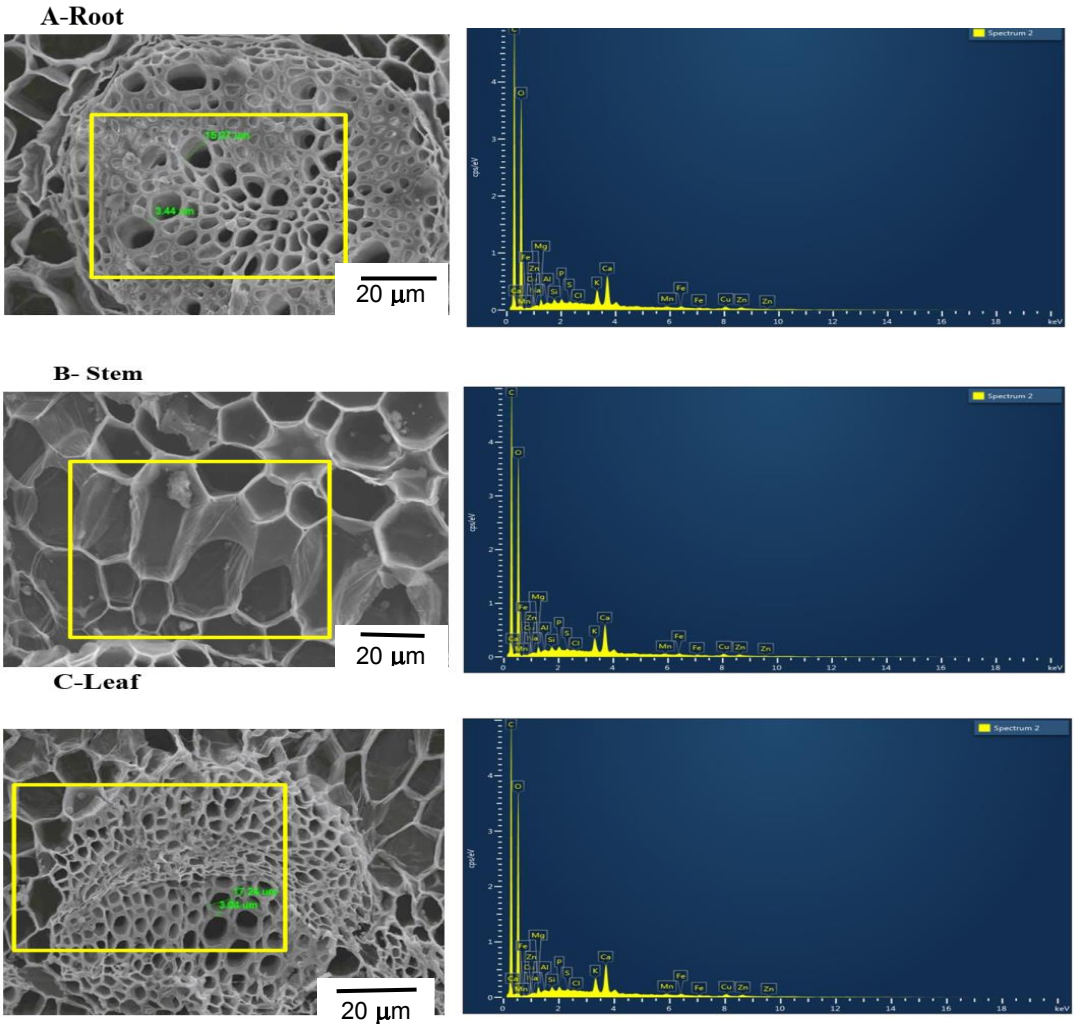


Fig. 27 Scanning Electron Micrographs and EDX spectrum of *Artemisia nilagirica* treated with iron.

In addition, stem tissues had more iron atoms than the control (Fig.27-B &Table.13). Magnesium and copper were slightly elevated in the stem tissues. Level of all other elements were dropped. Iron levels in leaf tissues were higher than in the control group whereas it is lesser than in root and stem tissues. While the distribution of other atoms stayed almost unchanged (Fig.27-C &Table.14).

EFFECT OF MERCURY

A. nilagirica treated with mercury, detected Hg atoms distributed in the root tissues which is not present in control root (Fig.28-A&Table.12).

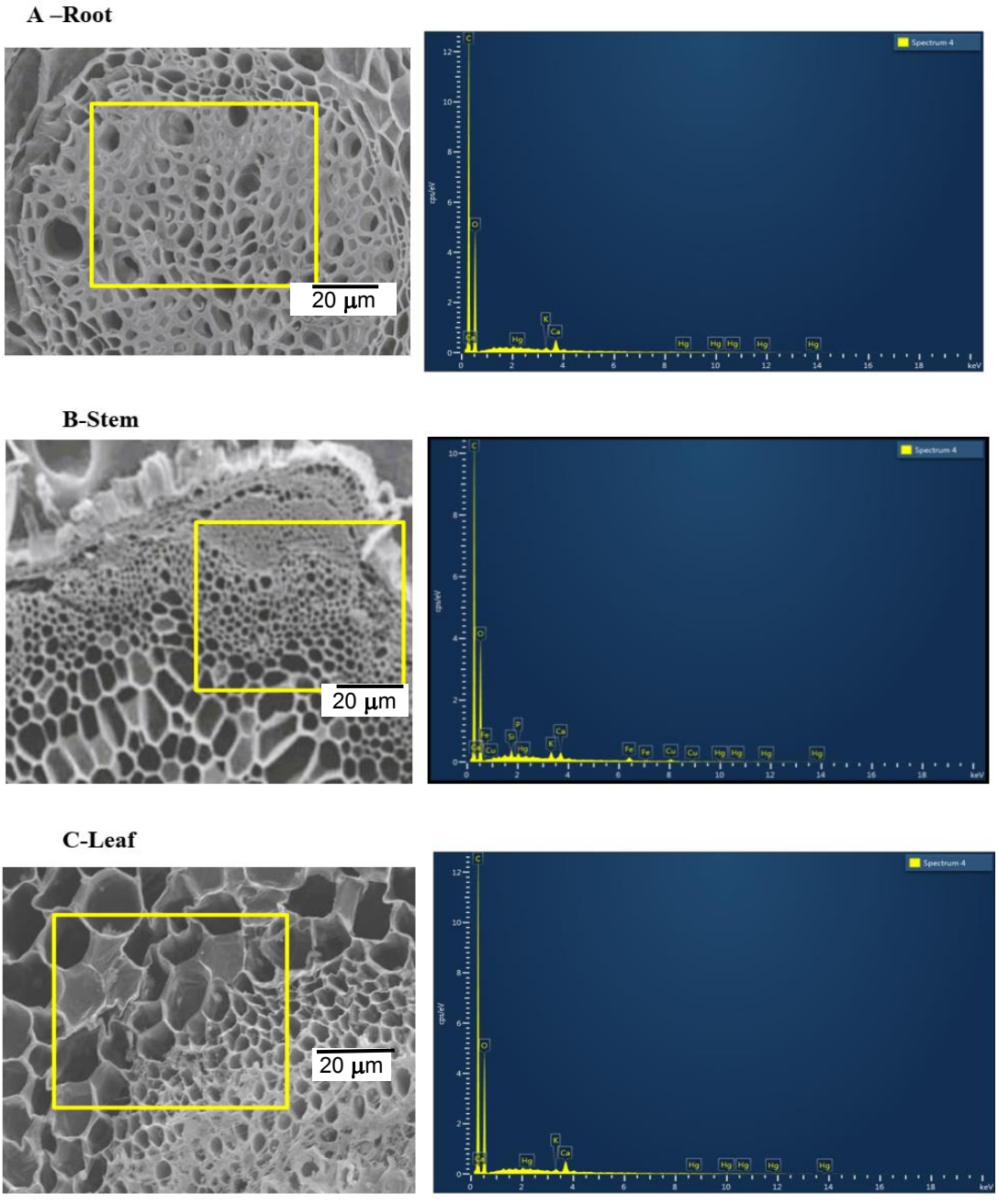


Fig. 28 Scanning Electron Micrographs and EDX spectrum of *Artemisia nilagirica* treated with mercury

Significant increase of oxygen, potassium, magnesium and also the decrease of carbon were observed after the treatment. Distribution of mercury atoms were seen in the stem tissues of Hg treated plant (Fig.28-B&Table.13). Maximum mercury atoms were present in the leaf tissue treated with Hg. There is an increase in the distribution of sodium in all plant parts while level of copper decreased (Fig.28-C&Table.14).

DRY WEIGHT PERCENTAGE

Distribution of dry weight percentage of plant parts of *Artemisia nilagirica* of various treatments are shown in Table.15 & Fig. 29-31. Dry weight percentage of plant parts of *A. nilagirica* exhibited gradual decrease during each interval of growth.

Table 15. Effect of aluminium, chromium, copper, iron and mercury on dry weight percentage in *Artemisia nilagirica.*

Treatments	Tissues	Interval - Days					
		0	4	8	12	16	20
Control	Root	8.4±0.92	9.1±1.12	9.6±0.98	10.5±1.24	12.10±0.90	13.60±1.20
	Stem	25.2±0.5	26.8±0.4	28.5±0.9	29±0.52	30.9±0.54	32.4±0.68
	Leaf	18.3±1.4	18.9±1.2	20.6±0.8	23.6±1.30	24.4±1.12	25.3±0.10
Aluminium (80 µM)	Root	8.6±1.31	9.2±0.65	9.5±1.32	10.3±1.01	10.82±1.22	11.04±2.03
	Stem	24.5±.09	25.3±1.2	26.8±0.2	28.2±.16	28.5±0.34	29.31±1.04
	Leaf	18.2±0.12	18.6±0.24	19.4±0.2	21.1±0.43	22.4±1.01	23.8±0.40
Chromium (70 µM)	Root	8.7±1.02	8.91±1.3	9.3±0.89	9.7±1.05	10.3±0.86	11.4±1.34
	Stem	24.8±0.9	25.3±1.1	25.8±.82	26.7±1.2	27.3±0.83	28.6±0.86
	Leaf	18.3±0.8	18.7±1.2	19.1±.92	20.3±.89	21.2±1.03	21.8±1.11
Copper (50 µM)	Root	8.2±1.12	8.5±1.42	8.6±1.09	8.8±1.4	8.9±1.23	9.3±1.45
	Stem	22.6±2.1	24.4±2.3	24.8±1.7	25.0±2.3	25.4±1.38	25.8±2.31
	Leaf	18.4±.96	18.5±1.2	18.7±.84	19.1±1.5	19.4±1.02	19.5±0.06
Iron (70 µM)	Root	8.7±.1.6	8.82±1.43	9.2±1.25	9.5±2.24	10.1±1.26	11.6±1.87
	Stem	24.3±2.3	24.8±1.4	25±2.17	25.3±1.3	26.1±0.98	27.4±0.81
	Leaf	18.5±2.2	18.6±2.8	18.8±1.9	19.3±1.5	19.6±0.83	19.6±1.43
Mercury (10 µM)	Root	8.2±1.44	8.3±0.96	8.5±1.32	8.8 ±1.40	8.8±0.90	9.2±1.25
	Stem	22.3±2.3	23.4±1.8	23.7±1.6	24.2±2.3	24.9±1.134	25.1±1.56
	Leaf	18.3±1.3	18.1±1.27	18.3±1.6	18.4±2.04	18.7±1.32	18.8±1.4

Values given are mean of 5 replicates ±S.E

Roots, stem and leaf of control plant showed similar pattern. Whereas, dry weight percentage of plant parts of *A. nilagirica* varied significantly among different treatments with metals. Dry weight of roots of Al treated plant registered an increase during first three intervals and thereafter a steady decline wasobserved (Fig.29). Same trend was observed in the case of stem and leaves also. (Fig.30, Fig. 31). Roots, stem and leaf of plants treated with Cu and Hg showed an initial increase in dry weight percentage up to 4th day and thereafter a gradual decrease was exhibited. Compared to the control, dry weight of root, stem and leaf of plants treated with Cr and Fe showed gradual increase during all intervals.

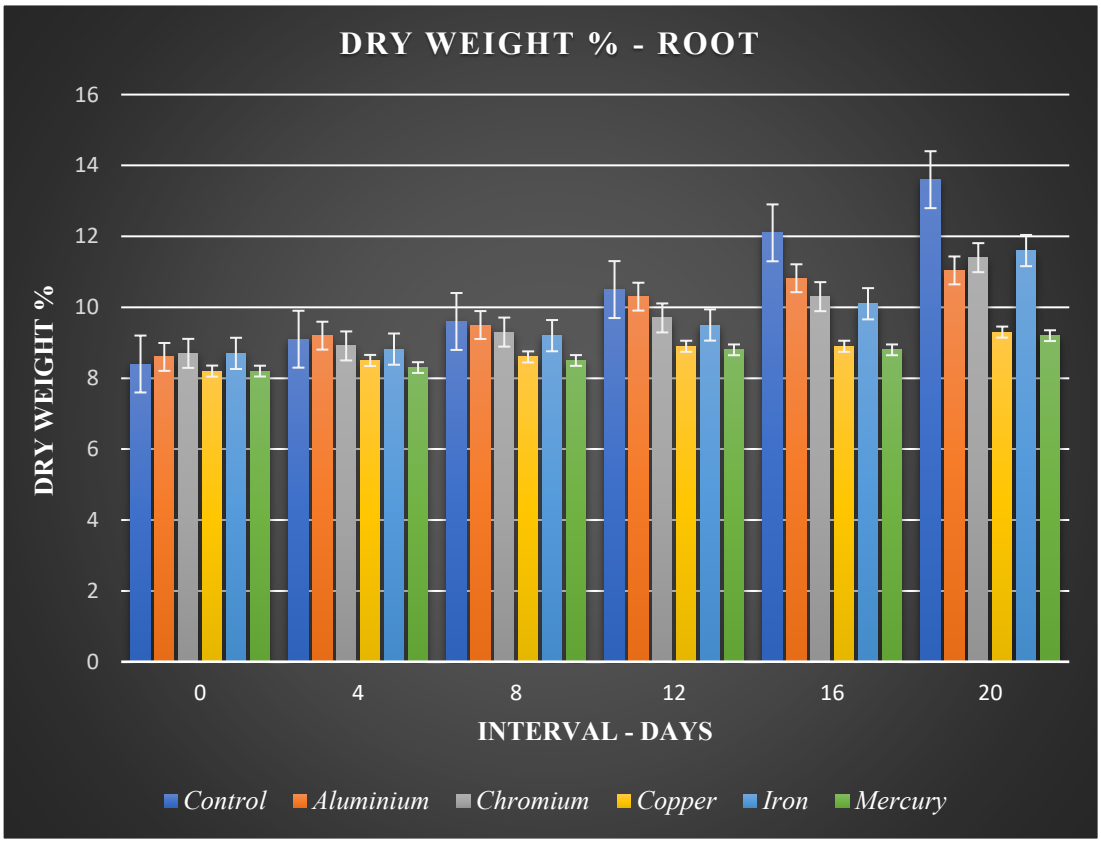


Fig. 29 Effect of aluminium, chromium, copper, iron and mercury on dry weight percentage of root in *Artemisia nilagirica.*

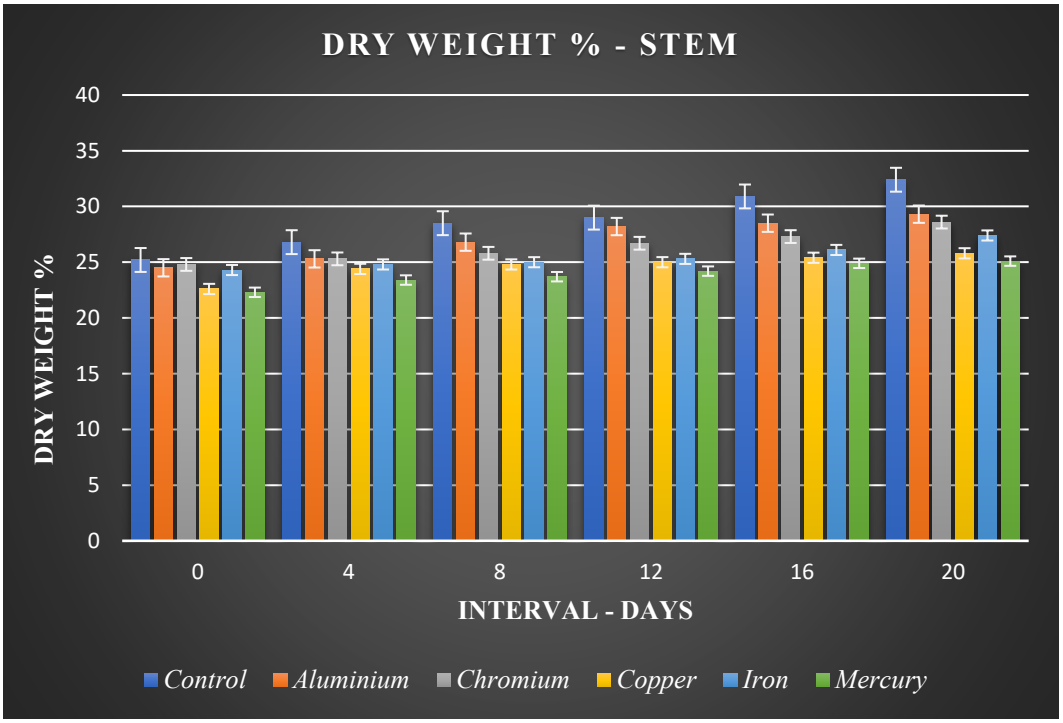


Fig. 30 Effect of aluminium, chromium, copper, iron and mercury on dry weight percentage of stem in *Artemisia nilagirica.*

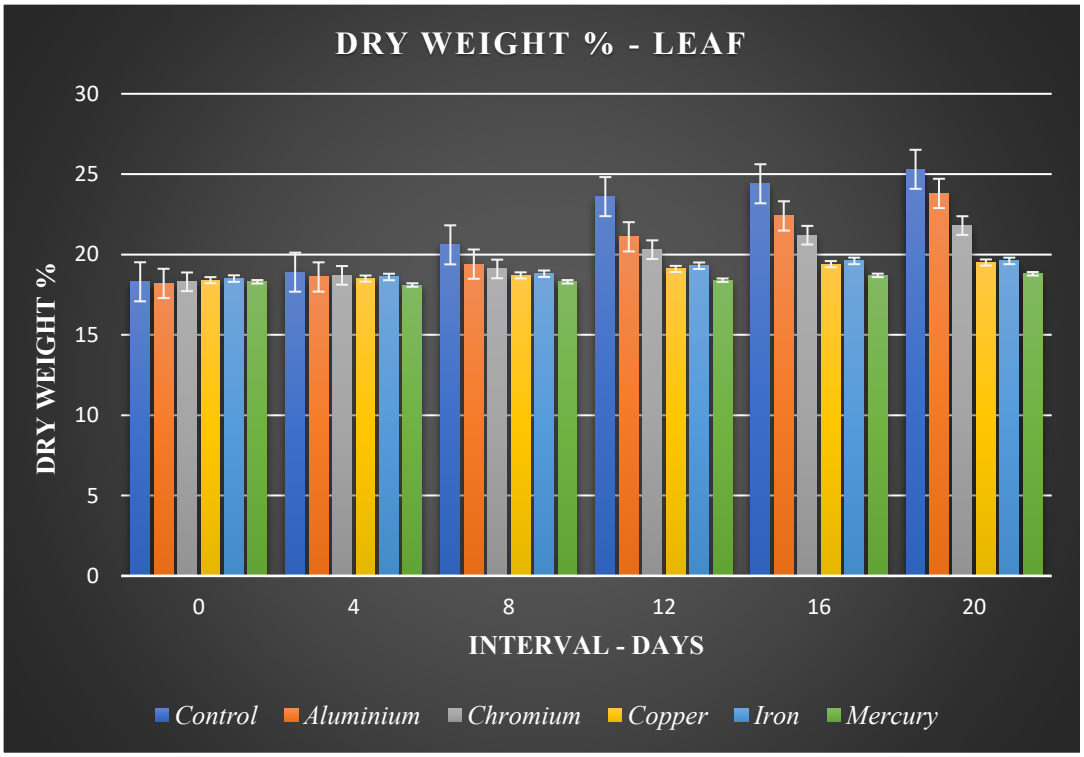


Fig. 31 Effect of aluminium, chromium, copper, iron and mercury on dry weight percentage of leaf in *Artemisia nilagirica.*

PIGMENT DISTRIBUTION

Table. 16 depicts the effects of heavy metals, such as Al, Cr, Cu, Fe and Hg, on the amount of carotenoid and chlorophyll (Chl *a*, Chl *b* and total chlorophyll) in *A. nilagirica* leaves over a 20 - day period. The results show varied degrees of pigment depletion, underscoring the negative impact of heavy metal stress on photosynthetic pigments, which are vital for plant development and metabolism.

Chlorophyll *a* Content (Fig. 32)

Chl *a* steadily increased under controlled circumstances, reaching 6.12 ± 0.005 mg/g DW by day 20. Chl *a* levels were, however, considerably impacted by heavy metal treatments.

Aluminium: In comparison to other metals, the amount of Chl *a* elevated lightly, peaking at 5.02 ± 0.033 mg/g DW, suggesting less severe disruption in photosynthetic machinery.

Chromium: After initially encouraging a little rise, the level of Chl *a* decreased to 4.74 ± 0.112 mg/g DW by day 20, most likely as a result of enzyme inhibition and oxidative stress.

Copper: Because copper is toxic to the pathways involved in chlorophyll production, there was a significant drop in levels, which fell to 2.05 ± 0.031 mg/g DW.

Iron: In keeping with role of iron in enhancing oxidative stress, Chl *a* exhibited a significant drop after day 8, reaching 2.25 ± 0.016 mg/g DW.

Mercury: The most noticeable decrease, showing significant harm to chloroplast integrity, with levels falling from 2.01 ± 0.106 mg/g DW initially to 1.96 ± 0.013 mg/g DW.

Chlorophyll *b* Content (Fig. 33)

Chromium and aluminium: Chl *b* levels showed moderate declines, ending at 2.02 ± 0.11 and 2.24 ± 0.18 mg/g DW, respectively, indicating partial resistance to these metals.

Iron and Copper: Chl *b* levels sharply decreased, suggesting that chlorophyll synthesis was inhibited, especially when exposed to iron stress (0.99 ± 0.150 mg/g DW) and copper stress (0.91 ± 0.040 mg/g DW).

Mercury: The worst drop, with Chl *b* dropping to 0.56 ± 0.141 mg/g DW by day 20, indicating significant chloroplast enzyme impairment.

Total Chlorophyll Content (Fig. 34)

Trends in the total chlorophyll content were in line with those of the individual chlorophyll components. The quantities of heavy metals produced varied degrees of decrease, while control samples showed a consistent increase to 8.71 ± 0.026 mg/g DW:

The moderate inhibition of chromium (6.76 ± 0.215 mg/g DW) and aluminium (7.26 ± 0.115 mg/g DW) was probably caused by oxidative stress that affects the production of chlorophyll.

There were notable decreases in copper (2.96 ± 0.025 mg/g DW), iron (3.24 ± 0.124 mg/g DW) and mercury (2.52 ± 0.116 mg/g DW), which indicated significant harm to photosynthetic enzymes and chloroplasts.

Carotenoid Content (Fig. 35)

By day 20 under control conditions, carotenoid levels increased to 3.52 ± 0.014 mg/g DW, confirming their function in photoprotection. However, heavy metals led to decreases:

Chromium and aluminium: Their function in reducing oxidative stress was demonstrated by their comparatively steady carotenoid levels.

Mercury and copper: Carotenoid levels dropped precipitously, reaching 2.03 ± 0.044 and 2.11 ± 0.320 mg/g DW, respectively, indicating decreased photoprotection and increased vulnerability to stress.

Iron: The final carotenoid concentration was 2.64 ± 0.021 mg/g DW, indicating moderate decreases.

The findings highlight how heavy metals negatively impact pigment content in *Artemisia nilagirica*. More noticeable reductions are brought on by metals like Cu, Fe and Hg, most likely as a result of oxidative damage, interference with chlorophyll production and decreased enzyme activity. Even though their effects are less severe, Al and Cr nevertheless affect photosynthetic efficiency by preventing the formation of pigment. These results highlight how important pigments are for sustaining photosynthesis in stressful situations and how heavy metal pollution must be reduced to protect plant health.

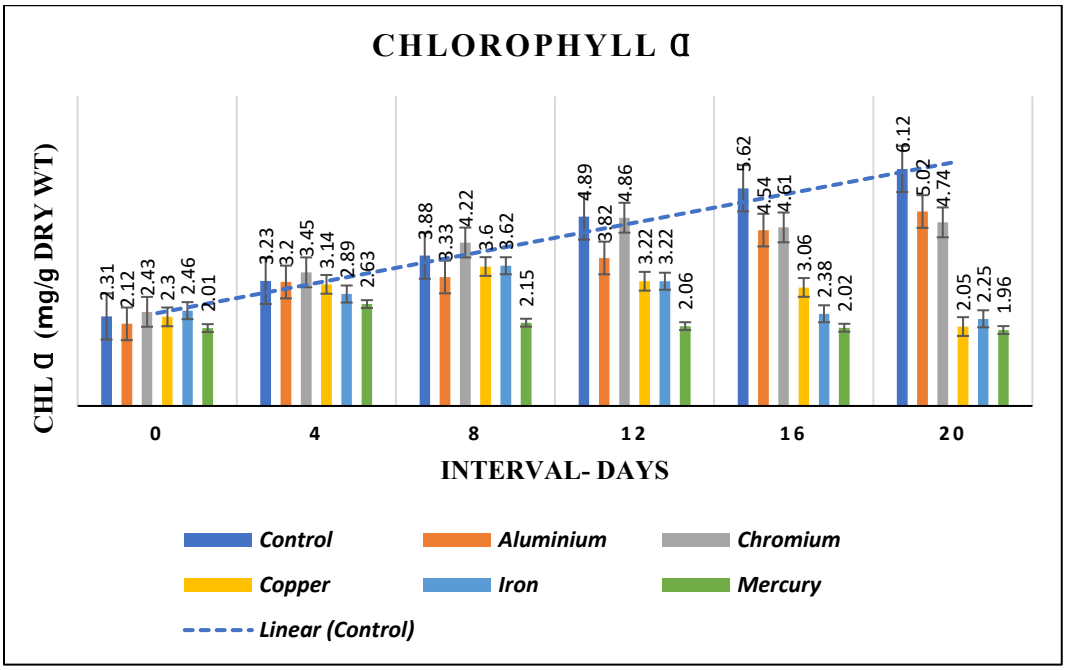


Fig. 32 Effect of aluminium, chromium, copper, iron and mercury on Chl *a* of leaf in *Artemisia nilagirica.*

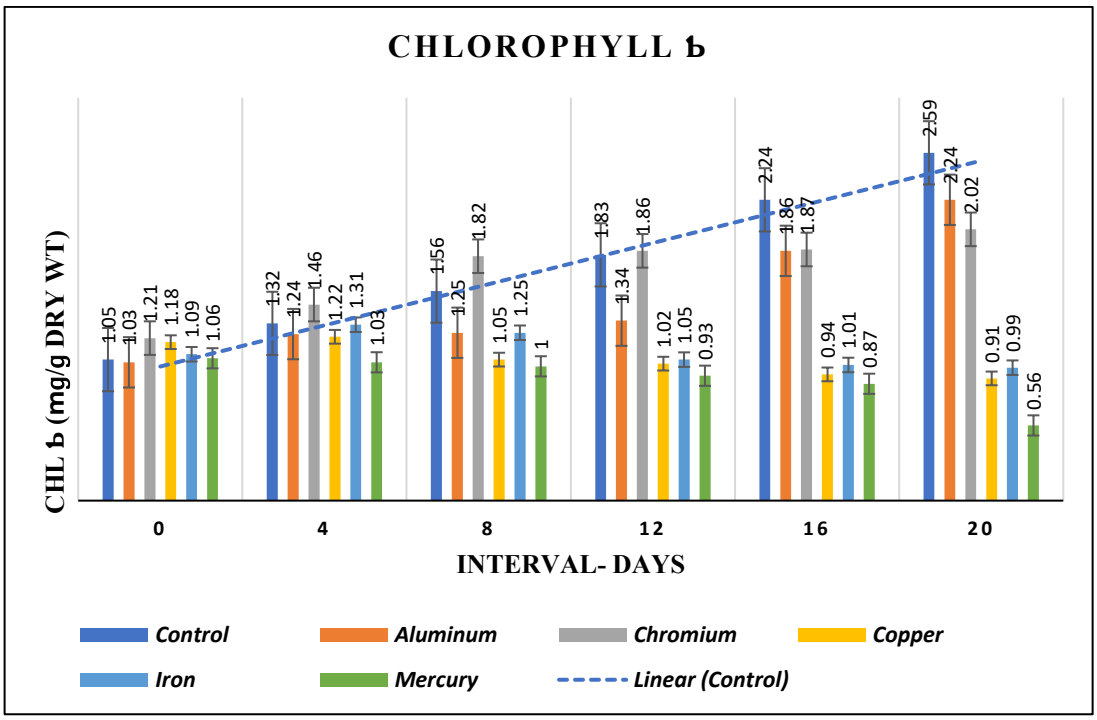


Fig. 33 Effect of aluminium, chromium, copper, iron and mercury on Chl *b* of leaf in *rtemisia nilagirica.*

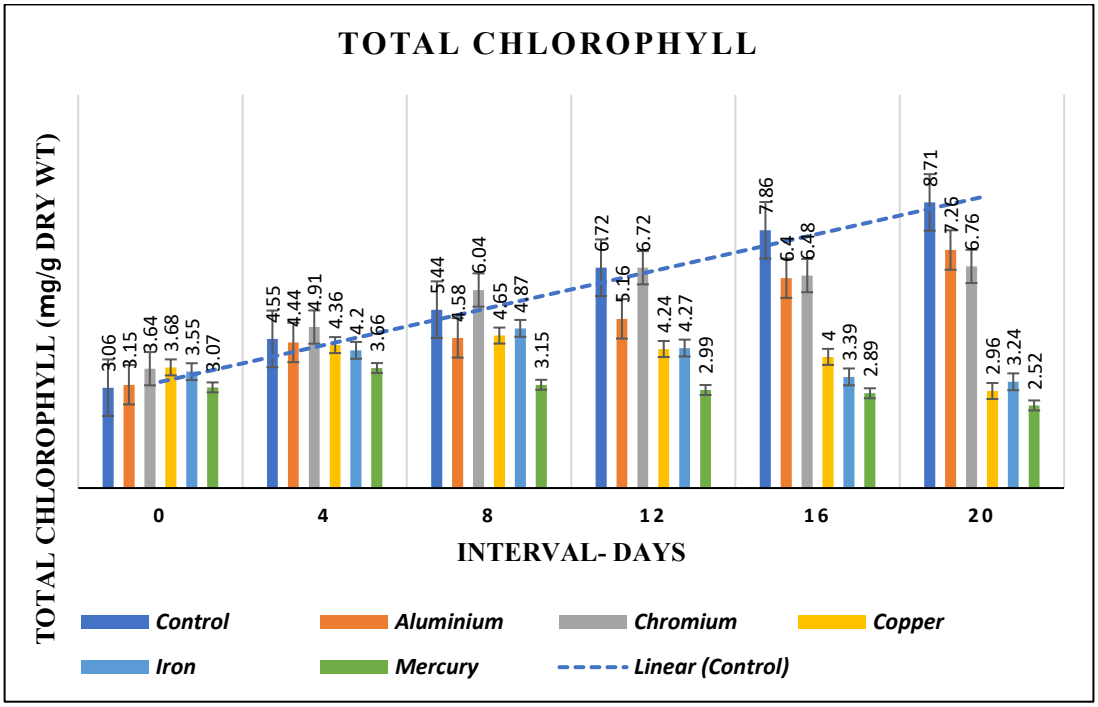


Fig. 34 Effect of aluminium, chromium, copper, iron and mercury on total chlorophyll of leaf in *Artemisia nilagirica.*

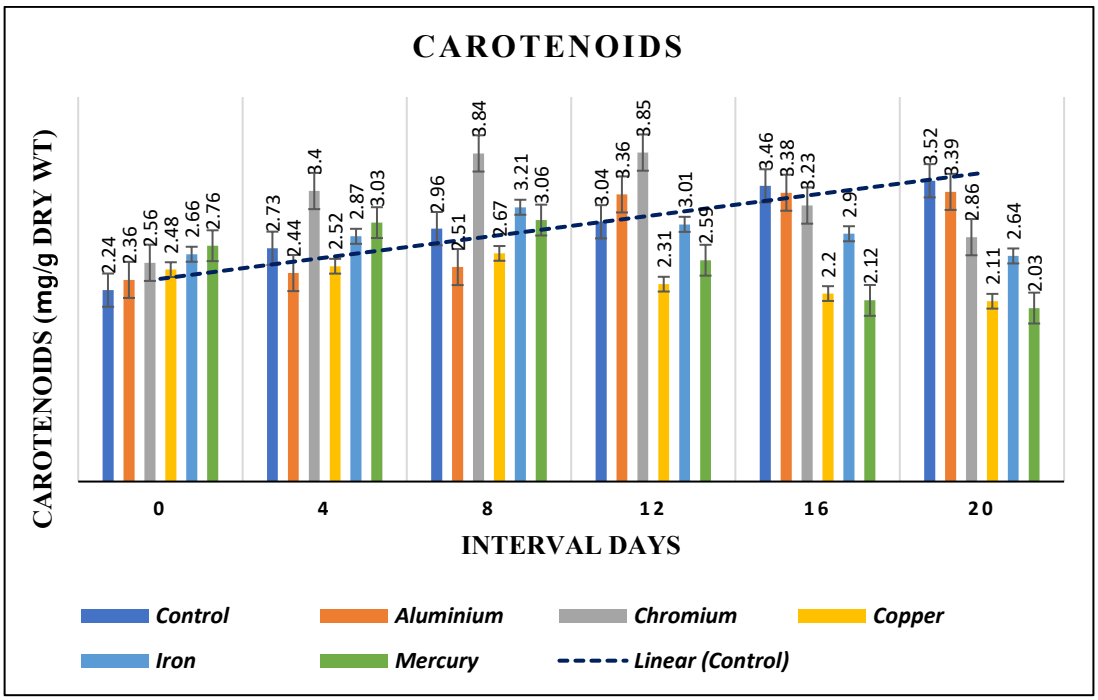


Fig. 35 Effect of aluminium, chromium, copper, iron and mercury on carotenoids of leaf in *Artemisia nilagirica.*

Table 16. Effect of aluminium, chromium, copper, iron and mercury on pigment distribution of leaf in *Artemisia nilagirica.* Chlorophyll content mg/g DW.

Treatments		Interval - Days					
		0	**4**	**8**	**12**	**16**	**20**
Control	Chl *a*	2.31± 0.012	3.23±0.002	3.88±0.024	4.89±0.010	5.62±0.022	6.12±0.005
	Chl *b*	1.05±0.023	1.32±0.011	1.56±0.004	1.83±0.112	2.24±0.024	2.59±0.016
	Total Chl	3.06±0.033	4.55±0.002	5.44±0.114	6.72±0.020	7.86±0.010	8.71±0.026
	Carotenoid	2.24±0.110	2.73±0.120	2.96±0.011	3.04±0.024	3.46±0.006	3.52±0.014
Aluminium (80 µM)	Chl *a*	2.12±0.120	3.20±0.142	3.33±0.012	3.82±0.004	4.54±0.104	5.02±0.033
	Chl *b*	1.03±0.004	1.24±0.120	1.25±0.005	1.34±0.016	1.86±0.002	2.24±0.18
	Total Chl	3.15±0.002	4.44±0.012	4.58±0.104	5.16±0.214	6.4±0.026	7.26±0.115
	Carotenoid	2.36±0.130	2.44±0.016	2.51±0.021	3.36±0.035	3.38±0.101	3.39±0.003
Chromium (70 µM)	Chl *a*	2.43±0.104	3.45±0.105	4.22±0.224	4.86±0.003	4.61±0.042	4.74±0.112
	Chl *b*	1.21±0.142	1.46±0.045	1.82±0.116	1.86±0.105	1.87±0.002	2.02±0.11
	Total Chl	3.64±0.215	4.91±0.013	6.04±0.224	6.72±0.016	6.48±0.203	6.76±0.215
	Carotenoid	2.56±0.003	3.40±0.032	3.84±0.012	3.85±0.023	3.23±0.022	2.86±0.010
Copper (50 µM)	Chl *a*	2.50±0.002	3.14±0.032	3.60±0.107	3.22±0.062	3.06±0.011	2.05±0.031
	Chl *b*	1.18±0.130	1.22±0.121	1.05±0.104	1.02±0.045	0.94±0.001	0.91±0.040
	Total Chl	3.68±0.123	4.36±0.220	4.65±0.005	4.24±0.106	4.00±0.131	2.96±0.025
	Carotenoid	2.48±0.001	2.52±0.002	2.67±0.010	2.31±0.014	2.20±0.112	2.11±0.320

Treatments		Interval - Days					
		0	**4**	**8**	**12**	**16**	**20**
Iron (70 µM)	Chl *a*	2.46±0.024	2.89±0.044	3.62±0.007	3.22±0.102	2.38±0.005	2.25±0.016
	Chl *b*	1.09±0.022	1.31±0.243	1.25±0.103	1.05±0.002	1.01±0.013	0.99±0.150
	Total Chl	3.55±0.022	4.20±0.042	4.87±0.110	4.27±0.017	3.39±0.301	3.24±0.124
	Carotenoid	2.66±0.045	2.87±0.122	3.21±0.113	3.01±0.011	2.90±0.012	2.64±0.021
Mercury (10 µM)	Chl *a*	2.01±0.106	2.63±0.023	2.15±0.013	2.06±0.24	2.02±0.020	1.96±0.013
	Chl *b*	1.06±0.013	1.03±0.001	1.00±0.115	0.93±0.023	0.87±0.012	0.56±0.141
	Total Chl	3.07±0.014	3.66±0.042	3.15±0.240	2.99±0.331	2.89±0.005	2.52±0.116
	Carotenoid	2.76±0.004	3.03±0.024	3.06±0.022	2.59±0.014	2.12±0.011	2.03±0.044

Values given are mean of 5 replicates ±S. E

TOTAL SOLUBLE SUGAR

The information presented (Table.17 and Figures. 36-38) illustrates how heavy metals (Al, Cr, Cu, Fe and Hg) affect the amount of soluble sugar in root, stem and leaf tissues of *Artemisia nilagirica*. A comprehensive analysis of the findings is provided below:

The amount of soluble sugar in roots (Fig. 36) increased in all treatments as compared to the control, however the time and size of the peak accumulation varied. The biggest increase was due to mercury stress, which peaked on day 12 at 8.16 mg/g DW and then decreased to 6.88 mg/g DW on day 20. Although it was less noticeable, other metals also showed this pattern. By day 20, for instance, chromium and copper showed a consistent rise, reaching 6.87 and 7.01 mg/g DW, respectively. Values stabilized at 6.23 mg/g DW for iron and 7.14 mg/g DW for aluminium, indicating a slower growth.

Across all treatments, the amount of soluble sugar in the stems grew steadily (Fig 37). The highest levels were caused by mercury exposure; on day 12, values reached 16.48 mg/g DW and by day 20, they had slightly decreased to 15.13 mg/g DW. Plants treated with copper likewise showed a noticeable rise, reaching a peak of 13.27 mg/g DW on day 12 and then gradually declining. The sugar level increased steadily with chromium as well, reaching 11.12 mg/g DW on day 20. By the end of the experiment, the sugar levels in the iron and aluminium treatments had increased moderately and steadily, stabilizing at about 11.90 and 10.25 mg/g DW, respectively. This increasing pattern raises the possibility that stems could serve as a reservoir or channel for sugars under stress.

Particularly under mercury stress, leaves showed the most noticeable increases in soluble sugar content. On day 12, the sugar content of leaves treated with mercury reached a peak of 19.45 mg/g DW, but by day 20, it had dropped to 15.03 mg/g DW. Likewise, significant sugar buildup was brought on by copper and chromium treatments, which peaked at 13.58 mg/g DW and 12.93 mg/g DW, respectively, in the later intervals. By day 20, the levels of iron and aluminium increased somewhat, with the sugar content rising to 13.29 and 10.81 mg/g DW, respectively. In contrast to roots and stems, the leaves notably had the largest total accumulation, suggesting that they play a crucial role in managing stress-induced sugar dynamics (Fig. 38).

Table 17. Effect of aluminium, chromium, copper, iron and mercury on total soluble sugar content (mg/g DW) of root, stem and leaf in *Artemisia nilagirica* (Clarke) Pamp.

Treatments	Tissues	Interval - Days					
		0	4	8	12	16	20
Control	Root	1.53±0.24	2.20±0.16	3.38±0.02	4.47±0.72	5.69±0.05	5.84±1.01
	Stem	3.32±1.03	5.78±0.31	7.61±0.15	8.63±1.23	9.58±0.11	9.96±0.12
	Leaf	5.01±0.42	6.34±1.10	7.48±1.21	8.92±0.40	12.08±0.26	12.24±0.04
Aluminium (80 µM)	Root	1.21±0.14	3.36±1.02	5.02±0.06	5.78±0.43	6.12±0.32	6.23±1.15
	Stem	3.76±0.62	5.15±1.41	8.46±0.35	9.52±0.15	10.18±0.13	10.25±0.21
	Leaf	5.53±1.80	7.55±0.31	8.69±1.02	10.04±1.02	10.46±1.24	10.81±0.14
Chromium (70 µM)	Root	2.01±0.45	3.28±0.06	5.56±1.13	6.10±0.22	6.54±1.02	6.87±0.01
	Stem	3.28±0.16	5.44±0.20	9.26±1.13	10.35±0.25	10.79±0.34	11.12±0.23
	Leaf	4.86±1.04	5.86±0.24	9.62±1.30	12.52±0.22	12.85±1.10	12.93±1.08
Copper (50 µM)	Root	1.25±0.51	4.5±0.47	6.43±0.02	7.41±0.21	7.29±0.01	7.01±1.36
	Stem	3.54±1.10	7.53±0.35	11.05±0.26	13.27±0.32	11.44±0.14	11.03±0.11
	Leaf	5.65±0.02	8.61±0.25	12.33 ±0.16	13.58±1.30	12.72±1.04	12.94±0.14
Iron (70 µM)	Root	1.27±0.61	3.5±0.95	5.84±0.14	6.35±0.42	6.47±1.03	7.14±0.01
	Stem	3.52±0.22	5.63±0.05	10.43±0.28	11.60±0.40	11.87±0.12	11.90±0.55
	Leaf	6.03±1.05	6.92±0.43	10.45 ±0.50	12.94±1.32	13.21±1.46	13.29±0.10
Mercury (10 µM)	Root	1.46± 0.82	5.25±0.26	7.63±1.24	8.16±0.15	7.31±0.46	6.88±0.20
	Stem	4.85±0.20	9.24±0.14	13.65±0.32	16.48±0.10	15.26±1.23	15.13±0.04
	Leaf	6.19±1.03	10.51±0.13	15.07±1.20	19.45±1.34	16.36±1.37	15.03±1.22

Values given are mean of 5 replicates ±S. E

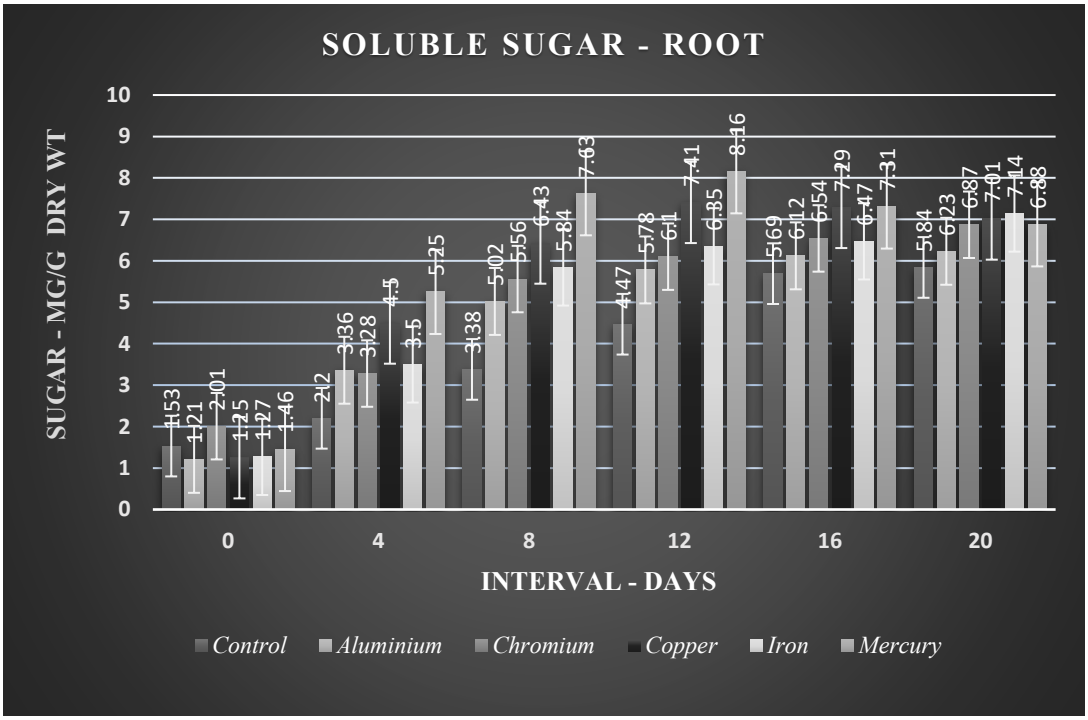


Fig. 36 Effect of aluminium, chromium, copper, iron and mercury on total sugar content (mg/g DW) in root of *Artemisia nilagirica* (Clarke) Pamp.

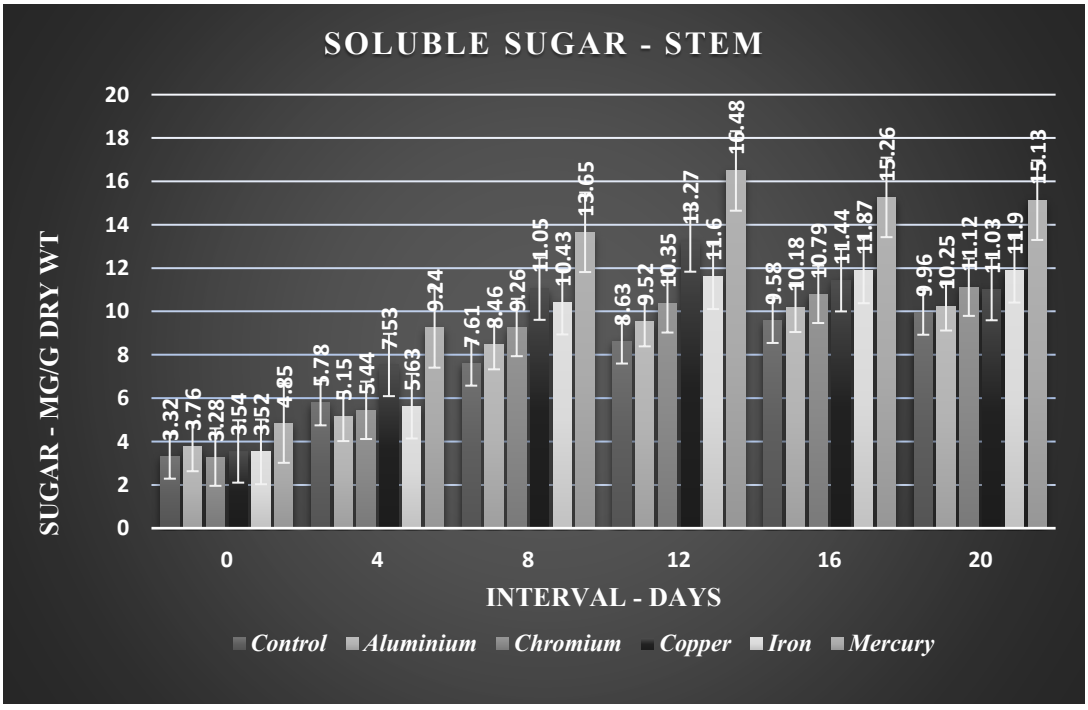


Fig. 37 Effect of aluminium, chromium, copper, iron and mercury on total sugar content (mg/g DW) in stem of *Artemisia nilagirica* (Clarke) Pamp.

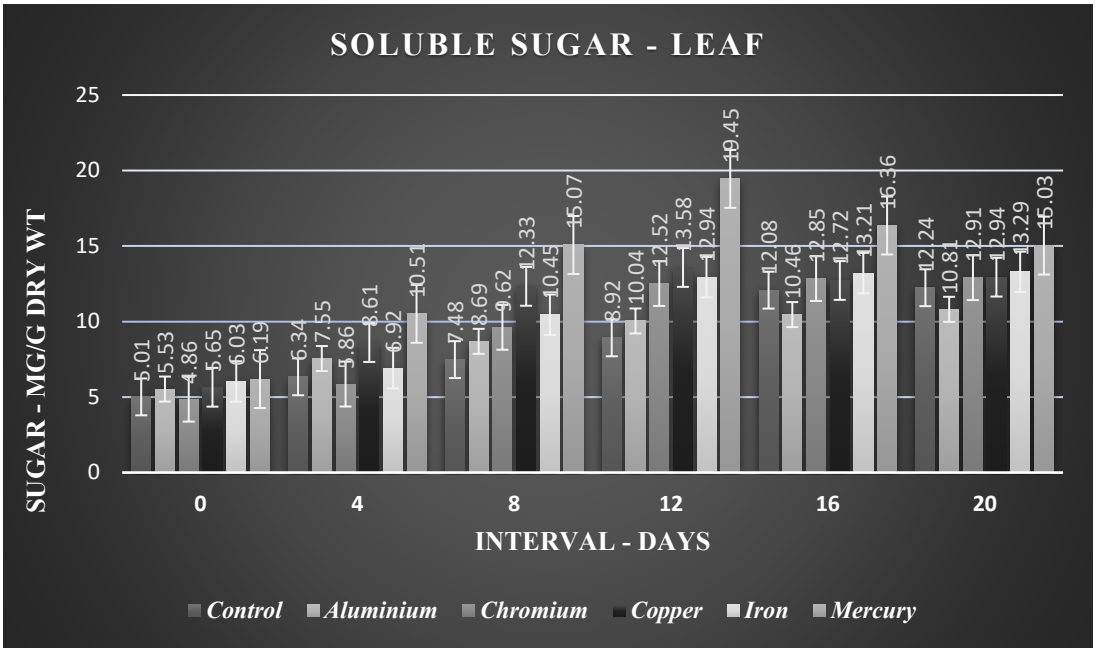


Fig. 38 Effect of aluminium, chromium, copper, iron and mercury on total sugar content (mg/g DW) in leaf of *Artemisia nilagirica* (Clarke) Pamp.

STARCH CONTENT

In comparison to the control, where starch accumulation showed a consistent increasing trend throughout the experimental period, the data in Table.18 & Fig. 39-41 show a considerable decrease in the amount of starch present in the roots, stems, and leaves of *Artemisia nilagirica* under heavy metal stress.

The starch concentration in roots steadily dropped from 2.55 ± 0.05 mg/g DW on day 0 to 2.25 ± 0.12 mg/g DW on day 20 under aluminium stress (80 µM), indicating either accelerated degradation or hindered starch synthesis. The trend for chromium (70 µM) stress was similar, with a minor recovery in the later days. Sharper decreases were caused by stress due to copper (50 µM), iron (70 µM) and mercury (10 µM). Mercury had the most extreme reduction, going from 2.84 ± 1.40 mg/g DW on day 0 to 2.04 ± 0.06 mg/g DW on day 20 (Fig. 39). This decrease is a result of stress-induced oxidative damage caused by heavy metals interfering with starch hydrolysis and glucose metabolism.

Under all treatments, the amount of starch in the stems decreased (Fig. 40). While Cr induced a greater fall, especially after day 8, with the ultimate concentration at 4.36 ± 0.04 mg/g DW, Al decreased starch from 4.58 ± 0.43 mg/g DW on day 0 to 4.28 ± 0.05 mg/g DW on day 20. The most significant stresses were Cu and Hg, which decreased from 5.25 ± 0.18 mg/g DW to 3.78 ± 0.13 mg/g DW and from 4.62 ± 0.58 mg/g DW to 3.63 ± 0.15 mg/g DW, respectively. Enzyme inhibition, which prevents both production and storage, may be the cause of toxicity effects of Hg on starch metabolism.

The starch content of leaves under substantial metal stress was also reduced (Fig. 41). Starch was considerably decreased by Al and Cr, with final concentrations of 6.28 ± 1.42 mg/g DW and 5.84 ± 0.09 mg/g DW, respectively. The most notable decrease in leaf starch was induced by Hg stress, which went from 6.61 ± 0.24 mg/g DW on day 0 to 4.63 ± 0.52 mg/g DW on day 20. The reductions were more noticeable in Cu and Fe. Increased respiratory demands and the mobilization of starch reserves during stressors may be the cause of the decrease of starch content on leaves.

The interruption of regular metabolic processes under heavy metal stress is reflected in the decrease of starch in all tissues. Under stress, these metals increase the breakdown of starch for energy, hinder photosynthesis, and decrease the enzyme activity necessary for starch production. Due to its high toxicity, mercury had the strongest inhibitory effect on starch content, highlighting its negative effects on the metabolic health of the plant. According to this pattern, heavy metals seriously compromise the physiological stability of plants by interfering with the processes that allocate and store carbohydrates.

Table 18. Effect of aluminium, chromium, copper, iron and mercury on total starch content (mg/g DW) of root, stem and leaf in *Artemisia nilagirica* (Clarke) Pamp.

Treatments	**Interval-Days**						
	Tissues	0	4	8	12	16	**20**
Control	Root	2.32±0.41	3.68±0.12	3.96±0.03	4.20±0.32	4.32±0.43	4.56±0.55
	Stem	4.21±0.13	5.46±0.56	5.85±0.14	6.46±1.32	7.01±0.24	7.43±0.02
	Leaf	5.46±0.25	6.78±0.15	7.03±1.24	7.25±0.16	8.43±1.25	8.82±0.42
Aluminium (80 µM)	Root	2.55±0.05	2.34±0.13	2.31±0.10	2.28±0.35	2.16±1.20	2.25±0.12
	Stem	4.58±0.43	4.42±0.10	4.28±0.17	4.18±0.14	4.21±1.23	4.28±0.05
	Leaf	5.86±0.10	5.74±0.23	5.62±0.04	5.51±0.32	5.76±1.05	6.28±1.42
Chromium (70 µM)	Root	3.04±0.21	3.01±0.24	2.86±0.16	2.62±0.53	2.81±0.11	2.93±0.08
	Stem	4.63±0.51	4.54±0.03	4.40±0.36	4.32±0.12	4.15±0.52	4.36±0.04
	Leaf	5.96±1.02	5.85±1.07	5.63±0.04	5.56±0.38	5.37±1.02	5.84±0.09
Copper (50 µM)	Root	2.82±0.34	2.67±0.42	2.53±0.10	2.37±1.31	2.23±0.45	2.15±1.06
	Stem	5.25±0.18	5.08±1.14	4.36±0.26	4.20±0.14	3.96±0.10	3.78±0.13
	Leaf	5.87±0.23	5.59±0.53	5.34±1.22	5.16±0.05	5.04±1.14	4.78±0.15
Iron (70 µM)	Root	3.41±0.35	3.22±0.15	3.06±0.11	3.01±0.01	2.63±0.24	2.96±0.14
	Stem	4.65±1.26	4.52±0.20	4.23±0.14	4.01±0.45	3.98±0.16	4.05±0.28
	Leaf	6.36±0.15	6.08±0.17	5.96±1.52	5.67±0.20	5.52±1.43	5.75±0.25
Mercury (10 µM)	Root	2.84± 1.40	2.58±0.16	2.32±0.23	2.21±0.35	2.14±0.31	2.04±0.06
	Stem	4.62±0.58	4.37±0.43	4.20±0.08	3.84±1.02	3.74±0.25	3.63±0.15
	Leaf	6.61±0.24	5.53±1.03	5.31±1.31	5.12±0.72	4.93±1.34	4.63±0.52

Values given are mean of 5 replicates ±S. E

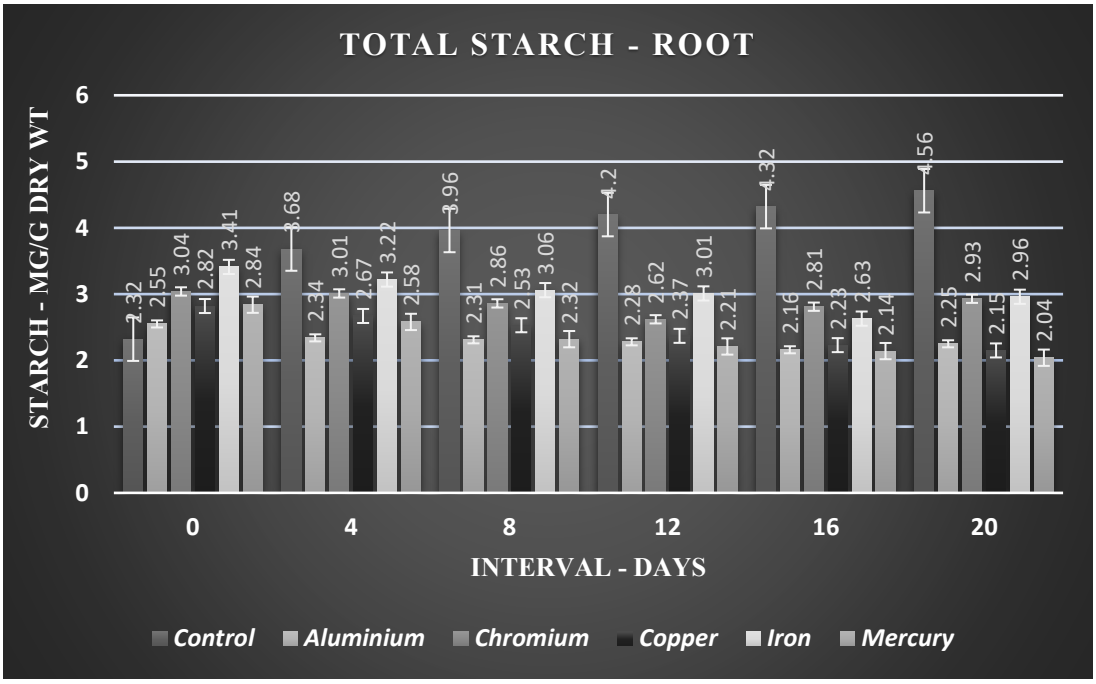


Fig. 39 Effect of aluminium, chromium, copper, iron and mercury on total starch content (mg/g DW) in root of *Artemisia nilagirica* (Clarke) Pamp.

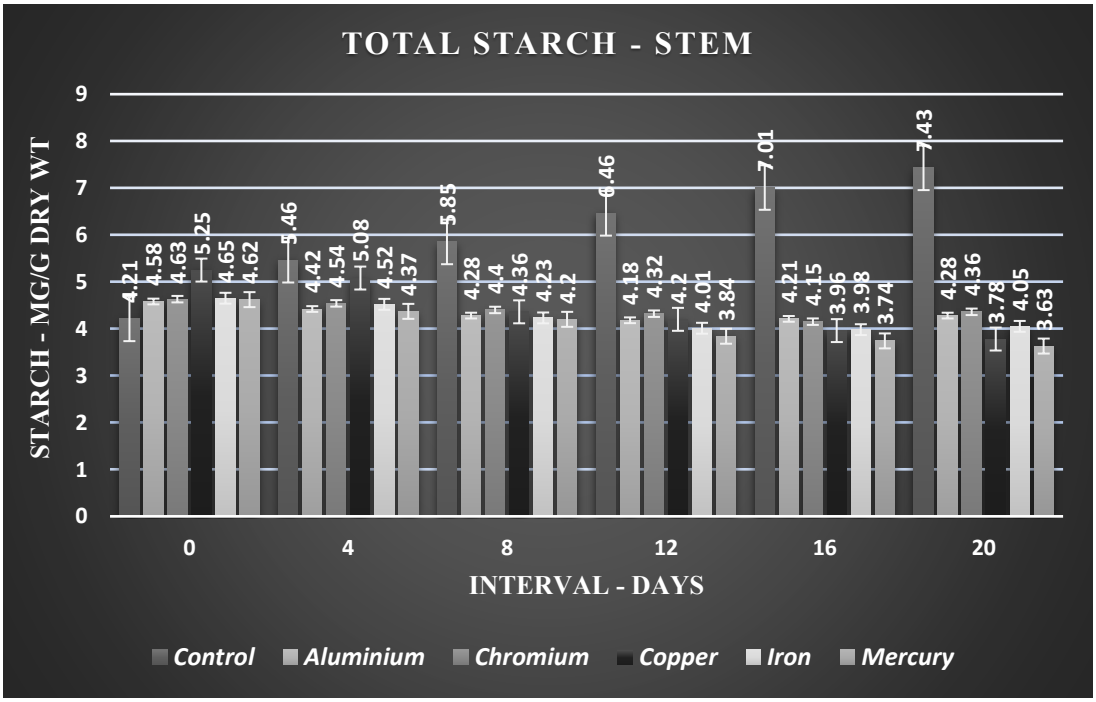


Fig. 40 Effect of aluminium, chromium, copper, iron and mercury on total starch content (mg/g DW) in stem of *Artemisia nilagirica* (Clarke) Pamp.

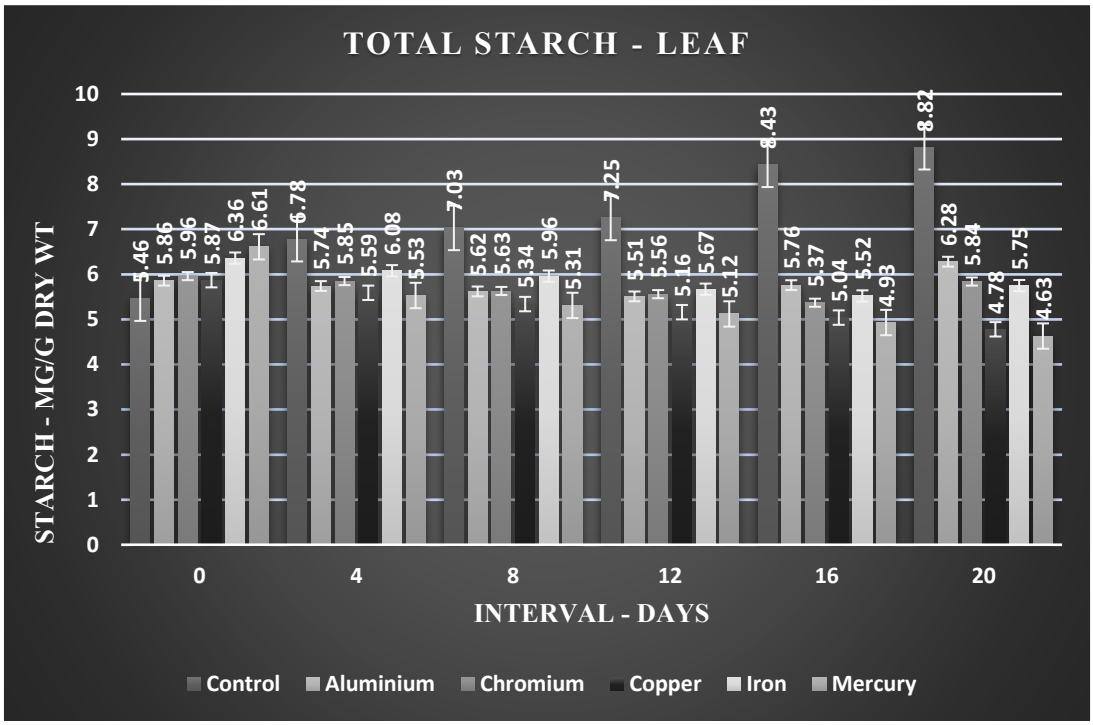


Fig. 41 Effect of aluminium, chromium, copper, iron and mercury on total starch content (mg/g DW) in leaf of *Artemisia nilagirica* (Clarke) Pamp.

TOTAL PROTEIN

Table. 19 presents the effects of heavy metals (Al, Cr, Cu, Fe and Hg) on the overall protein content of roots, stems, and leaves of *Artemisia nilagirica.* Different physiological reactions to heavy metal-induced stress were reflected in the protein levels, which differed across tissues and treatment periods.

Across all treatments, the protein content of roots first elevated and peaked at various times before falling (Fig. 42). On 8th day, the maximum protein concentration was generated by mercury treatment (23.62 ± 0.15 mg/g DW), indicating a strong early stress response. On the other hand, protein levels dramatically decreased by day 20 (15.26 ± 0.45 mg/g DW), suggesting oxidative stress and protein breakdown. While copper and iron had intermediate impacts, Al and Cr showed a similar trend but smaller overall reductions.

The most notable protein increases in stems were triggered by Hg exposure, which peaked on day 8 at 25.34 ± 1.21 mg/g DW (Fig. 43). This pattern emphasizes how stress-related proteins, such heat-shock proteins, are induced. Protein levels steadily decreased after the first spike, but by day 20, they remained greater than in other treatments. Comparable effects with lower values were caused by Cu exposure (11.87 ± 1.30 mg/g DW on day 20). After day 8, protein concentration significantly decreased as a result of Al and Cr treatments, indicating tissue injury and decreased metabolic activity.

Across all treatments, leaves had the highest protein concentration, indicating their crucial function in metabolism and photosynthesis. Protein levels peaked at 29.74 ± 0.14 mg/g DW on day 8 after mercury treatment, and then declined to 22.45 ± 0.01 mg/g DW on day 20. While Al and Cr generated large drops, Cu and Fe caused similar early rises followed by moderate reductions, resulting in final protein concentrations of 13.52 ± 0.11 and 12.36 ± 0.12 mg/g DW, respectively (Fig. 44).

Protein content was impacted by heavy metals through processes like metal chelation, which alters protein structures, and oxidative stress, which breaks down proteins and prevents synthesis. Although initially high protein levels indicate the initiation of stress-responsive pathways, extended exposure resulted in structural damage and metabolic inhibition. The most vulnerable tissue was found to be the roots, although the leaves first exhibited a robust stress response before succumbing to oxidative damage. Whereas Al and Cr had less severe but still noticeable impacts, Hg had the most noticeable effect, causing notable early protein increases. These results highlight how intricately heavy metal stress and protein metabolism interact in *A. nilagirica.*

Table 19. Effect of aluminium, chromium, copper, iron and mercury on total protein content (mg/g DW) of root, stem and leaf in *Artemisia nilagirica* (Clarke) Pamp.

Treatments	Tissues	Interval - Days					
		0	4	8	12	16	20
Control	Root	5.94±0.08	6.85±0.03	9.67±1.01	10.55±1.12	12.01±0.22	12.20±0.01
	Stem	7.24±0.14	7.91±0.45	11.62±0.73	13.42±1.06	16.53±0.01	16.95±0.12
	Leaf	8.04±0.23	8.56±0.34	13.82±1.25	17.86±1.34	22.31±0.13	24.8±0.05
Aluminium (80 µM)	Root	5.56±0.26	8.76±0.15	11.84±0.85	9.32±0.01	8.64±0.58	7.83±0.51
	Stem	8.09±1.45	10.78±0.20	14.35±0.26	10.39±0.07	7.43±0.13	7.28±0.52
	Leaf	8.94±0.10	13.76±0.32	21.65±0.14	16.83±0.07	12.53±1.04	12.36±0.12
Chromium (70 µM)	Root	6.07±0.64	9.51±0.30	12.68±0.22	8.94±0.15	7.36±0.41	7.32±0.59
	Stem	8.02±0.13	12.40±1.16	16.33±0.02	11.76±0.27	10.51±1.21	9.03±0.14
	Leaf	9.37±0.64	14.65±0.24	23.58±0.31	23.96±0.27	19.33±0.08	13.52±0.11
Copper (50 µM)	Root	5.98±0.23	10.94±0.18	16.47±0.62	12.06±0.24	9.24±1.02	8.51±0.05
	Stem	7.76±0.26	14.45±0.01	20.48±0.46	14.23±0.61	12.83±0.73	11.87±1.30
	Leaf	8.24±0.51	16.64±0.12	26.72±0.03	24.94±0.60	21.26±0.15	19.03±0.52
Iron (70 µM)	Root	5.94±0.24	10.34±0.25	14.53±0.03	11.16±0.2	8.21±0.14	7.86±0. 54
	Stem	7.86±1.06	13.62±0.01	24.27±0.02	12.18±0.35	11.43±0.02	11.02±0.56
	Leaf	9.13±0.01	14.98±0.56	25.11±0.42	23.04±0.02	20.38±0.33	18.20±0.43
Mercury (10 µM)	Root	6.04±0.13	12.86±0.03	23.62±0.15	21.1±0.62	15.74±0.49	15.26±0.45
	Stem	8.10±0.35	20.05±0.31	25.34±1.21	23.35±0.05	21.53±0.31	20.82±1.02
	Leaf	8.98±0.16	22.69±0.55	29.74±0.14	27.42±0.06	23.92±0.12	22.45±0.01

Values given are mean of 5 replicates ±S. E

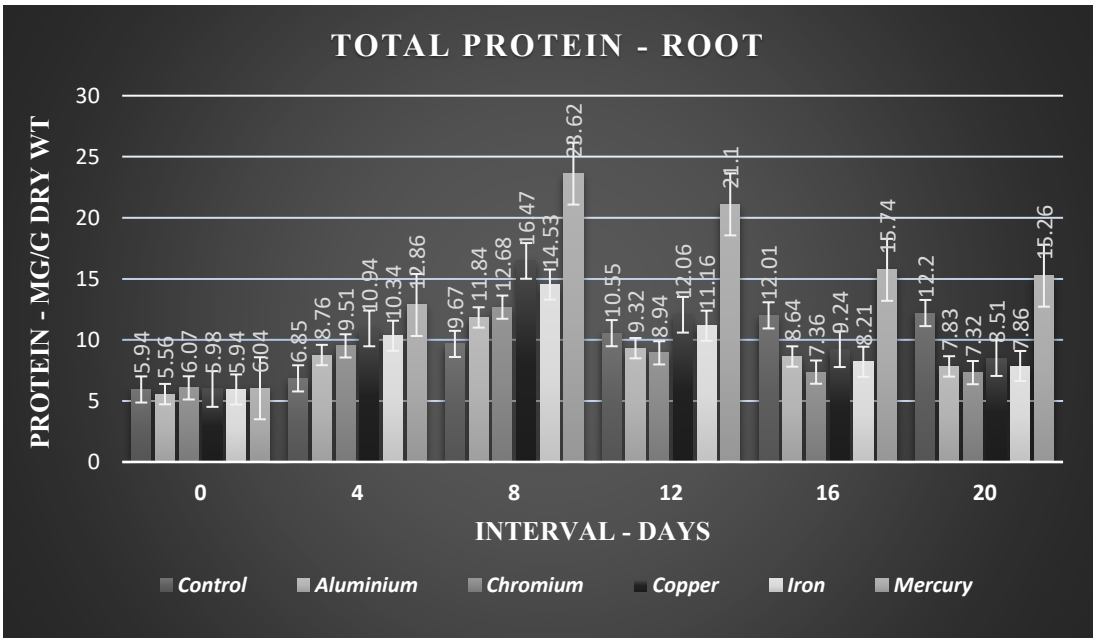


Fig. 42 Effect of aluminium, chromium, copper, iron and mercury on total protein content (mg/g DW) in root of *Artemisia nilagirica* (Clarke) Pamp.

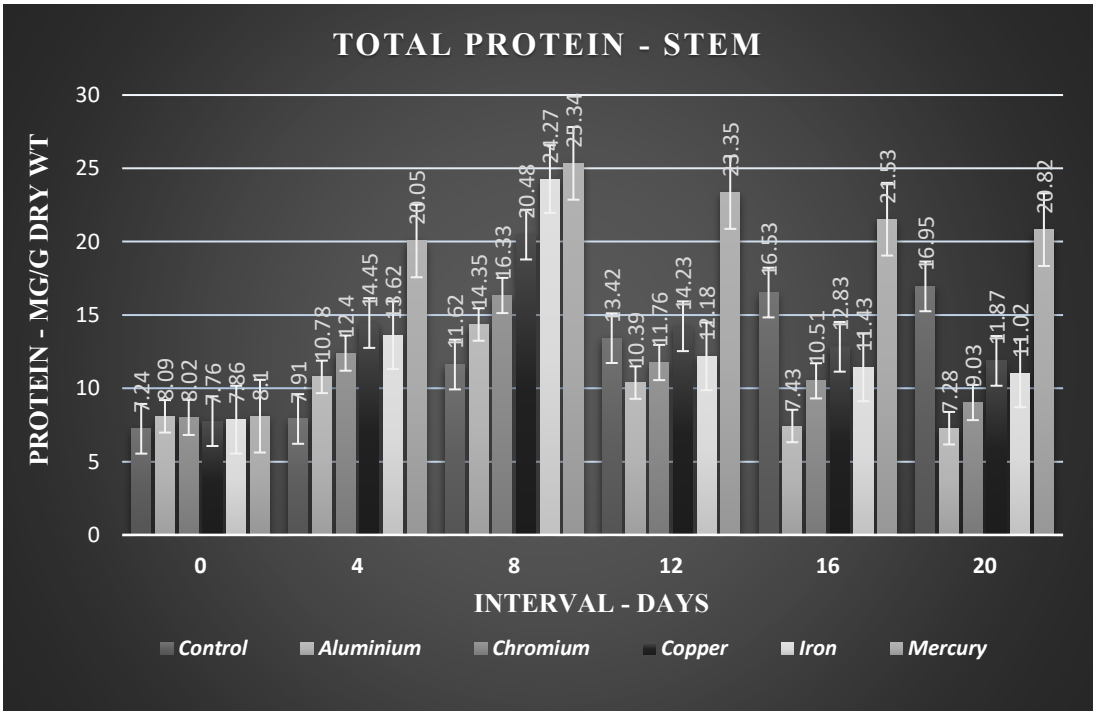


Fig. 43 Effect of aluminium, chromium, copper, iron and mercury on total protein content (mg/g DW) in stem of *Artemisia nilagirica* (Clarke) Pamp.

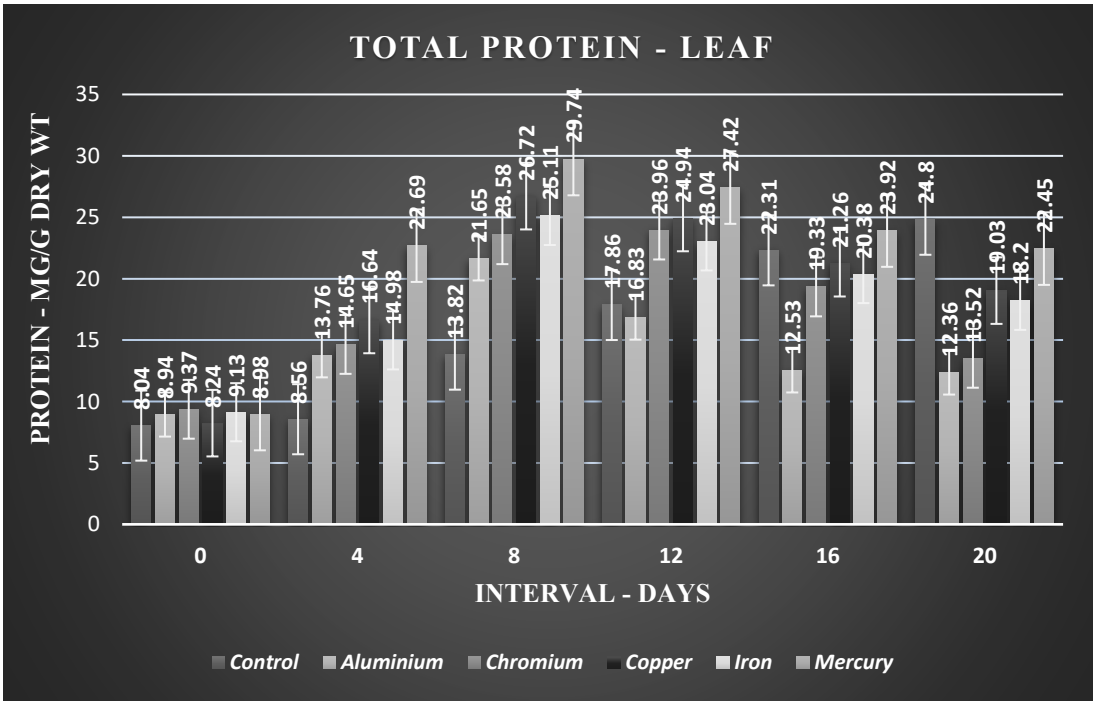


Fig. 44 Effect of aluminium, chromium, copper, iron and mercury on total protein content (mg/g DW) in leaf of *Artemisia nilagirica* (Clarke) Pamp.

PROLINE

Aluminium, chromium, copper, iron and mercury all have an impact on the amount of proline in *Artemisia nilagirica* roots, stems, and leaves, as shown in Table 20. A well-known Osmo protectant, proline, accumulates in plants in response to abiotic stressors, indicating the adaptive responses of plant to heavy metal toxicity.

Over the course of the experiment, the proline level in the roots of control plants remains constant and low at approximately 0.28 mg/g DW (Fig. 45). On the other hand, proline content rises significantly in response to heavy metal exposure. The strongest effect is seen with mercury (10 μM), with proline level peaking at 1.680 ± 0.12 mg/g DW on day 20. Likewise, proline levels are markedly increased by copper (50 μM) and chromium (70 μM), reaching 1.264 ± 1.43 and 0.964 ± 0.36 mg/g DW, respectively, at the conclusion of treatment. Treatments with iron and aluminium result in modest increases, with final values of 0.993 ± 0.25 mg/g DW and 0.816 ± 0.07 mg/g DW, respectively. These findings imply that increased levels of oxidative and osmotic stress caused by copper and mercury cause roots to produce more proline as a protective mechanism.

Throughout the trial, stems show comparable patterns, with low proline levels in the control group (Fig. 46). Treatment with heavy metals causes a significant increase, with mercury accumulating the most (1.869 ± 0.41 mg/g DW on day 20). Significant proline synthesis is also triggered by copper, reaching 1.563 ± 0.34 mg/g DW by day 20. Chromium follows (1.243 ± 0.32 mg/g DW). Over the course of the trial, proline levels were 0.760 ± 0.065 for aluminium and 0.923 ± 1.30 mg/g DW for iron, indicating modest impacts. The increased proline buildup is indicative of the function of stem in preserving osmotic equilibrium and reducing oxidative damage brought on by heavy metal stress.

Among all tissues, leaves accumulate the most proline, particularly when exposed to mercury stress, reaching an extraordinary peak of 3.518 ± 0.13 mg/g DW on day 20. Significant increases are likewise brought about by copper and chromium treatments; final proline levels were 2.104 ± 0.62 and 1.874 ± 0.60 mg/g DW, respectively. Proline buildup from iron and aluminium treatments is mild and peaks

at 1.932 ± 1.06 mg/g DW and 0.962 ± 1.03 mg/g DW, respectively. Because of their active metabolic and photosynthetic functions, which necessitate increased defence against oxidative damage and ionic imbalance under heavy metal stress, leaves have higher proline levels (Fig. 47).

Mercury causes the greatest proline accumulation, followed by copper and chromium, according to the trend observed in all tissues, but iron and aluminium have rather mild impacts. By stabilizing proteins, scavenging reactive oxygen species, and preserving cellular turgor, functions as an osmolyte and antioxidant are essential in reducing the stress caused by heavy metals.

Proline content in *Artemisia nilagirica* is markedly increased by heavy metal exposure, with tissue-specific variations indicating varying capacity for stress tolerance. The significance of proline as a biomarker for evaluating plant reactions to heavy metal toxicity is highlighted by these results.

Table 20. Effect of aluminium, chromium, copper, iron and mercury on total proline content (mg/g DW) of root, stem and leaf in *Artemisia nilagirica* (Clarke) Pamp.

Treatments	Tissues	Interval - Days					
		0	4	8	12	16	20
Control	Root	0.281±1.10	0.282±0.15	0.280±0.23	0.281±0.03	0.280±0.14	0.282±0.52
	Stem	0.203±0.15	0.203±0.16	0.206±0.15	0.202±0.20	0.210±0.14	0.206±0.20
	Leaf	0.376±0.24	0.377±0.16	0.379±0.42	0.373±0.23	0.373±0.05	0.375±1.21
Aluminium (80 µM)	Root	0.269±1.22	0.365±0.23	0.427±1.26	0.604±1.20	0.726±0.30	0.816±0.07
	Stem	0.198±0.12	0.242±0.15	0.282±1.60	0.358±0.24	0.639±0.52	0.76±0.065
	Leaf	0.369±0.43	0.568±0.44	0.637±0.12	0.738±0.05	0.794±0.26	0.962±1.03
Chromium (70 µM)	Root	0.265±0.45	0.385±0.27	0.532±0.43	0.652±1.01	0.738±0.43	0.964±0.36
	Stem	0.203±1.25	0.389±0.54	0.534±0.28	0.642±0.51	0.837±0.91	1.243±0.32
	Leaf	0.401±0.13	0.684±0.18	0.795±1.40	1.268±0.65	1.532±1.14	1.874±0.60
Copper (50 µM)	Root	0.283±0.18	0.568±1.62	0.781±0.31	0.894±0.04	0.965±1.24	1.264±1.43
	Stem	0.242±0.042	0.486±1.52	0.704±0.32	0.787±1.04	0.936±0.92	1.563±0.34
	Leaf	0.318±0.05	0.765±1.12	0.885±0.43	1.563±0.81	1.8621±0.42	2.104±0.62
Iron (70 µM)	Root	0.243±0.16	0.446±1.41	0.621±0.34	0.654±0.82	0.802±1.60	0.993±0.25
	Stem	0.240±1.02	0.427±0.63	0.652±0.80	0.702±0.46	0.865±0.41	0.923±1.30
	Leaf	0.348±0.18	0.689±0.25	0.845±0.33	1.447±1.64	1.650±0.83	1.932±1.06
Mercury (10 µM)	Root	0.260±0.06	0.640±0.53	0.863±0.65	0.947±1.61	1.364±1.14	1.680±0.12
	Stem	0.224±0.26	0.537±0.28	0.874±1.02	0.904±0.10	0.953±0.43	1.869±0.41
	Leaf	0.423±1.03	0.982±0.46	1.68±0.42	1.829±1.35	2.644±0.74	3.518±0.13

Values given are mean of 5 replicates ± S.E

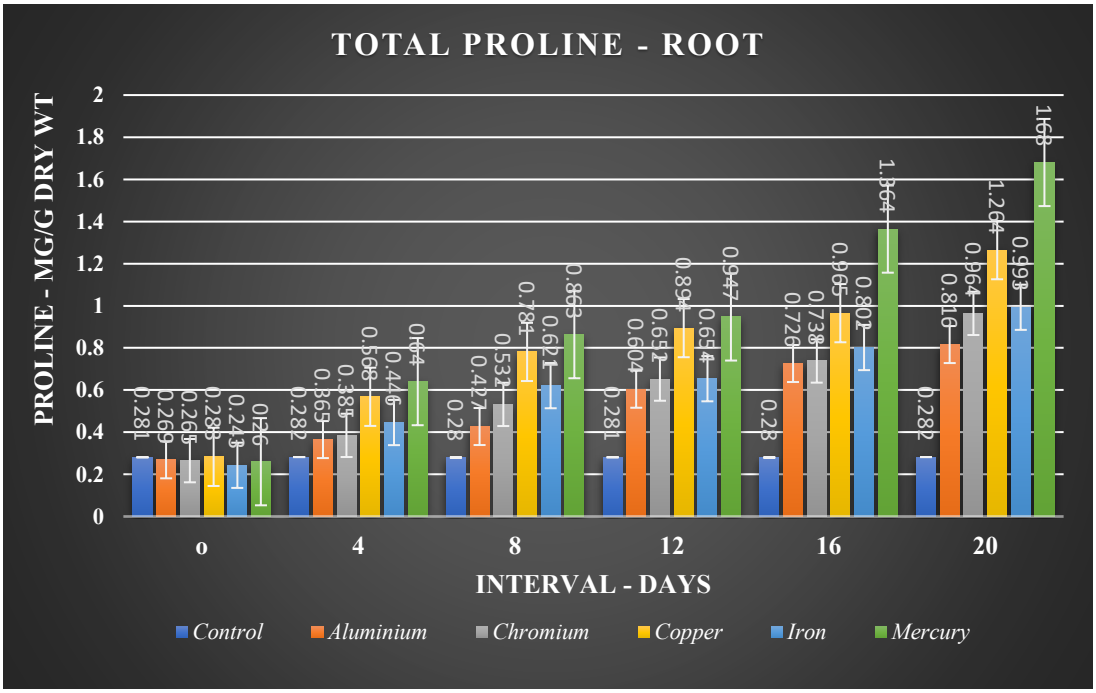


Fig. 45 Effect of aluminium, chromium, copper, iron and mercury on total proline content (mg/g DW) in root of *Artemisia nilagirica* (Clarke) Pamp.

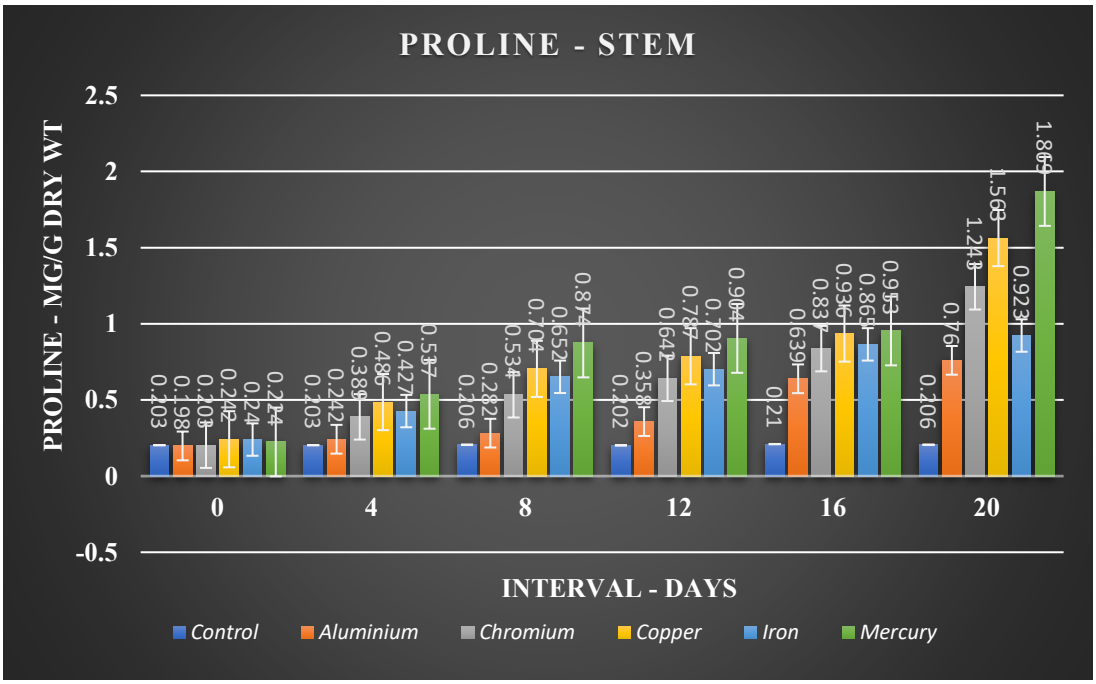


Fig. 46 Effect of aluminium, chromium, copper, iron and mercury on total proline content (mg/g DW) in stem of *Artemisia nilagirica* (Clarke) Pamp.

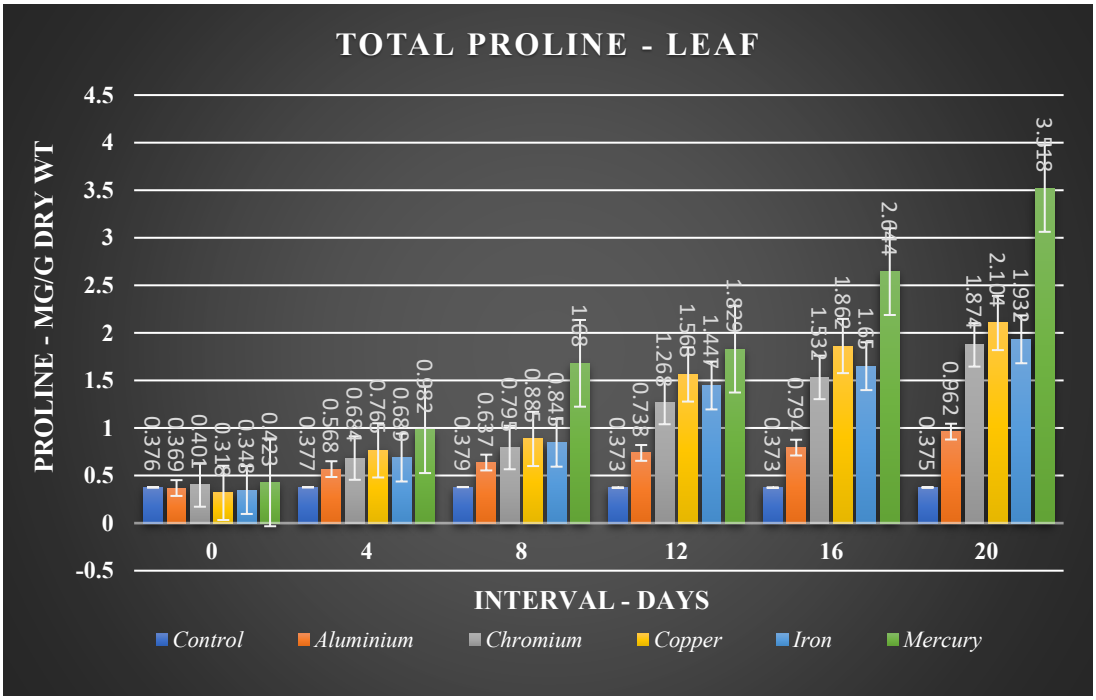


Fig. 47 Effect of aluminium, chromium, copper, iron and mercury on total proline content (mg/g DW) in leaf of *Artemisia nilagirica* (Clarke) Pamp.

PHENOLICS

Table. 21 shows how the total phenolic concentration of *Artemisia nilagirica* roots, stems, and leaves is affected by the heavy metals Al, Cr, Cu, Fe and Hg. Phenolics are essential in the fight against heavy metal-induced oxidative damage. Phenolics content rose dramatically with exposure duration and metal concentration across all treatments, suggesting that the plant was adapting to lessen oxidative damage.

In the roots of *A. nilagirica*, phenolic content significantly increased when exposed to heavy metals, indicating the systemic antioxidative defence by plant. Phenolic buildup in roots was greatest when mercury poisoning was present, reaching a peak of 29.59 mg/g dry weight by day 20 (Fig. 48). A strong phenolic reaction was also brought on by copper exposure, whereas minor increases were seen with chromium and iron. The function of root as the main site of heavy metal uptake and its subsequent defence against oxidative stress was highlighted by the noticeable increase in phenolic levels caused by aluminium toxicity, albeit less aggressive.

Under heavy metal treatments, the stems showed intermediate levels of phenolic content, which is in line with their role in metal translocation. The maximum phenolic synthesis was observed in stems after exposure to mercury, with levels rising to 27.56 mg/g by day 20 (Fig. 49). Due to their capacity to produce oxidative stress, copper and chromium also resulted in notable increases. Exposure to aluminium had the least noticeable effect, but iron poisoning produced phenolics in a significant amount. These findings demonstrate how stems contribute to the systemic circulation of phenolics as a defence mechanism for the plant. Throughout all heavy metal treatments, leaves continuously had the highest phenolic content, which is indicative of their direct exposure to oxidative stress and crucial function in photosynthesis.

The leaves that were exposed to mercury showed the greatest rise in phenolic content, reaching a peak of 37.48 mg/g dry weight on day 20 (Fig. 50). A notable phenolic response was also induced by copper poisoning, reaching 33.44 mg/g by day 20. Iron and chromium caused significant increases in phenolic content (27.46 mg/g and 28.06 mg/g, respectively), although aluminium caused very mild increases. The increased production of phenolic compounds in leaves highlights how important they are for reducing oxidative damage by means of antioxidative processes.

These results show that *A. nilagirica* responds to heavy metal stress in a tissue-specific manner, with phenolic content rising in roots, stems and leaves. Because of their functional significance in preventing oxidative stress, leaves had the greatest levels; roots, which serve as the main site of contact for heavy metals, showed significant increases; and stems, which correspond to their function in translocation, demonstrated intermediate levels. The adaptability and potential of plant for use in phytoremediation are demonstrated by this adaptive phenolic response.

Table 21. Effect of aluminium, chromium, copper, iron and mercury on total phenolics content (mg/g DW) of root, stem and leaf in *Artemisia nilagirica* (Clarke) Pamp.

Treatments	Tissues	Interval - Days					
		0	4	8	12	16	**20**
Control	Root	3.56±0.01	5.23±0.12	7.66±1.34	8.33±0.05	10.15±0.25	10.36±0.16
	Stem	2.23±1.05	3.52±1.052	5.15±0.04	5.98±0.03	7.24±0.12	7.86±1.21
	Leaf	6.59±0.12	7.65±0.14	9.88±0.22	11.4±0.02	13.02±1.26	14.05±0.41
Aluminium (80 µM)	Root	3.73±0.06	7.76±1.20	11.85±0.04	12.56±0.01	13.6±0.17	13.56±0.03
	Stem	2.35±0.11	8.45±0.24	11.25±1.01	13.65±0.14	14.28±0.18	15.26±0.06
	Leaf	6.51±1.14	9.89±0.11	13.58±1.26	15.56±1.03	19.87±0.04	21.73±0.42
Chromium (70 µM)	Root	4.03±0.33	8.24±0.41	12.06±0.22	15.22±1.24	16.79±1.42	16.92±0.10
	Stem	2.56±0.24	10.01±1.23	11.95±0.34	14.42±1.04	17.24±0.52	20.13±0.06
	Leaf	6.34±0.12	10.88±0.24	16.25±0.18	19.24±0.08	23.7±0.35	27.46±1.52
Copper (50 µM)	Root	3.85±0.26	12.46±0.50	15.78±1.02	17.85±0.46	20.25±0.01	21.13±0.15
	Stem	2.22±0.56	11.16±1.05	15.56±0.90	18.84±0.04	21.93±0.11	23.96±0.26
	Leaf	5.96±0.07	13.85±0.03	21.36±0.12	24.52±0.06	29.63±0.24	33.44±0.12
Iron (70 µM)	Root	4.12±0.02	10.61±0.41	13.34±1.66	15.46±0.07	17.86±0.08	18.02±0.17
	Stem	2.42±0.35	10.38±1.05	13.22±0.40	17.84±0.04	20.55±1.13	21.36±0.54
	Leaf	5.99±0.40	11.87±1.04	18.70±0.23	20.48±0.04	26.55±1.35	28.06±0.05
Mercury (10 µM)	Root	3.65±0.10	15.63±0.22	19.45±2.23	24.40±0.46	28.65±0.14	29.59±0.02
	Stem	2.63±1.40	12.48±0.5	18.35±0.03	22.6±1.28	26.68±0.04	27.56±1.62
	Leaf	6.72±0.31	14.38±2.04	25.9±0.08	29.88±1.10	34.56±0.61	37.48±0.02

Values given are mean of 5 replicates ±S. E

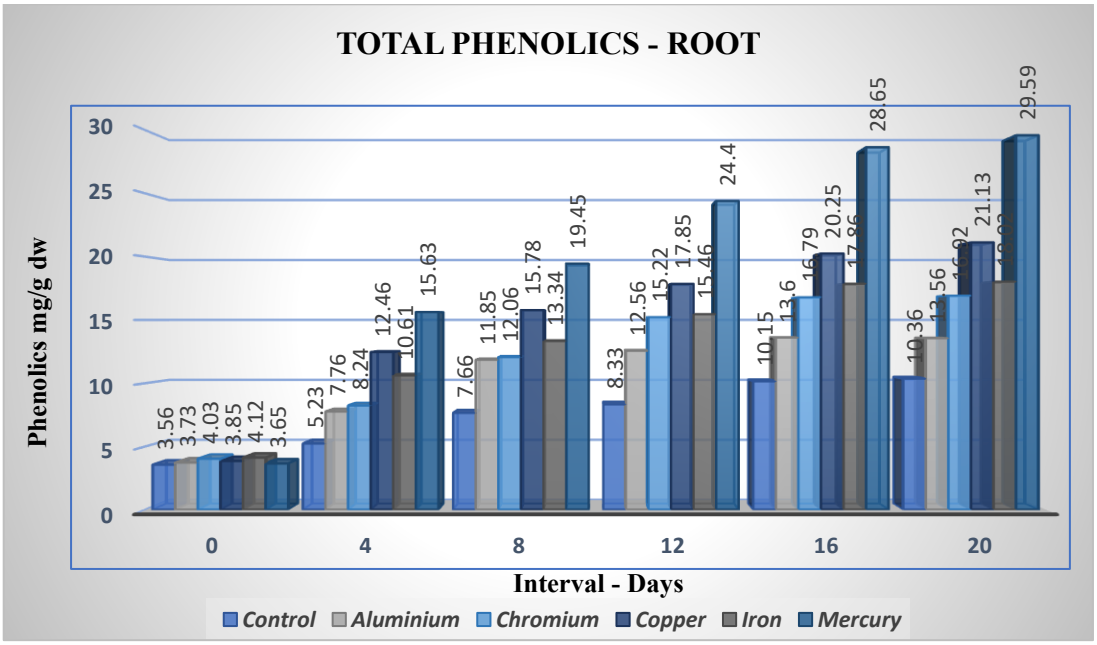


Fig. 48 Effect of aluminium, chromium, copper, iron and mercury on total phenolics content (mg/g DW) in root of *Artemisia nilagirica* (Clarke) Pamp.

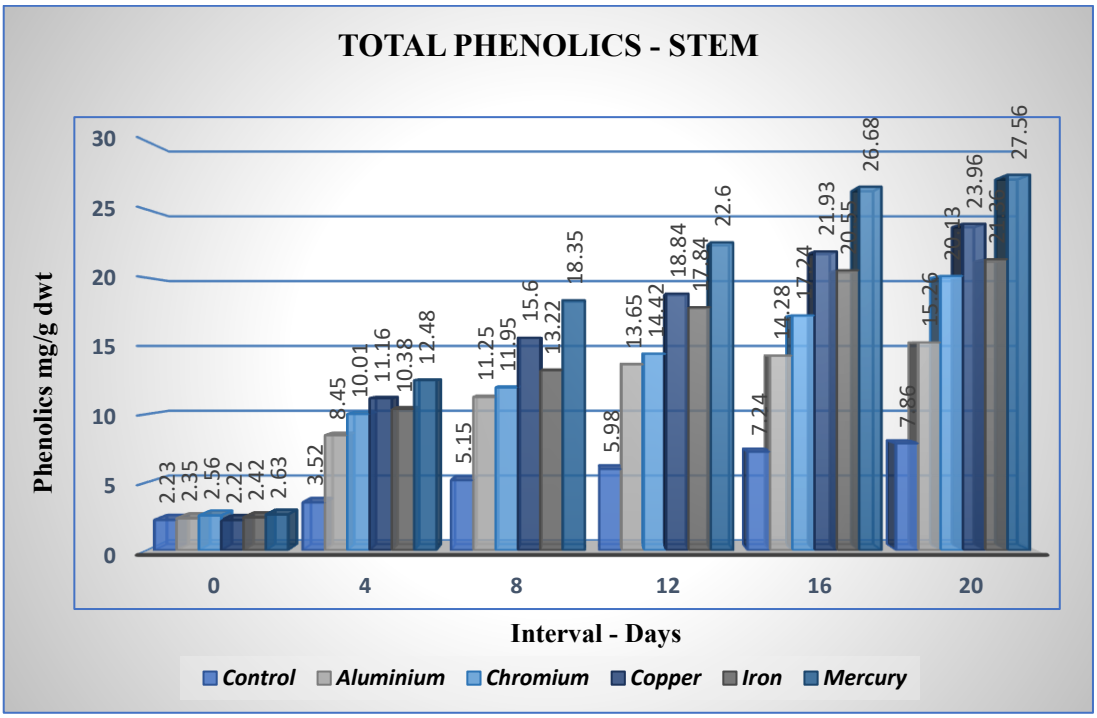


Fig. 49 Effect of aluminium, chromium, copper, iron and mercury on total phenolics content (mg/g DW) in stem of *Artemisia nilagirica* (Clarke) Pamp.

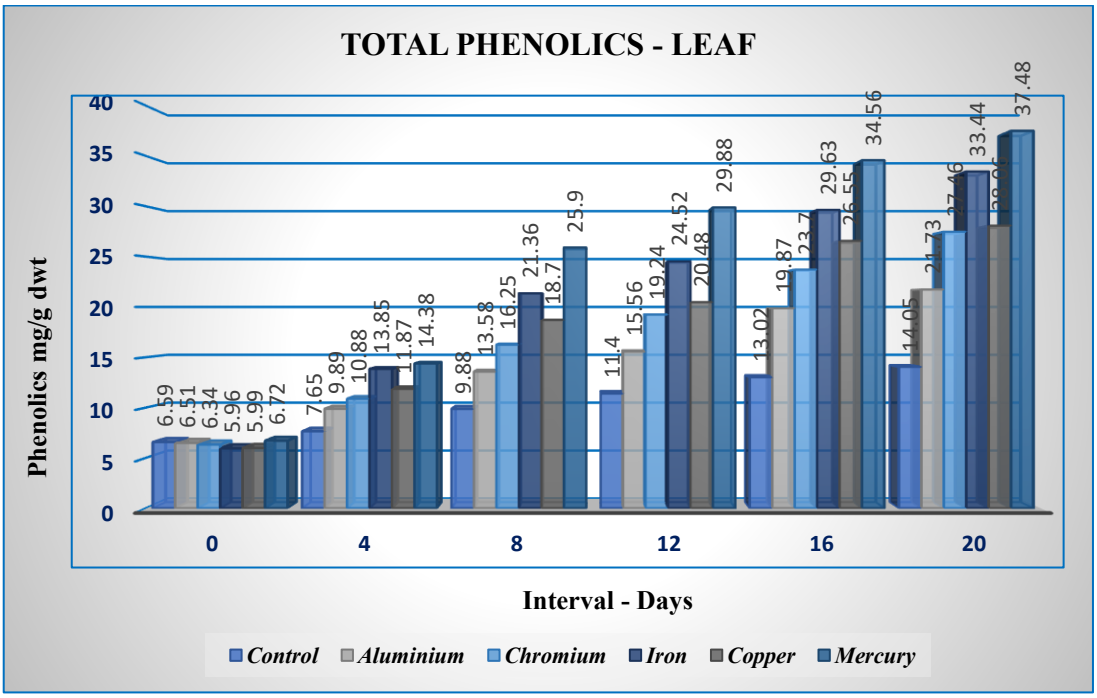


Fig. 50 Effect of aluminium, chromium, copper, iron and mercury on total phenolics content (mg/g DW) in leaf of *Artemisia nilagirica* (Clarke) Pamp.

MDA CONTENT

Table. 22 displays the effects of heavy metal toxicity on malondialdehyde (MDA) content in the tissues of its roots, stem, and leaves of *A. nilagirica* under different treatments. One important sign of oxidative stress in plant cells is MDA, a marker of lipid peroxidation. The findings showed that the MDA level varied significantly among tissues in response to stress from Al, Cr, Cu, Fe and Hg.

Under severe metal exposure, the amount of MDA in roots elevated drastically, indicating increased oxidative stress (Fig. 51). The highest MDA levels were generated by exposure to mercury (49.02 µg/g at 20 hours), followed by chromium (36.34 µg/g) and copper (46.65 µg/g). Moderate increases were caused by iron and aluminium, with maximum levels of 37.53 µg/g and 21.40 µg/g, respectively. These findings demonstrate how the root serves as the first point of interaction for lipid peroxidation and metal uptake.

Oxidative stress brought on by metal translocation in stem was shown by intermediate MDA levels (Fig. 52). Chromium (37.78 µg/g), copper (44.01 µg/g), and mercury (45.15 µg/g) caused the largest rise. Additionally, MDA levels were raised by iron and aluminium, which hit 40.64 µg/g and 35.56 µg/g, respectively. The oxidative damage linked to heavy metal transportation is seen in this. The highest MDA levels have been observed in leaves, which is in line with their metabolic activity and oxidative stress exposure (Fig. 53). Mercury was the most significant cause of lipid peroxidation, as evidenced by the peak MDA levels of 51.22 µg/g. Significant increases were also caused by chromium (40.55 µg/g) and copper (49.63 µg/g). Aluminium (39.57 µg/g) and iron (44.03 µg/g) produced somewhat smaller but still noticeable increases, highlighting how susceptible leaves are to oxidative damage.

The MDA concentration of *Artemisia nilagirica* was markedly elevated by heavy metal exposure in all tissues, with leaves exhibiting the highest values, followed by stems and roots. Diverse metabolic functions of these tissues and susceptibilities to oxidative stress are reflected in this trend. While chromium, iron and aluminium had gradually less of an impact, copper and mercury increased lipid peroxidation the

greatest. These findings highlight the oxidative load that heavy metals place on *A. nilagirica* and how it affects membrane stability and cellular integrity.

Table 22. Effect of aluminium, chromium, copper, iron and mercury on total MDA content (µg/g DW) of root, stem and leaf in *Artemisia nilagirica.* (Clarke) Pamp.

Treatments	Tissues	Interval - Days					
		0	4	8	12	16	20
Control	Root	5.82±0.04	6.56±1.01	6.89±0.05	7.58±0.13	8.77±1.30	9.48±0.22
	Stem	9.42±0.02	10.25±0.11	11.48±1.32	15.06±0.33	18.14±0.01	18.46±1.23
	Leaf	13.31±0.21	14.34±0.04	16.92±0.44	24.75±0.09	25.32±0.15	26.05±0.16
Aluminium (80 µM)	Root	5.89±0.13	8.45±0.02	12.03±0.41	13.96±0.06	19.57±0.03	21.40±0.05
	Stem	9.45±0.16	12.45±0.12	26.09±0.02	29.15±0.12	33.98±0.33	35.56±0.08
	Leaf	13.56±0.42	22.11±0.56	28.82±0.42	35.42±0.40	38.83±0.02	39.57±0.55
Chromium (70 µM)	Root	5.63±0.11	10.87±0.45	19.75±0.03	22.43±0.16	35.65±0.22	36.34±0.05
	Stem	9.56±0.14	19.44±0.25	34.52±0.05	39.02±0.16	35.65±0.22	37.78±0.42
	Leaf	14.04±0.33	25.24±0.54	29.03±0.11	38.36±0.15	40.28±1.03	40.55±0.10
Copper (50 µM)	Root	5.99±0.05	11.94±0.05	25.43±0.08	30.22±0.24	45.15±0.12	46.65±0.01
	Stem	9.55±0.09	23.36±0.17	33.45±1.04	40.21±0.03	43.94±0.56	44.01±0.22
	Leaf	13.85±0.19	30.65±0.05	35.21±1.24	42.45±0.25	48.63±0.25	49.63±0.16
Iron (70 µM)	Root	6.01±0.26	11.24±0.09	22.88±0.02	28.37±0.11	36.68±0.33	37.53±0.04
	Stem	10.01±0.44	20.64±0.18	30.04±0.52	39.81±0.02	40.54±1.14	40.64±2.20
	Leaf	14.23±0.32	27.23±0.16	32.56±1.23	40.21±1.02	43.62±1.08	44.03±0.14
Mercury (10 µM)	Root	5.70±0.12	12.08±0.22	26.14±0.06	33.15±0.91	46.44±0.50	49.02±0.01
	Stem	9.65±1.24	24.95±0.33	39.36±0.22	42.48±0.08	44.92±0.62	45.15±0.05
	Leaf	13.69±0.43	30.96±0.22	36.54±1.30	44.54±0.55	50.24±0.36	51.22±1.02

Values given are mean of 5 replicates ±S. E

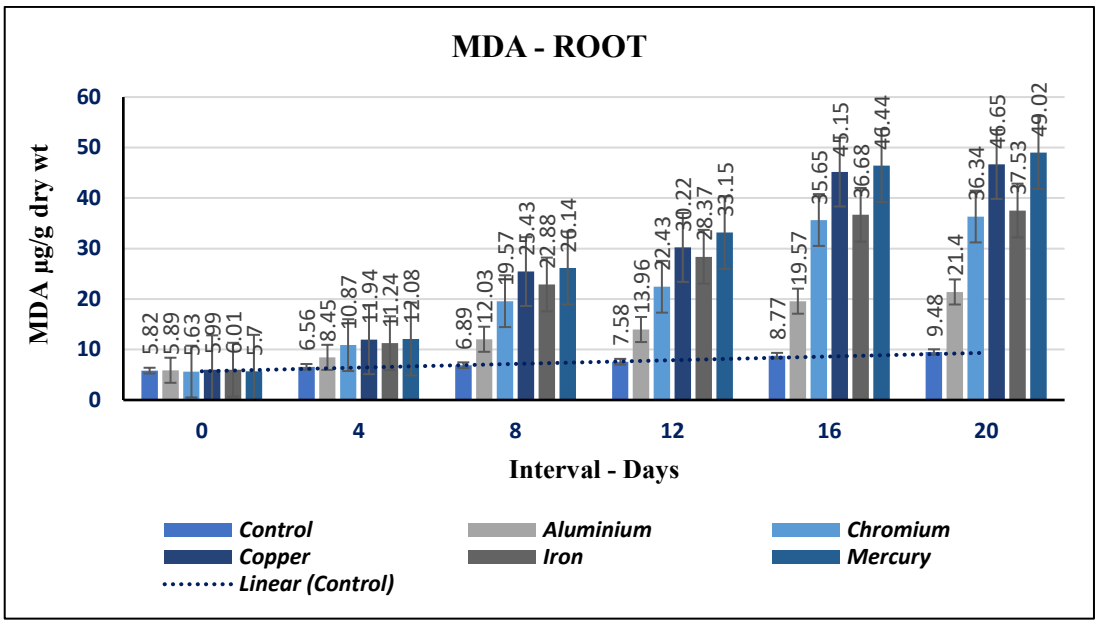


Fig. 51 Effect of aluminium, chromium, copper, iron and mercury on total MDA content (µg/g DW) in root of *Artemisia nilagirica* (Clarke) Pamp.

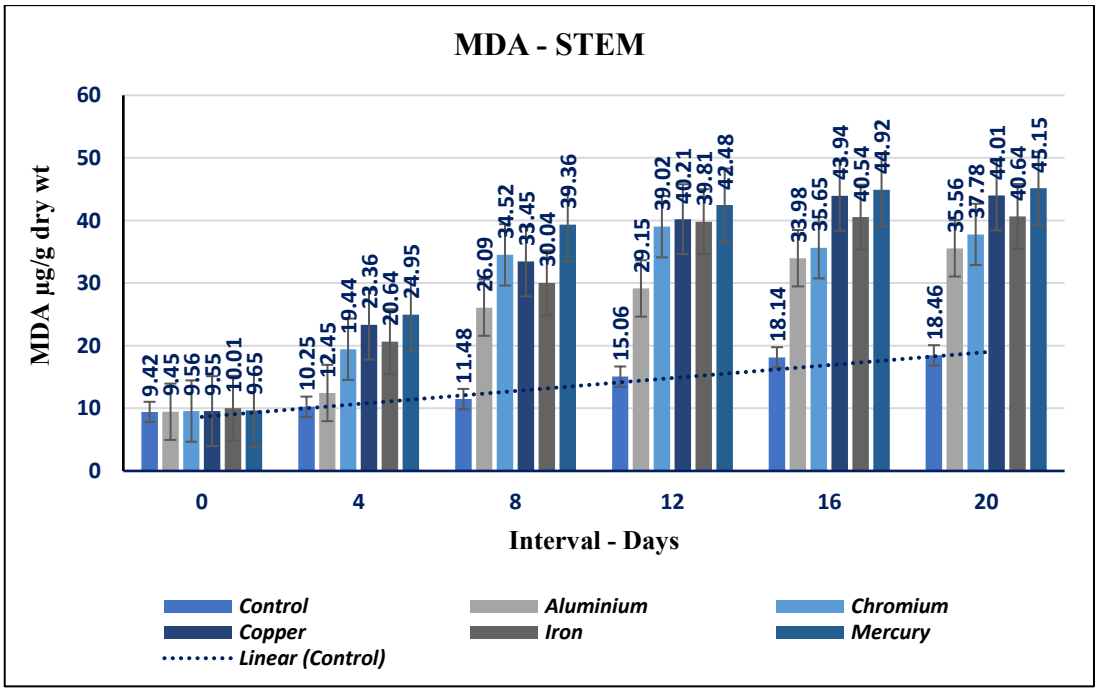


Fig. 52 Effect of aluminium, chromium, copper, iron and mercury on total MDA content (µg/g DW) in stem of *Artemisia nilagirica* (Clarke) Pamp.

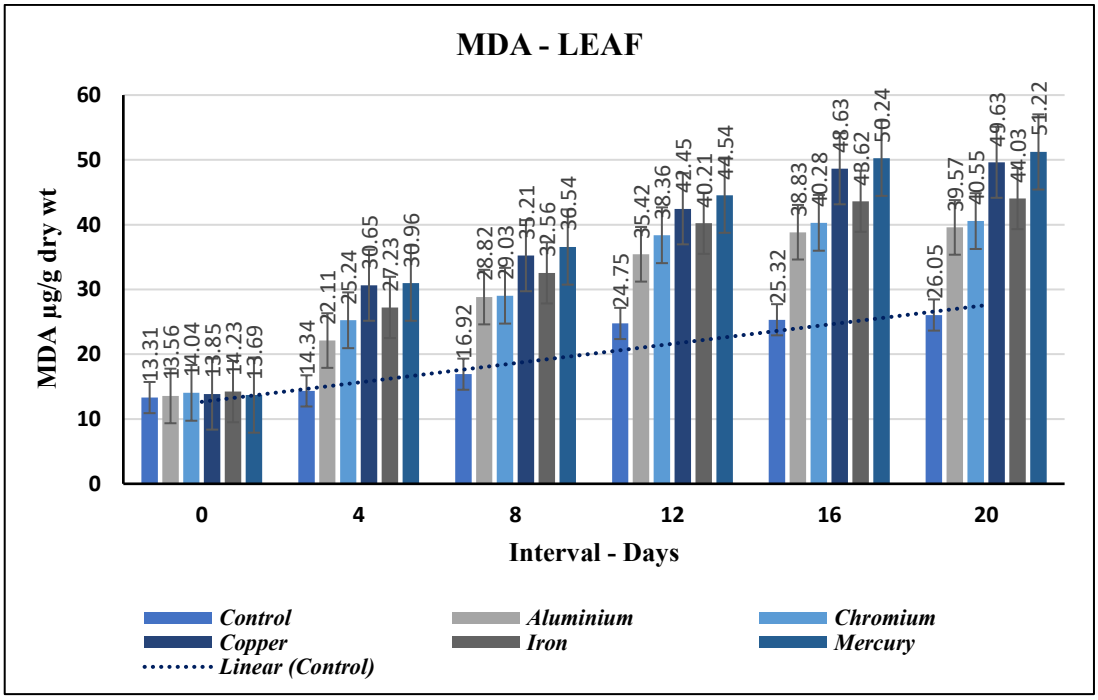


Fig. 53 Effect of aluminium, chromium, copper, iron and mercury on total MDA content (µg/g DW) in leaf of *Artemisia nilagirica* (Clarke) Pamp.

Table 23. Bioaccumulation patterns (µg/g dry weight) of aluminium, chromium, copper, iron and mercury on *Artemisia nilagirica* (Clarke) Pamp. grown in Hoagland solution containing standardized concentrations of heavy metals.

Treatments	Tissues	Bioaccumulation (ppm)		
		4th day	12th day	20th day
Aluminium (80 µM)	Root	0.452±0.30	0.864±0.12	1.204±0.02
	Stem	0.016±0.33	0.201±0.15	0.368±0.12
	Leaf	0.335±0.12	0.425±0.21	0.578±0.11
	Residue	1.355±0.11	0.668±0.20	0.008±0.02
Chromium (70 µM)	Root	0.384±0.03	1.562±0.03	2.241±0.01
	Stem	0.075±2.40	0.348±0.15	0.752±0.23
	Leaf	0.086±0.05	0.153±0.01	0.563±0.01
	Residue	3.094±0.24	1.576±0.05	0.117±0.30
Copper (50 µM)	Root	0.385±1.02	0.568±0.04	1.283±0.11
	Stem	0.094±0.15	0.325±0.13	0.812±0.05
	Leaf	0.241±0.01	0.651±0.15	1.040±0.23
	Residue	2.456±0.01	1.631±0.10	0.04±0.32
Iron (70 µM)	Root	0.868±0.14	1.480±0.11	2.002±0.02
	Stem	0.564±0.13	0.865±0.06	1.056±0.05
	Leaf	0.814±0.20	0.316±0.02	0.751±0.01
	Residue	1.663±0.15	1.248±0.40	0.10±0.23
Mercury (10 µM)	Root	0.064±0.15	0.242±0.33	0.860±0.30
	Stem	0.103±0.22	0.356±0.02	0.950±0.01
	Leaf	0.015±0.11	0.056±0.15	0.195±0.22
	Residue	1.824±0.02	1.302±0.42	0.0009±0.01

Values are mean of 5 replicates ±S.E

BIOCONCENTRATION FACTOR AND TRANSLOCATION FACTOR

Bioaccumulation was examined using two variables, namely BCF and TF. The response of *A. nilagirica* plants to the various metals exhibited notable differences in both values when comparing the Bioconcentration factor, or BCF (ratio of metal content of the roots to the medium), and the Translocation factor, or TF (ratio of metal concentration in the shoot to the root). Table. 24 presents the Bio-concentration Factor (BCF) and Translocation Factor (TF) values for *A. nilagirica* subjected to different metals at 4-day, 12-day, and 20-day intervals. These two factors give insights into how the plant accumulates metals internally (BCF) and translocate them from the roots to the above-ground parts (TF). Here is an analysis of the BCF and TF values for each metal.

Aluminium exhibits a moderate accumulation pattern in *A. nilagirica*. The BCF initially increases at 12 days, reaching its peak (1.29), but decreases at 20 days (0.557), suggesting that the plant initially absorbs and accumulates Al but does not retain its long-term. The TF values are consistently greater than 1, indicating some level of translocation of Al from the roots to the shoots, but the response of plant seems more limited over time, with a decline in translocation at the 20-day mark. The plant has a moderate response to Al, accumulating it to some extent but not in large quantities. Its translocation efficiency decreases over time, indicating a relatively weak or sensitive response.

Chromium shows a similar pattern to Cr, with an increase in BCF at the 12-day mark (0.99) before declining again at 20 days (0.615). The TF values are relatively stable, showing moderate translocation to the above-ground parts of the plant. However, like Al, Cr does not seem to be highly retained in the plant, suggesting that *A. nilagirica* may not be well-suited to accumulate this metal in large amounts. The plant shows moderate accumulation of Cr and while translocation occurs, the plant is not highly efficient at retaining or concentrating this metal, indicating it might be sensitive to Cr.

Copper stands out as a metal that is progressively accumulated by *A. nilagirica*. The BCF values steadily increase from 0.156 at 4 days to 0.404 at 20 days,

suggesting that the plant is more efficient at bioaccumulating Cu over time. The TF values also increase, indicating that Cu is not only being absorbed but also translocated to the above-ground parts efficiently. The rising trend in both BCF and TF over time highlights that Cu is a metal that the plant can accumulate and translocate with increasing efficiency. *A. nilagirica* is a hyperaccumulator of Cu, as it exhibits both significant bioaccumulation and effective translocation, with the TF remaining consistently above 1. This indicates that Cu may be hyperaccumulated by the plant, making it a candidate for further studies on phytoremediation or Cu bioaccumulation.

Iron shows TF greater than 1 at the 4-day and 12-day intervals, but the TF value drops below 1 at 20 days, indicating reduced translocation over time. The BCF values initially increase at 12 days, but then decrease at 20 days, suggesting that the plant accumulates Fe in the early stages but may not retain it as efficiently in the later stages. *A. nilagirica* shows a moderate response to iron, with bioaccumulation peaking around 12 days before it diminishes. It is sensitive to iron, indicating that while the plant initially accumulates iron, it is not well-suited for prolonged retention of this metal.

Mercury consistently has TF greater than 1 at all intervals, with the highest value at 4 days (2.60), indicating that the plant is effectively translocating Hg from the roots to the shoots. The BCF values steadily increase over time, from 0.035 at 4 days to 0.428 at 20 days, demonstrating that the plant progressively accumulates Hg. *A. nilagirica* is a hyperaccumulator of Hg, as it accumulates and translocate Hg efficiently, with high TF values and increasing BCF over time. The plant demonstrates an exceptional ability to accumulate and translocate mercury, with both BCF and TF showing strong evidence of mercury hyperaccumulation. This suggests that the plant could be a good candidate for phytoremediation of Hg-contaminated environments.

Table 24. Bio concentration factor (BCF) and translocation factor (TF).

Treatments	Interval - Days					
	4		12		20	
	BCF	**TF**	**BCF**	**TF**	**BCF**	**TF**
Aluminium	0.333	1.02	1.29	1.23	0.557	0.78
Chromium	0.124	1.19	0.99	1.22	0.615	0.631
Copper	0.156	1.24	0.348	1.57	0.404	**1.47**
Iron	0.521	1.64	1.18	1.58	0.537	0.859
Mercury	0.035	2.60	0.185	2.47	0.428	**1.33**

BCF: metal concentration ratio of plant roots to medium

TF: metal concentration ratio of plant shoots to roots.

SOD

The Table.25; Figs. 54-56 show how *Artemisia nilagirica* responds to different heavy metals by increasing superoxide dismutase (SOD) activity across root, stem, and leaf tissues over time. Here is a more concise analysis of each metals impact:

Aluminium exposure results in a significant increase in SOD activity across all tissues, with a noticeable rise over time. The plant responds to Al-induced oxidative stress by enhancing SOD production, particularly in the leaf (12.46 ± 1.02 at day 20), likely due to higher ROS production in the aerial tissues. This suggests an effective antioxidant defence mechanism to counteract Al toxicity.

Chromium exposure also elevates SOD activity in all tissues, with the root showing the highest increase (10.05 ± 0.23 at day 20). This indicates that *A. nilagirica* activates SOD production to combat Cr-induced oxidative stress, especially in the leaf and root, where oxidative damage is more likely to occur.

Copper toxicity induces a strong increase in SOD activity, especially in the root (12.04 ± 0.23 at day 20) and leaf (13.41 ± 0.25 at day 20). The data suggest that

Cu-induced ROS are efficiently neutralized by the antioxidant response of plant, with the leaf tissues experiencing the highest oxidative stress and therefore requiring the most robust defence.

Mercury results in the highest increase in SOD activity, especially in the stem (17.88 ± 1.14 at day 20) and leaf (18.72 ± 0.02 at day 20). This suggests that Hg induces substantial oxidative stress, and the plant responds with a significant upregulation of SOD to mitigate the damage, particularly in tissues exposed to the highest levels of stress.

All heavy metals (Al, Cr, Cu, Fe and Hg) elevate SOD activity in *A. nilagirica*, indicating a strong antioxidant response to counteract oxidative damage caused by metal toxicity. The leaf and stem tissues generally show the highest increases in SOD activity, reflecting their role in dealing with oxidative stress and maintaining cellular function under heavy metal stress.

Table. 25 Effect of aluminium, chromium, copper, iron and mercury on SOD activity (Unit activity/mg protein) of root, stem and leaf in *Artemisia nilagirica* (Clarke) Pamp.

	Tissues	Interval - Days		
		4	**12**	**20**
Control	Root	4.16 ± 0.01	4.87 ±0.32	**5.73 ±0.04**
	Stem	3.58 ± 0.21	4.22 ±0.24	**5.06 ±0.02**
	Leaf	4.04 ±0.11	6.95 ±0.05	**7.11 ±1.21**
Aluminium (80 µm)	Root	5.13 ± 0.20	7.84 ± 0.22	**8.43 ±1.23**
	Stem	6.52 ±0.01	9.34 ± 0.06	**10.56 ±0.29**
	Leaf	8.54 ± 0.16	11.78 ±0.42	**12.46 ±1.02**
Chromium (70 µm)	Root	6.84 ±1.32	8.45 ±0.02	**10.05 ±0.23**
	Stem	5.56 ±1.02	8.98 ±0.13	**9.95 ±1.14**
	Leaf	9.03 ± 0.25	11.56 ± 1.01	**13.02 ±0.04**
Copper (50 µm)	Root	7.83 ±1.15	11.60 ± 0.02	**12.04 ± 0.23**
	Stem	8.73 ±1.34	10.51 ±1.01	**12.40 ±0.06**
	Leaf	10.24±1.11	12.52 ±1.02	**13.41 ± 0.25**
Iron (70 µm)	Root	5.54 ±1.50	7.41 ±0.56	**8.87 ± 0.03**
	Stem	6.45 ±1.06	6.98 ± 0.71	**7.55 ±0.84**
	Leaf	8.26 ±1.02	8.92 ±1.01	**10.74 ± 0.92**
Mercury (10 µm)	Root	8.94 ±0.36	12.50 ±0.52	**10.36 ± 0.04**
	Stem	8.52 ±1.41	13.46± 0.30	**17.88 ±1.14**
	Leaf	**14.87 ±0.02**	**17.51 ±0.05**	**18.72 ± 0.02**

Values given are mean of 5 replicates ± S.E

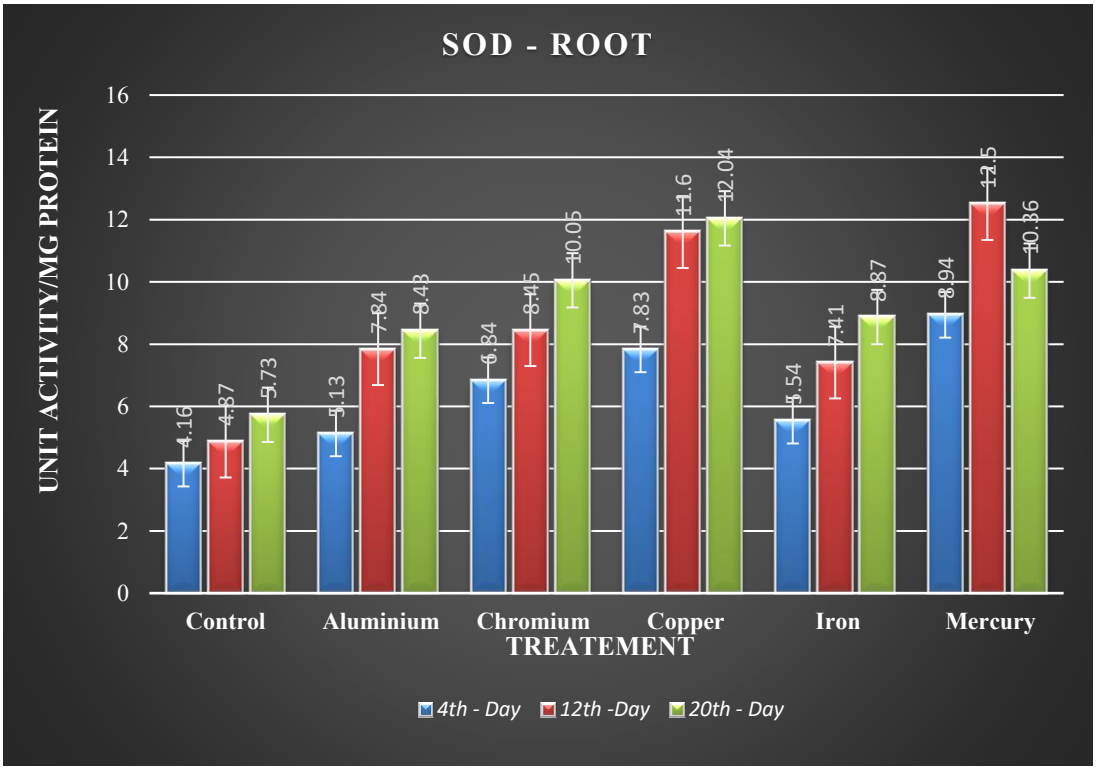


Fig. 54 Effect of aluminium, chromium, copper, iron and mercury on SOD activity on root of *Artemisia nilagirica* (Clarke) Pamp.

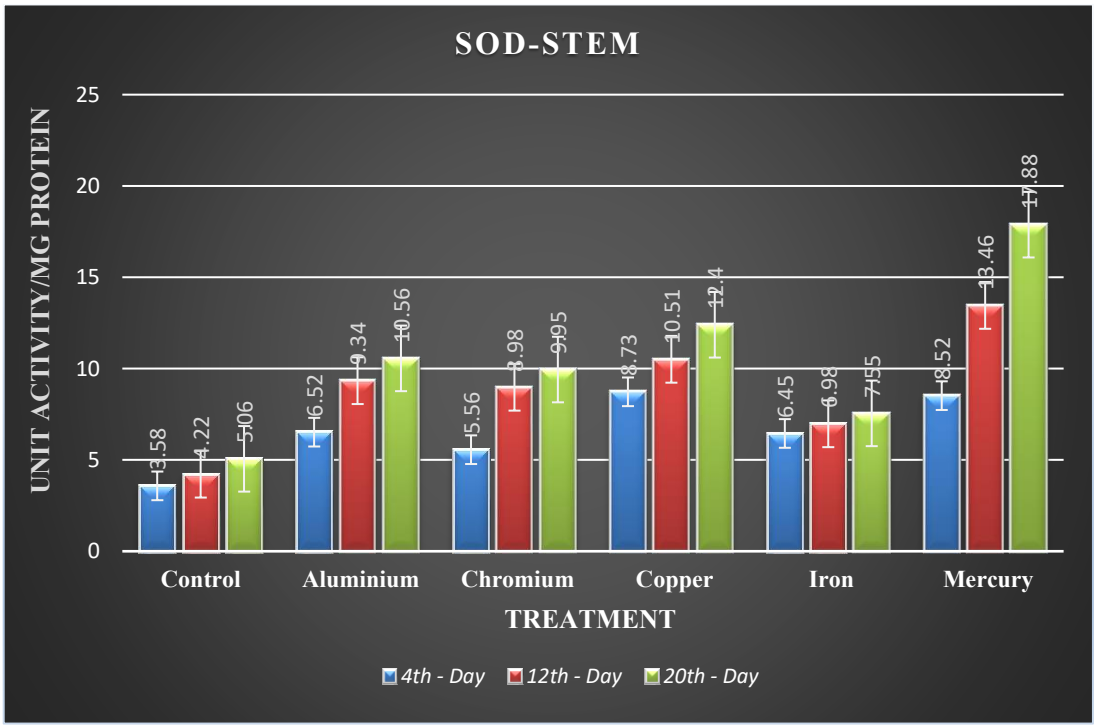


Fig. 55 Effect of aluminium, chromium, copper, iron and mercury on SOD activity on stem of *Artemisia nilagirica* (Clarke) Pamp.

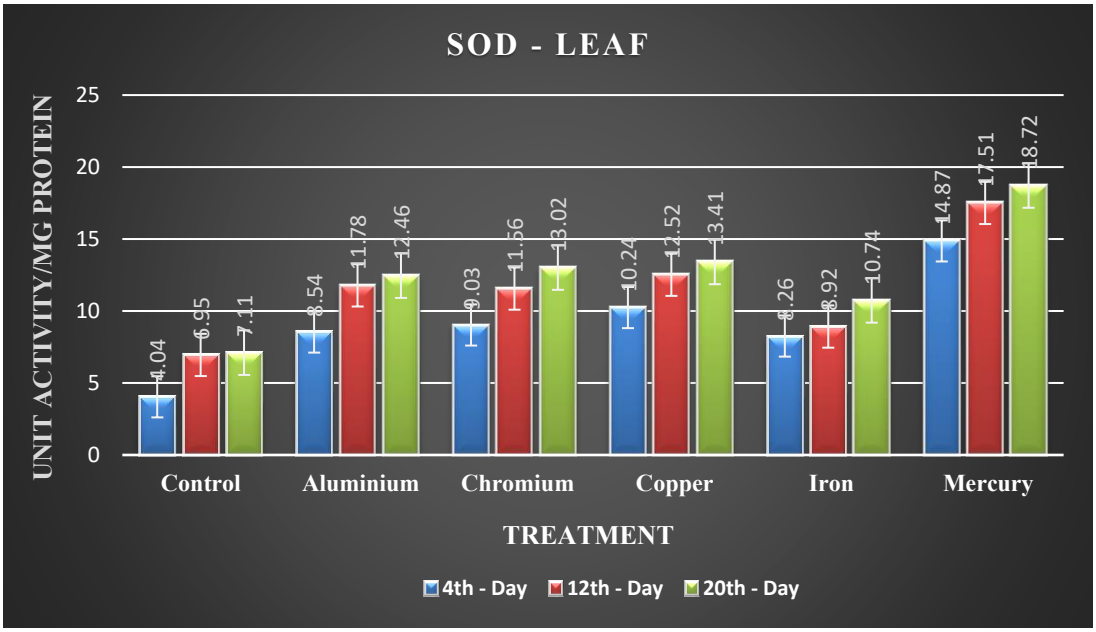


Fig. 56 Effect of aluminium, chromium, copper, iron and mercury on SOD activity on stem of *Artemisia nilagirica* (Clarke) Pamp.

CATALASE ACTIVITY

Catalase activity of *A. nilagirica* showed an increasing trend in control plants (Table 26; Figs. 57-59) during growth up to 20 days and the trend of activity was more or less uniform. Aluminium exposure caused an increase in catalase activity across all tissues, with the root showing a gradual rise from 0.87 ± 0.20 to 2.56 ± 0.25 over 20 days. The stem also exhibited an increase, though less pronounced, from 0.89 ± 1.21 to 2.46 ± 0.21. In leaf tissue, catalase activity initially peaked at 2.40 ± 0.01 on day 12 but slightly declined by day 20, indicating a transient stress response followed by potential adaptation or toxicity.

Chromium caused a moderate increase in catalase activity, especially in the root and stem. The root showed a steady rise in catalase activity, indicating a mild stress response. The stem demonstrated a consistent increase, suggesting an adaptive response to the metal. In the leaf, catalase activity showed a more significant rise, indicating a stronger oxidative stress response to Cr exposure. The root showed an increase from 0.64 ± 0.42 (day 4) to 1.23 ± 0.14 (day 20), suggesting a mild stress

response. The stem exhibited a steady rise from 0.85 ± 0.11 to 2.88 ± 0.31, indicating the ability of plant to adapt to chromium exposure. Leaf tissue displayed a more significant increase, from 1.22 ± 0.30 to 2.96 ± 0.15, highlighting oxidative impact of Cr on the plant.

Copper exposure resulted in a notable increase in catalase activity, particularly in the leaf and stem tissues. The root showed a moderate increase, indicating a moderate stress response to Cu. Both the stem and leaf displayed substantial rises in catalase activity, suggesting that these tissues are highly affected by Cu-induced oxidative stress and are actively responding with enhanced catalase production.

Iron exposure resulted in a notable increase in catalase activity in all tissues. The root catalase activity rose from 0.80 ± 0.012 to 2.98 ± 0.42, showing moderate stress over the 20-day period. The catalase activity of stem increased from 1.62 ± 2.02 to 3.25 ± 0.50, indicating adaptation to iron stress. Similarly, the leaf showed a substantial rise from 2.01 ± 1.14 to 3.57 ± 0.24, reflecting the plant defence mechanism against Fe-induced oxidative damage.

Mercury exposure resulted in the highest catalase activity across all tissues, indicating severe oxidative stress. The root, stem, and leaf tissues all showed dramatic increases in catalase activity, with the leaf exhibiting the most significant rise. This suggests that mercury induces the strongest oxidative stress response in *A. nilagirica*, with the plant activating its antioxidant defence mechanisms, particularly catalase, to mitigate the effects of the heavy metal.

Table. 26 Effect of aluminium, chromium, copper, iron and mercury on Catalase activity (Unit activity/mg protein) of root, stem and leaf in *Artemisia nilagirica* (Clarke) Pamp.

	Tissues	Interval - Days		
		4	12	20
Control	Root	0.84 ±0.50	1.53 ±0.13	**1.86 ±0.05**
	Stem	0.92 ±0.11	1.55 ± 1.04	**2.43 ±0.01**
	Leaf	1.03 ±0.24	2.86 ± 0.05	**2.88 ±0.30**
Aluminium (80 µM)	Root	0.87 ±0.20	2.30 ±0.01	**2.56 ±0.25**
	Stem	0.89 ± 1.21	1.67 ±0.23	**2.46 ±0.21**
	Leaf	1.36 ±1.50	2.40 ±0.01	**2.32 ±1.15**
Chromium (70 µM)	Root	0.64 ±0.42	1.47 ±0.02	**1.23 ±0.14**
	Stem	0.85 ±0.11	1.56 ±1.06	**2.88 ±0.31**
	Leaf	1.22 ±0.30	2.58 ±0.01	**2.96 ±0.15**
Copper (50 µM)	Root	0.56 ±0.01	0.93 ±0.04	**1.28 ±1.24**
	Stem	0.94 ±0.24	2.48 ±1.50	**2.93 ±0.01**
	Leaf	2.53 ±1.03	3.89 ±0.16	**3.94 ±0.05**
Iron (70 µM)	Root	0.80 ±0.012	2.87 ±0.15	**2.98 ±0.42**
	Stem	1.62 ±2.02	1.99 ±1.03	**3.25 ±0.50**
	Leaf	2.01 ±1.14	3.24 ±0.06	**3.57 ±0.24**
Mercury (10 µM)	Root	4.15±0.01	3.42 ± 0.16	**4.76 ±0.18**
	Stem	4.54 ±1.30	4.63 ±1.02	**4.67 ±0.15**
	Leaf	**5.22 ±0.03**	**4.7 6±1.06**	**5.89 ±1.11**

Values given are mean of 5 replicates ± S.E

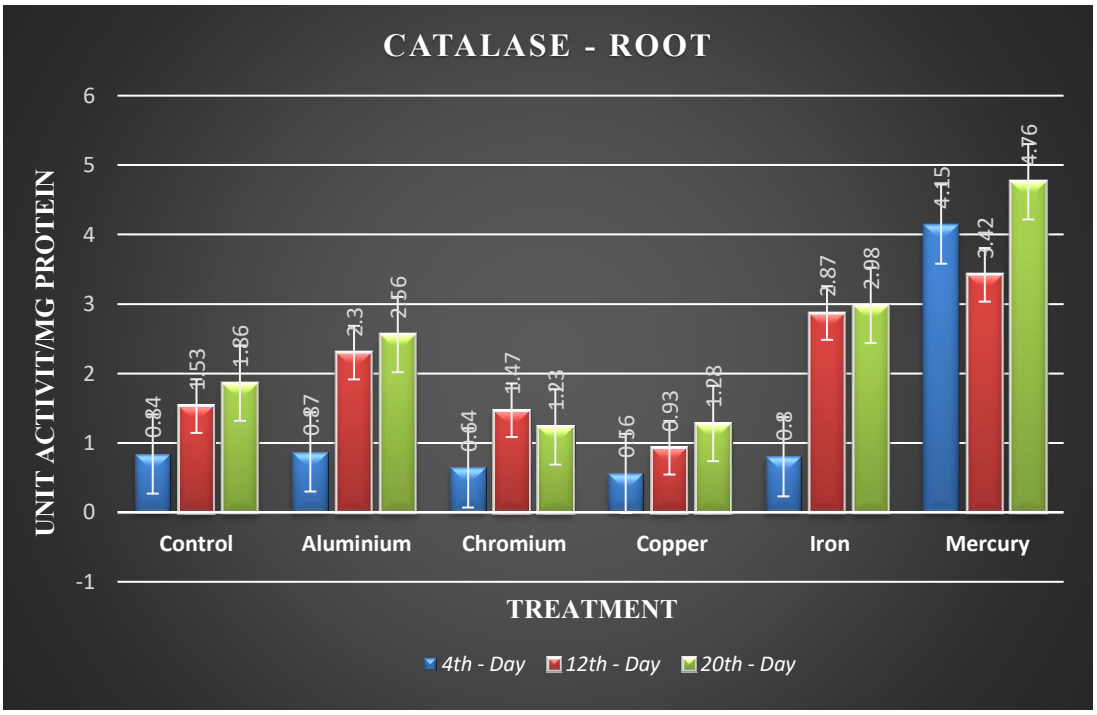


Fig. 57 Effect of aluminium, chromium, copper, iron and mercury on catalase activity in root of *Artemisia nilagirica* (Clarke) Pamp.

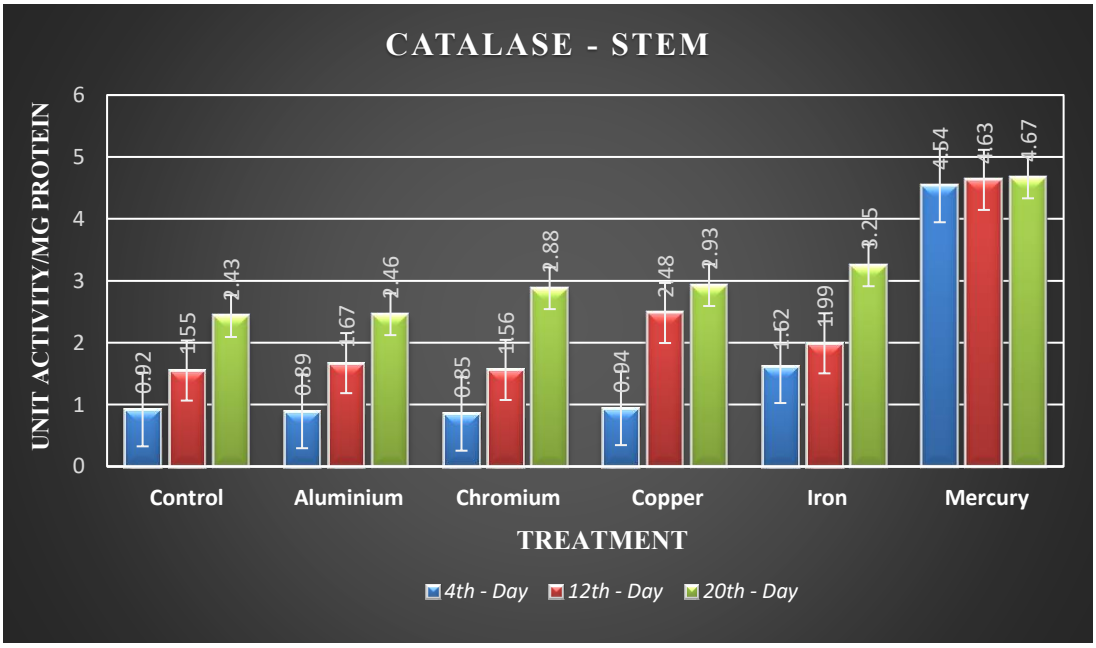


Fig. 58 Effect of aluminium, chromium, copper, iron and mercury on catalase activity in stem of *Artemisia nilagirica* (Clarke) Pamp.

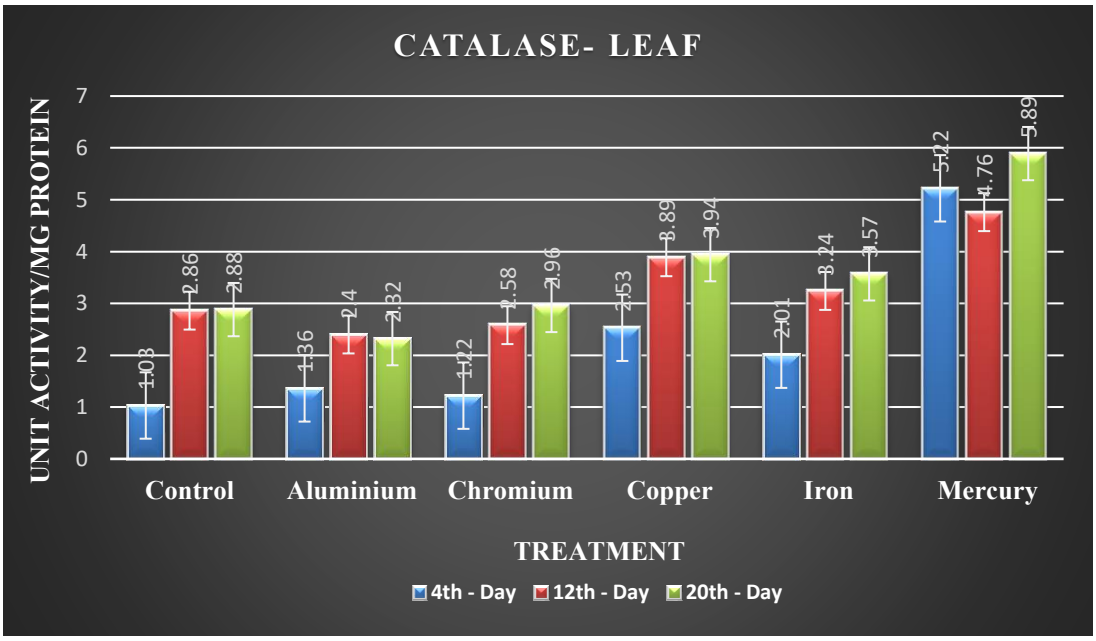


Fig. 59 Effect of aluminium, chromium, copper, iron and mercury on catalase activity in leaf of *Artemisia nilagirica* (Clarke) Pamp.

GCMS ANALYSIS OF *ARTEMISIA NILAGIRICA* (CLARKE) PAMP. LEAVES.

Twenty-six secondary metabolites were identified using GCMS analysis of the methanolic extract of *Artemisia nilagirica*; the area percentage of compounds indicates the quantity of the compounds (Table. 27). The GC-MS analysis reveals a diverse profile of compounds, with 3-Thujanol being the most abundant at 27.72%, followed by Phytol (21.37%) and Caryophyllene oxide (14.31%). Other notable compounds include (+)-Beta-thujone (6.99%), (-)-Beta-caryophyllene (2.30%), and Germacrene-D (0.61%), which contribute to the overall complexity of the sample. Compounds such as Alpha-humulene (0.47%) and Isoaromadendrene Epoxide (2.72%) are also present, though they occur in smaller amounts. A few compounds that appear in trace amounts include Andrographolide (1.80%) and 7-Decen-1-olacetate (0.41%). The control sample shows a well-balanced distribution of these compounds, reflecting a stable chemical composition without the influence of external stressors. (+)-Cedrol was the secondary metabolite that occurred least frequently in control plants based on area percentage, while 3-Thujanol was the component that occurred most abundantly. It is discovered that the distribution of these secondary metabolites is crucial to the pharmacological efficacy of the plant.

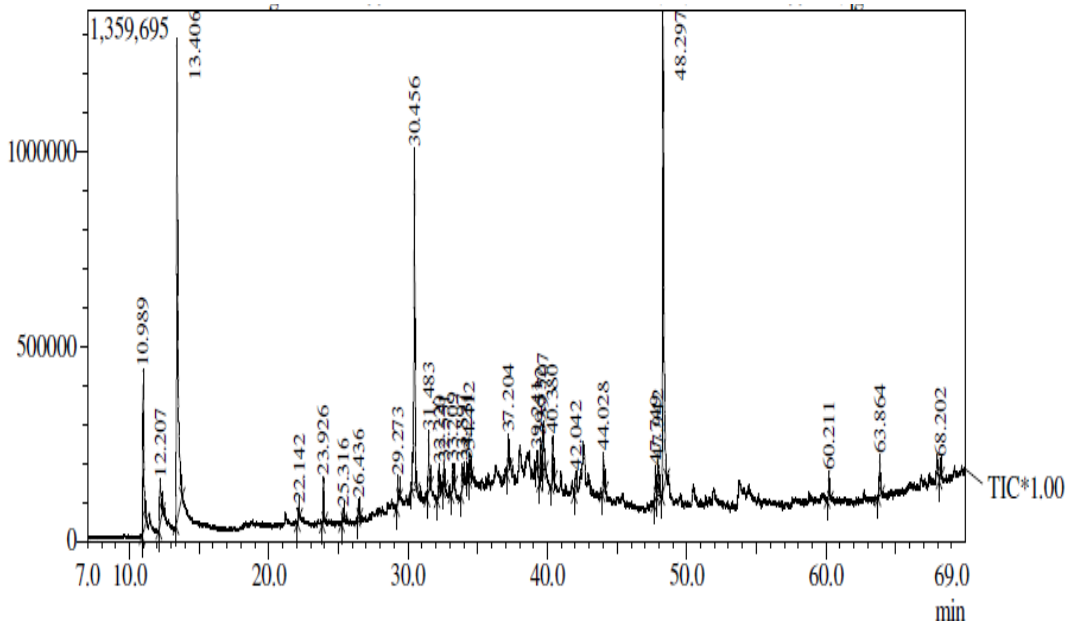


Figure. 60 GCMS Analysis spectrum of *Artemisia nilagirica* (Clarke) Pamp.

Table. 27 GCMS Analysis *Artemisia nilagirica* (Clarke) Pamp.

Sl.No	Compounds	Area %
1	(+) – Beta- thujone	**6.99**
2	3-Thujanol	**27.72**
3	(+)-Cedrol	**0.43**
4	(-)-, Beta, -caryophyllene	**2.30**
5	Alpha- humulene	**0.47**
6	Germacrene - D	**0.61**
7	Caryophyllene oxide	**14.31**
8	Humulene epoxide	**2.13**
9	Ledenoxide-(ii)	**1.18**
10	Alloaromadendrenoxide- (1)	**0.87**
11	Benzocycloheptaen-7-ol,2,3,4,5,6,7,8-octahydro-1,1,4a,7-tetramethyl	**1.06**
12	(-)-, Beta, -caryophyllene epoxide	**1.08**
13	7-Decen-1-olacetate	**0.41**
14	Isoaromadendrene Epoxide	**2.72**
15	4-Isopropenyl-4,7-dimethyl-1-oxaspiro [2.5] octane	**1.38**
16	Alpha, -terpinyl 3-methylbutanoate	**0.45**
17	Neophytadiene	**1.06**
18	Ethanocyclopenta[a]cyclopropa[e]cyclodecen-11-one,1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl	**2.38**
19	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	**1.90**
20	Aromadendrene oxide-(2)	**1.11**
21	9,12- Octadecadienoic acid, methyl ester	**1.07**
22	Andrographolide	**1.80**
23	Phytol	**21.37**
24	1,2- Benzene dicarboxylic acid	**1.10**
25	4-Caranol	**1.53**
26	Cyclohexane-1-Methylene	**2.57**
		100.00

DISTRIBUTION OF SECONDARY METABOLITES IN *ARTEMISIA NILAGIRICA* TREATED WITH ALUMINIUM

GCMS examination of plants treated with aluminium revealed the presence of twenty secondary metabolites (Table. 28). The GC-MS profile reveals several changes in the compound profile, reflecting the impact of aluminium toxicity. While Phytol (20.55%) remains the most abundant, 3-Thujanol decreases to 24.56%, and Caryophyllene oxide drops to 10.25%. Some compounds, such as (+)-Cedrol (0.43%) and Ledenoxide-(ii) (1.18%), are completely absent in the treated sample, indicating that aluminium toxicity may have caused their degradation or inhibited their synthesis. The compound Germacrene-D increases significantly to 3.61%, and (-)-Isocaryophyllene (3.18%) and Thunbergol (2.84%) emerge as new components, which were not present in the control. Additionally, compounds like Alpha-humulene (0.35%) and Andrographolide (0.87%) show reduced abundances, further supporting the notion that aluminium exposure disrupts normal metabolic pathways. These shifts in the compound profile suggest that aluminium toxicity may alter the biosynthesis of secondary metabolites, leading to the formation of new compounds and the reduction or loss of others.

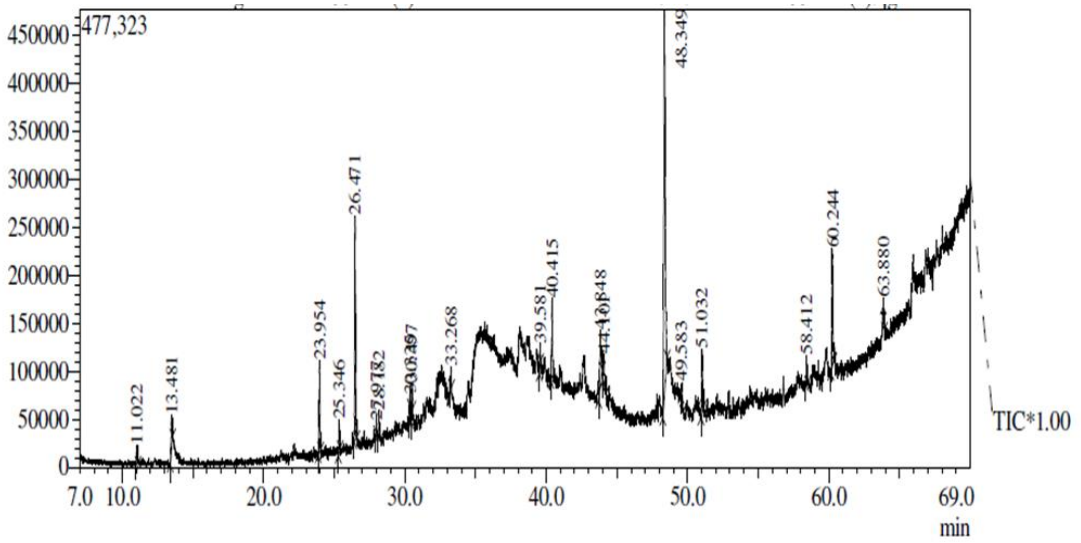


Figure. 61 GCMS Analysis spectrum of *Artemisia nilagirica* (Clarke) Pamp. treated with Aluminium.

Table. 28 GCMS analysis *Artemisia nilagirica* (Clarke) Pamp. treated with aluminium.

Sl. No.	Compounds	Area %
1	(+) – Beta- thujone	**7.32**
2	(-)- Alpha thujone	**4.48**
3	3-Thujanol	**24.56**
4	Isoaromadendrene epoxide	**1.43**
5	(-)-, Beta, -caryophyllene	**1.93**
6	Alpha- humulene	**0.35**
7	Germacrene - D	**3.61**
8	(-)-, Beta, -caryophyllene epoxide	**2.68**
9	Caryophyllene oxide	**10.25**
10	1,2-Benzenedicarboxylic acid	**1.02**
11	(-)- Isocaryophyllene	**3.18**
12	Andrographolide	**0.87**
13	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	**4.01**
14	9-Acetyl-S-octahydrophenanthrene	**1.24**
15	7-Decen-1-olacetate	**2.41**
16	Phytol	**20.55**
17	4-Isopropenyl-4,7-dimethyl-1-oxaspiro [2.5] octane	**2.38**
18	Alpha, -terpinyl 3-methylbutanoate	**3.66**
19	Neophytadiene	**1.23**
20	Thunbergol	**2.84**
		100.00

DISTRIBUTION OF SECONDARY METABOLITES IN *ARTEMISIA NILAGIRICA* TREATED WITH CHROMIUM

In comparing the GC-MS profiles of the control and plant treated with chromium, significant differences in the compound composition are evident. The control sample consists of 26 compounds, while the Cr-treated plant has only 15 compounds, showing a marked reduction in the number of metabolites due to Cr toxicity (Table. 29). Notably, Cedrol, Ledenoxide-(ii), Isoaromadendrene Epoxide, Andrographolide, and Thunbergol are absent in the Cr-treated plant, indicating that

chromium exposure may have caused the degradation or inhibition of these compounds. In contrast, new metabolites such as Cis-Z-alpha bisabolene epoxide (6.49%), Stigmasta-5,22-dien-3-ol (4.38%), and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (4.5%) appear in the chromium-treated plant, suggesting that Cr toxicity may lead to the formation of novel compounds. Other compounds such as 3-Thujanol (27.00%), Phytol (21.29%), and (-)-Beta-caryophyllene (10.01%) are still abundant in the Cr-treated plant, but their relative percentages are altered compared to the control. These results emphasize a notable decrease in the diversity of metabolites, with some compounds disappearing and others emerging, as a consequence of chromium-induced stress.

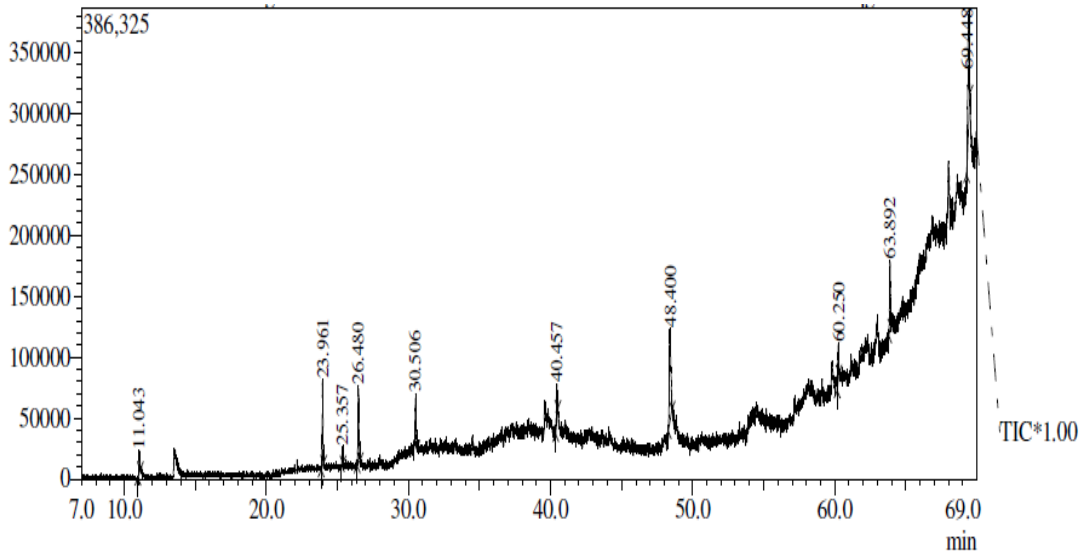


Figure. 62 GCMS Analysis spectrum of *Artemisia nilagirica* (Clarke) Pamp. treated with Chromium.

Table. 29 GCMS analysis *Artemisia nilagirica* (Clarke) Pamp. treated with chromium.

Sl. No	Compounds	Area %
1	(+) – Beta- thujone	**3.35**
2	3-Thujanol	**27.00**
3	(-)-, Beta, -caryophyllene	**10.01**
4	Alpha- humulene	**2.23**
5	(-)-, Beta, -caryophyllene epoxide	**1.09**
6	Germacrene - D	**1.28**
7	Phytol	**21.29**
8	Caryophyllene oxide	**3.76**
9	Alloaromadendrenoxide- (1)	**2.74**
10	Benzocycloheptaen-7-ol,2,3,4,5,6,7,8-octahydro-1,1,4a,7-tetramethyl	**2.65**
11	Cis-Z-alpha bisabolene epoxide	**6.49**
12	Stigmasta-5,22-dien-3-ol	**4.38**
13	4-Isopropenyl-4,7-dimethyl-1-oxaspiro [2.5] octane	**5.22**
14	Neophytadiene	**4.01**
15	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	**4.5**
		100.00

DISTRIBUTION OF SECONDARY METABOLITES IN *ARTEMISIA NILAGIRICA* TREATED WITH COPPER

GC-MS profile of *Artemisia nilagirica* treated with copper, a notable increase in the number of metabolites is observed due to Cu toxicity (Table. 30). The control plant contains 26 compounds, while the Cu-treated plant has 30 compounds, suggesting an increase in the diversity of metabolites in response to copper exposure. Despite this, the overall number of metabolites in the Cu-treated plant is relatively balanced, with some compounds showing significant changes in abundance. Compounds such as (-)-Alpha Thujone (0.88%), Caryophyllene oxide (5.22%), and Phytol (19.48%) remain abundant in the Cu-treated plant, although there are also some newly formed metabolites, such as Geranyl linalool isomer b (2.74%), Valerenol (1.22%), and 8,11,14-Docosatrienoic acid, Methyl ester (1.32%), which were not present in the control plant. However, compounds like Cedrol, Isoaromadendrene Epoxide, Thunbergol, and Andrographolide are absent in the Cu-treated plant,

indicating that copper exposure may have led to the degradation or inhibition of these compounds. Furthermore, there is a marked decrease in the presence of several sesquiterpenes like (-)-Beta-caryophyllene (2.06%) and Alpha-humulene (0.46%) in the Cu-treated plant. This suggests that Cu toxicity has a substantial impact on the biosynthesis of certain secondary metabolites, with some compounds being significantly reduced or deleted while others emerge or increase in response to the metal stress.

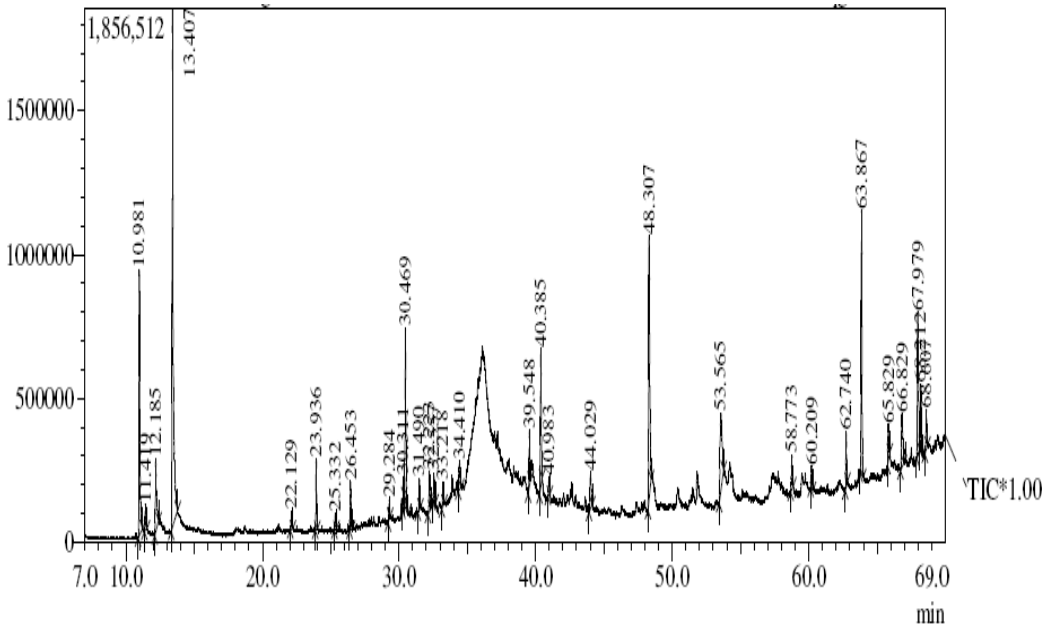


Figure. 63 GCMS Analysis spectrum of *Artemisia nilagirica* (Clarke) Pamp. treated with copper.

Table. 30 GCMS analysis *Artemisia nilagirica* (Clarke) Pamp. treated with copper.

Sl. No	Compounds	Area %
1	(+) – Beta- thujone	**8.62**
2	3-Thujanol	**25.35**
3	(-)-Alpha Thujone	**0.88**
4	(-)-, Beta, -caryophyllene	**2.06**
5	Alpha- humulene	**0.46**
6	Germacrene - D	**1.47**
7	(-)-, Beta, -caryophyllene epoxide	**1.36**
8	Caryophyllene oxide	**5.22**
9	Humulene epoxide	**0.94**
10	Ledenoxide-(ii)	**3.43**
11	Alloaromadendrenoxide- (1)	**0.94**
12	Benzocycloheptaen-7-ol,2,3,4,5,6,7,8-octahydro-1,1,4a,7-tetramethyl	**1.05**
13	Alpha-caryophyllene	**0.02**
14	Phytol	**19.48**
15	4-Isopropenyl-4,7-dimethyl-1-oxaspiro [2.5] octane	**1.31**
16	Alpha, -terpinyl 3-methylbutanoate	**2.31**
17	Neophytadiene	**1.57**
18	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	**4.25**
19	Aromadendrene oxide-(2)	**2.05**
20	Retinal	**1.40**
21	Spathulenol	**1.46**
22	Andrographolide	**1.71**
23	Ethanocyclopenta[a]cyclopropa[e]cyclodecen-11-one,1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl	**1.24**
24	Cyclohexane, -1-Methylene-3-(1-Methylethenyl)	**2.34**
25	2-Beutenal,2-methyl-4-(2,6,6-trimethyl)	**1.57**
26	Geranyl linalool isomer b	**2.74**
27	1,2 Benzene dicarboxylic acid	**0.70**
28	Valerenol	**1.22**
29	8,11,14- Docosatrienoic acid, Methyl ester	**1.32**
30	Alpha- terpinyl propanoate	**1.53**
		100.00

DISTRIBUTION OF SECONDARY METABOLITES IN *ARTEMISIA NILAGIRICA* TREATED WITH IRON

GCMS examination of plants treated with iron revealed the presence of 21 secondary metabolites (Table. 31), indicating a reduction in the diversity of metabolites. Several compounds present in the control plant, such as (-)-Alpha Thujone (0.88%), Caryophyllene oxide (5.22%), Cedrol (0.43%), and Isoaromadendrene Epoxide (2.72%), are either significantly reduced or absent in the Fe-treated plant, suggesting that iron toxicity impairs the biosynthesis of these compounds. New compounds, such as Pentanoic acid, 5-hydroxy-2,4-di-t-butylphenyl ester (0.42%), Spathulenol (2.58%), and 2-Beutenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl) (2.37%), emerge in the Fe-treated plant, indicating a shift in the metabolic pathways due to the stressor. The reduction in the total number of compounds and the disappearance of some bioactive metabolites suggest that Fe toxicity negatively impacts the medicinal potential of *A. nilagirica*.

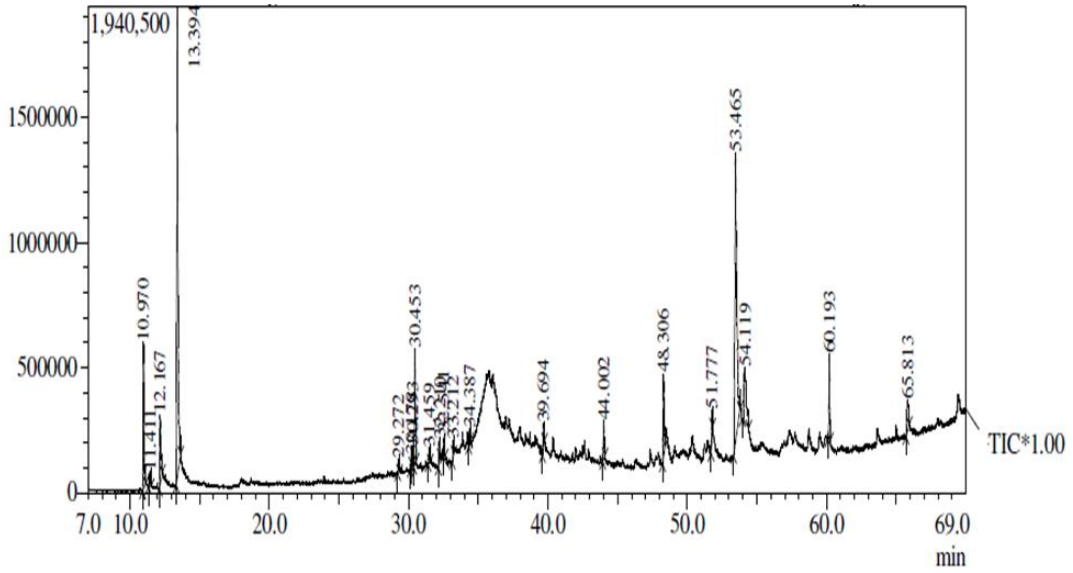


Figure. 64 GCMS Analysis spectrum of *Artemisia nilagirica* (Clarke) Pamp. treated with iron.

Table. 31 GCMS analysis *Artemisia nilagirica* (Clarke) Pamp. treated with iron.

Sl. No	Compounds	Area %
1	(+) – Beta- thujone	**9.24**
2	3-Thujanol	**24.80**
3	Caryophyllene oxide	**0.07**
4	(-)-, Beta, -caryophyllene	**5.40**
5	Alpha-caryophyllene	**1.81**
6	Germacrene - D	**1.24**
7	(-)-, Beta, -caryophyllene epoxide	**1.59**
8	Phytol	**19.57**
9	Humulene epoxide	**1.43**
10	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	**6.43**
11	Alloaromadendrenoxide- (1)	**4.64**
12	1,2 Benzene dicarboxylic acid	**0.94**
13	Spathulenol	**2.58**
14	7-Decen-1-olacetate	**1.66**
15	Isoaromadendrene Epoxide	**4.23**
16	2-Beutenal,2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	**2.37**
17	Alpha, -terpinyl 3-methylbutanoate	**3.42**
18	Neophytadiene	**1.81**
20	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	**6.35**
21	Pentanoic acid, 5-hydroxy- 2,4-di-t-butylphenyl ester	**0.42**
		100.00

DISTRIBUTION OF SECONDARY METABOLITES IN *ARTEMISIA NILAGIRICA* TREATED WITH MERCURY

The GCMS profile of *A. nilagirica* treated with mercury showed a significant alteration in its secondary metabolite composition compared to the control plant (Table. 32). A total of 32 compounds were detected in the Hg-treated plant, whereas the control plant exhibited 26 compounds. Despite the increased number of compounds, there was a remarkable decrease in the overall diversity of metabolites, with several key compounds absent in the Hg-treated plant. Compounds such as Caryophyllene oxide, Spathulenol, and Andrographolide, which were present in the control, were lost under Hg stress. On the other hand, new compounds such as Isothujol (0.07%), Globulol (0.73%), and 9-Acetyl-S-octahydrophenanathrene

(1.50%) emerged in the Hg-treated plant. The reduction in the number of bioactive compounds, along with the introduction of novel metabolites, suggests that mercury toxicity significantly alters the metabolic pathways in *A. nilagirica*, potentially decreasing its medicinal efficacy.

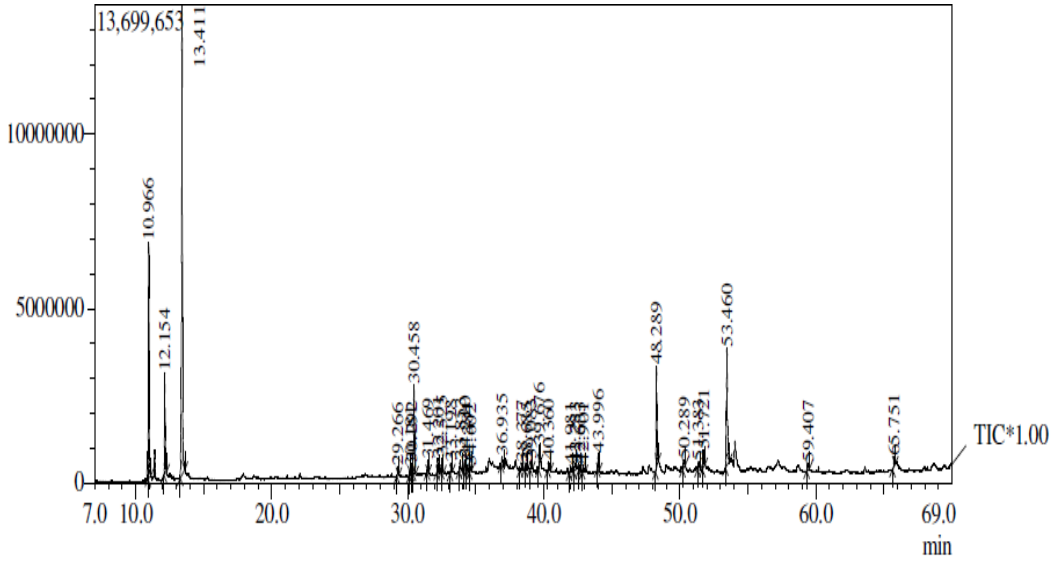


Figure. 65 GCMS Analysis spectrum of *Artemisia nilagirica* (Clarke) Pamp. treated with mercury.

Table. 32 GCMS Analysis *Artemisia nilagirica* (Clarke) Pamp. treated with mercury.

Sl. No	Compounds	Area %
1	(+) – Beta- thujone	**6.12**
2	3-Thujanol	**21.42**
3	Isothujol	**0.07**
4	(-)-, Beta, -caryophyllene	**2.63**
5	Alpha- humulene	**1.09**
6	Germacrene - D	**3.48**
7	(-)-, Beta, -caryophyllene epoxide	**2.45**
8	Caryophyllene oxide	**3.76**
9	Humulene epoxide	**3.87**
10	Ledenoxide-(ii)	**1.77**
11	Retinal	**2.00**
12	Humulene oxide	**0.68**
13	Phytol	**9.50**
14	Spathulenol	**0.72**
15	Alloaromadendrenoxide- (1)	**2.74**
16	Benzocycloheptaen-7-ol,2,3,4,5,6,7,8-octahydro-1,1,4a,7-tetramethyl	**4.95**
17	7-Decen-1-olacetate	**1.66**
18	Isoaromadendrene Epoxide	**4.23**
19	Neophytadiene	**2.62**
20	Ethanocyclopenta[a]cyclopropa[e]cyclodecen-11-one,1a,2,5,5a,6,9,10,10a-octahydro-5	**5.66**
21	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	**2.34**
22	Aromadendrene oxide-(2)	**1.31**
23	6-Epi-shypbunol	**1.72**
24	9-Acetyl-S-octahydrophenanathrene	**1.50**
25	Globulol	**0.73**
26	Andrographolide	**1.78**
27	1,2 Benzene dicarboxylic acid	**2.53**
28	8,11,14- Docosatrienoic acid, Methyl ester	**1.65**
29	Alpha- terpinyl propanoate	**1.72**
30	Cyclohexane, -1-Methylene-3-(1-Methylethenyl)	**1.03**
31	2-Beutenal,2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yi)	**0.86**
32	Humulane-1,6-dien-3-ol	**1.41**
		100.00

FOURIER TRANSFORM INFRARED (FTIR) - ANALYSIS

Control/ Al - Treated Plant (Fig. 66).

3397cm^{-1} and 2923cm^{-1}: The absorption bands at 3397 cm^{-1} and 2923 cm^{-1}, corresponding to the **O-H** and **C=O** stretches, show no significant shifts between the control and aluminium-treated plants. This indicates that major structural components of the plant cell wall, such as cellulose, hemicellulose, and pectins, remain largely unaffected by Al exposure.

1635cm^{-1}: The absorption band at 1635 cm^{-1}, associated with **N-H** and **C=O** stretches found in peptides and proteins, also remains consistent between the control and Al-treated plants. This suggests that Al does not significantly impact the protein composition or synthesis in the plants.

The FTIR analysis demonstrates that aluminium exposure does not significantly alter the functional groups associated with structural polysaccharides or proteins in the plant. While the overall biochemical composition appears preserved, subtle molecular-level effects and adaptive responses to aluminium stress may still occur.

Control/ Cr -Treated Plant (Fig. 67).

3397 cm^{-1}: FTIR spectrum of control plant demonstrated an absorption band at 3397 cm^{-1}. It represents the OH group, which is commonly connected to proteins, carbohydrates, and polyphenols. These molecules include hydroxyl groups, which are indicated by the presence of this band.

3400 cm^{-1}: In the Cr-treated plant, this band is slightly shifted compared to the control plant (from 3397 cm^{-1} to 3400 cm^{-1}). This shift could be due to changes in the molecular interactions in the plant cells caused by Cr treatment. It still corresponds to **OH** groups (carbohydrates, proteins, and polyphenols).

1635 cm^{-1}: This band corresponds to the **N-H** (amine) and **C=O** (carbonyl) stretches, typical of **peptides** (proteins). This indicates the presence of peptide bonds and amino groups in the plant tissues.

1020 cm^{-1}: This is a new band that appears only in the chromium-treated plant. It corresponds to the **SiO_2 (silica)** stretching vibration. The presence of this band suggests that Cr treatment might have induced silica incorporation or a change in silica content in the plant, which is not found in the control.

According to the FTIR analysis, the Cr treatment has somewhat changed the molecular structure of the plant. This includes modifications to the OH band and the emergence of a new silica band (SiO_2) that is not present in the control plant. This would suggest that the metabolic profile of the plant has changed, presumably in reaction to chromium stress.

Control/ Cu -Treated Plant (Fig. 68).

The study of the control and copper-treated plant samples using FTIR spectroscopy shows a number of substantial spectral alterations, suggesting that copper treatment significantly affects molecular structure of the plant. The following are the specifics of these changes.

1635 cm^{-1}: which is attributed to the bending vibration of N-H and **C=O** in peptide bonds, typical of proteins or peptides. This band is crucial for identifying the secondary structure of proteins, particularly the amide I band associated with the stretching of the **C=O** bond.

1610 cm^{-1}: The shift indicates that copper may bind to unsaturated **C=C** bonds, which could be part of aromatic rings, alkenes, or other unsaturated compounds present in the plant. The shift could also reflect a change in the conformation of peptide bonds or an interaction between the copper ions and the amide or carbonyl groups. Specifically, copper binding may alter the electronic environment around the peptide bonds, causing a change in the vibrational frequency.

1385 cm^{-1}: The 1385 cm^{-1} absorption band, attributed to the C-H bending vibration of the terminal methyl groups in aliphatic ketones or other similar structures, was observed in both the control and copper-treated plants. This band was not significantly affected by copper treatment, suggesting that the copper ions did not

significantly alter the structure or concentration of aliphatic ketones or related compounds in the plant tissues. This indicates that effect of copper on the plant may be more selective, targeting certain functional groups like amines and amides, while leaving others, such as aliphatic ketones, unaffected.

Disappearance of the 3397 cm^{-1} Band: In the FTIR spectrum of the control plant, a prominent absorption band is observed at 3397 cm^{-1}, which is typically associated with the **NH-** stretching vibration in amines or amides. This band reflects the presence of amino or amide groups in proteins or peptides.

The disappearance of the N-H stretching band is a strong indicator that copper ions are affecting the protein or peptide structure, potentially by binding to amino acids, peptides, or other nitrogen-containing components in the plant cells. This could result in a change in the protein conformation, reducing the availability of free amino or amide groups that would normally exhibit the 3397 cm^{-1} absorption.

The FTIR analysis reveals that copper toxicity significantly alters the functional groups of key biomolecules, particularly proteins. The disappearance of the **N-H** stretching band and shifts in amide I and II bands indicate copper binding to nitrogen and carbonyl groups, causing conformational changes and potential disruption of protein function. In parallel, the unchanged methyl **C-H** bending vibration at 1385 cm^{-1} suggests that copper selectively impacts biomolecules involved in metal binding and stress responses, while sparing others, like lipids.

Control/ Fe -Treated Plant (Fig. 69).

Several noteworthy characteristics in the control plant spectrum that were either noticeably absent or not considerably changed in the Fe-treated plant spectrum are revealed by the FTIR analysis of the control and iron (Fe)-treated plants. These findings shed light on the biochemical effects of iron treatment on the molecular structure of the plant, especially in relation to the main functional groups present in proteins and polysaccharides in the cell wall.

3397 cm^{-1} and 2923 cm^{-1}: In the FTIR spectrum of the control plant, the absorption bands observed at 3397 cm^{-1} and 2923 cm^{-1} are characteristic of hydroxyl (**OH**) and carboxyl (**COOH**) groups, respectively. The band at 3397 cm^{-1} is attributed to the hydrogen-bonded hydroxyl stretching vibrations typically present in cellulose, hemicellulose, and other polysaccharides, which are major components of the plant cell wall. The band at 2923 cm^{-1} corresponds to the **C-H** stretching vibrations found in the methyl groups of hemicelluloses and pectins, which are also integral parts of the cell wall structure.

1635 cm^{-1}: The 1635 cm^{-1} absorption band in the control plant is typically attributed to the bending vibration of the **N-H** and **C=O** groups in the peptide bonds of proteins. This band is part of the **amide I** and **II** vibrations, which reflect the secondary structure of proteins, such as the folding of polypeptide chains in the proteome of plant. The presence of this band in the control plant indicates the normal presence of proteins and their associated structures within the plant cells. In contrast, the Fe-treated plant does not show any significant absorption at 1635 cm^{-1}, suggesting that iron treatment does not significantly affect the protein content or structure in the same way as some other metals (such as copper) might. The absence of this absorption band in the Fe-treated plant suggests that iron treatment does not cause major alterations in protein conformation or in the structure of peptide bonds, at least in the functional groups that are detectable by FTIR analysis.

The absence or reduction of the characteristic absorption bands at 3397 cm^{-1}, 2923 cm^{-1}, and 1635 cm^{-1} in the Fe-treated plant suggests that iron has a limited impact on the plant cell wall composition and protein structure compared to other heavy metals. The hydroxyl and carboxyl groups associated with polysaccharides and pectins in the cell wall appear to be less affected by iron treatment. This might indicate that iron does not significantly disrupt primary cellular structure or metabolism of the plant. Furthermore, the lack of changes in protein-related absorption bands suggests that iron does not directly interact with peptide bonds or protein secondary structures in a way that would be detectable by FTIR.

Control/ Hg -Treated Plant (Fig. 70).

Significant variations in the absorption bands corresponding to important functional groups in the plant cell wall and proteins were seen in the FTIR spectra of the control and mercury-treated plants.

3397 cm^{-1} and 2923 cm^{-1}: The band at 3397 cm^{-1} is attributed to the hydroxyl (**OH**) stretching vibrations, which are characteristic of the cellulose, hemicellulose, and pectins in the plant cell wall. Similarly, the band at 2923 cm^{-1} is associated with **C-H** stretching vibrations from alkyl groups such as methyl and methylene groups present in polysaccharides. In the mercury-treated plants, these bands show slight shifts or reduced intensities compared to the control plants. This suggests that mercury treatment might cause alterations in the cell wall composition or structure, potentially affecting the polysaccharide matrix and water-binding capacity. Such changes could be indicative of modifications in the structural integrity of cell wall due to mercury exposure.

1635 cm^{-1}: The band at 1635 cm^{-1} corresponds to amide I and amide II bands, which are associated with the **N-H** bending and **C=O** stretching vibrations of peptide bonds in proteins. In the mercury-treated plants, this band shows a reduction in intensity, suggesting that mercury exposure may alter the protein structure or reduce the presence of proteins in the plant. This could reflect conformational changes or denaturation of proteins, potentially due to the toxicity of mercury affecting protein stability and functionality.

1385 cm^{-1}: The band at 1385 cm^{-1} is attributed to C-H bending vibrations from terminal methyl groups found in aliphatic ketones and other organic compounds. The mercury-treated plants exhibited a shift or decrease in intensity of this band, indicating that mercury treatment may influence lipid metabolism or membrane composition, potentially affecting the cell membrane structure and function.

The findings of FTIR research show that treatment with mercury alters the functional groups connected to proteins and the cell wall. It is possible that the harmful effects of mercury on the structural integrity of the plant cellular components are

reflected in the observed shifts and intensity variations in the bands, which point to changes in the polysaccharide and protein structure of the plant.

FOURIER TRANSFORM INFRARED (FTIR) - ANALYSIS

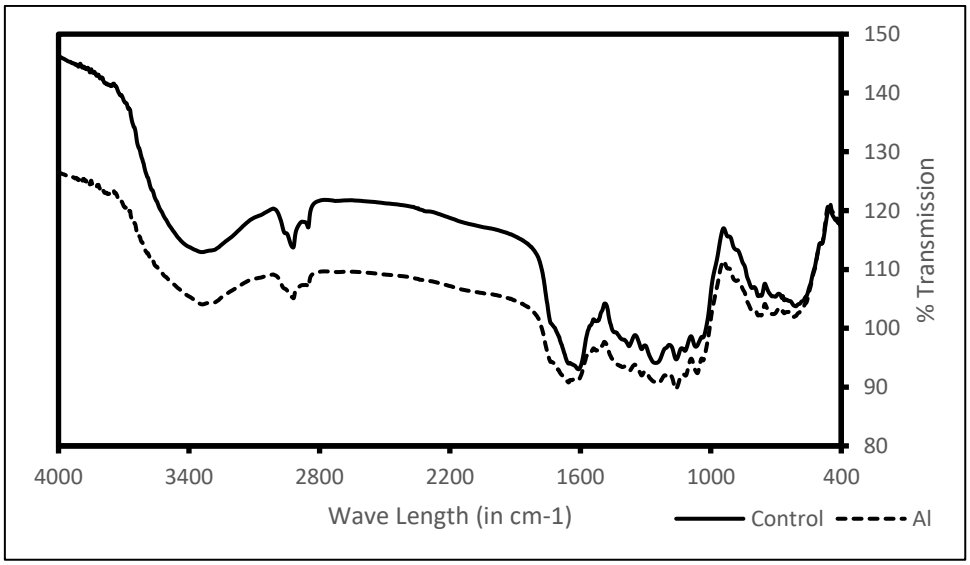


Figure. 66 FTIR spectrum - Control / Al

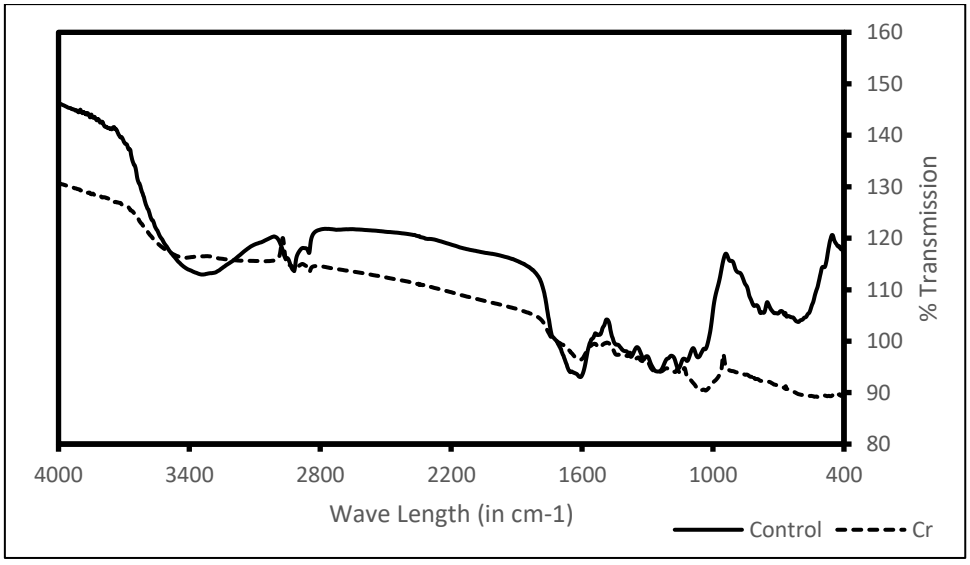


Figure. 67 FTIR spectrum - Control / Cr

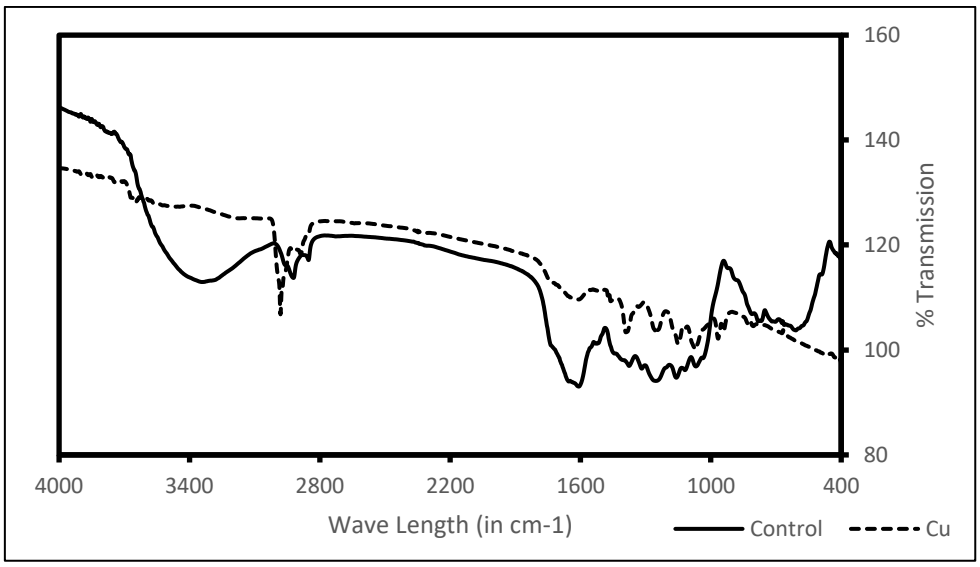


Figure. 68 FTIR spectrum - Control / Cu

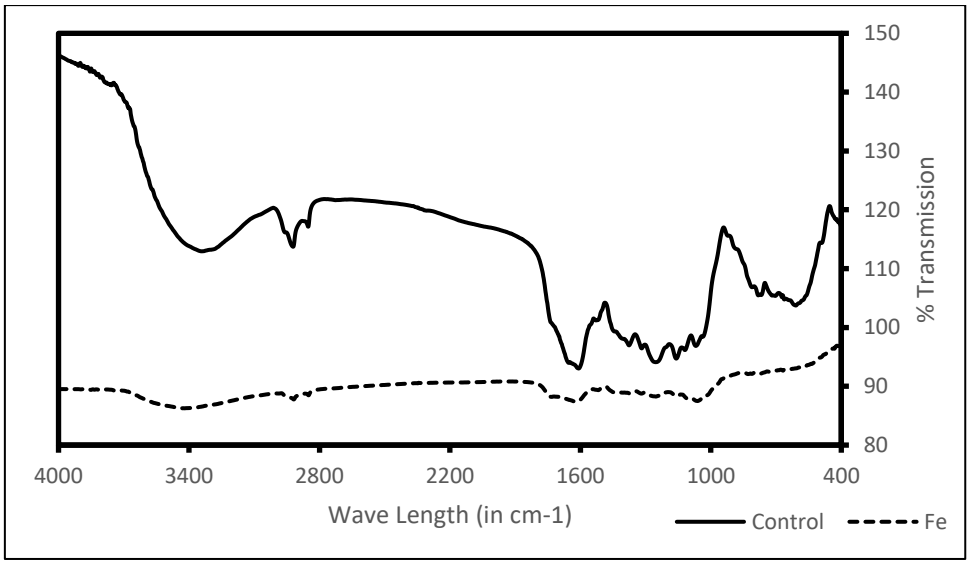


Figure. 69 FTIR spectrum - Control / Fe

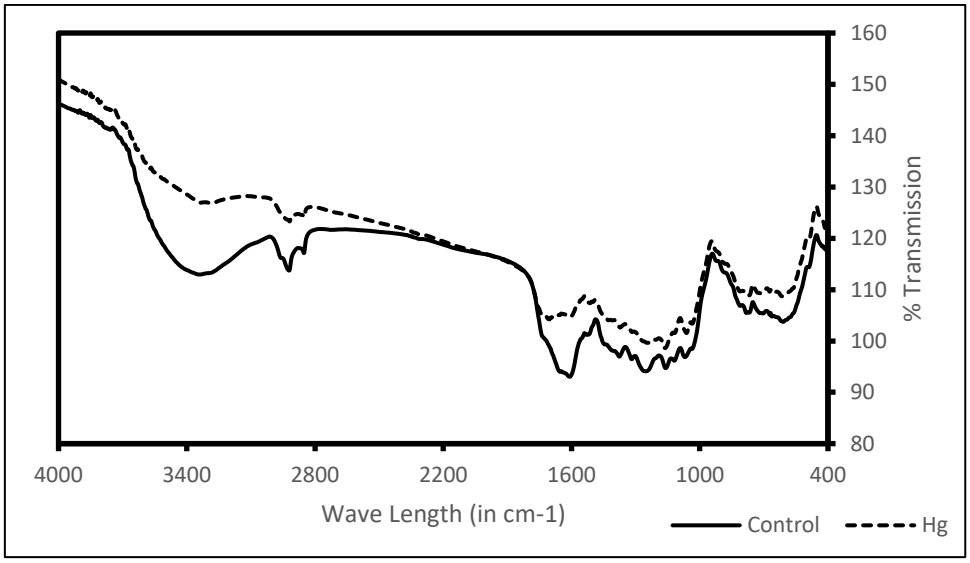


Figure. 70 FTIR spectrum - Control / Hg

IN VITRO CYTOTOXICITY STUDIES

The cytotoxic potential of *Artemisia nilagirica* leaf extract is examined in this study under various metal toxicity conditions (aluminium, chromium, copper, iron and mercury). In a controlled experimental setup, the percentage of cell death at different drug concentrations (12.5 µg/mL to 200 µg/mL) was used to evaluate the cytotoxicity. The information shows how each metal affects the cytotoxic effect of the plant extract and is presented as mean cell death percentages ± standard error (S.E.) (Table.33; Fig.71).

Control Group

Cell death increased in a dose-dependent manner in the control group, which was exposed to no metals and merely received the leaf extract. The cell death rate was 89.4% at the maximum dose (200 µg/mL), demonstrating the intrinsic cytotoxicity of *A. nilagirica* leaf extract. This implies that the extract itself possesses potent anti-cancer effects that intensify with increasing concentration (Fig. 72).

Effect of Metals

Aluminium (Al): Cytotoxic capability of *A. nilagirica* is obviously impaired by Al. With cytotoxicity values of 8.52% and 13.1%, respectively, Al exposure

significantly reduces cell death at lower concentrations (12.5 and 25 µg/mL) as compared to the control. Even at the maximum studied concentration (200 µg/mL), Al only produced 81.6% cytotoxicity, which is less than the 89.4% shown in the control. However, this inhibition becomes less noticeable at higher concentrations. It is possible that Al interferes with active compounds in *A. nilagirica*, that cause cell death by interacting with cellular components (Fig. 73).

Chromium (Cr): Like Al, Cr also showed a decrease in the cytotoxic effect at lower doses. At 12.5 µg/mL (9.97%) and 25 µg/mL (13.9%), the cytotoxicity of the leaf extract was reduced; however, at 150 µg/mL, it increased to 32.0%. However, Cr produced less cell death (72.1%) than the control, even at the maximum dosage (200 µg/mL). Through its interactions with cell receptors or enzymes essential for cell death, Cr may decrease the bioavailability or effectiveness of active phytochemicals of plant (Fig. 74).

Copper (Cu): In contrast to Al and Cr, Cu had a less severe adverse effect on cytotoxicity. At lower concentrations (12.5 µg/mL at 4.46% and 25 µg/mL at 4.68%), Cu did decrease cell death; nevertheless, even at greater concentrations, the cytotoxicity remained relatively moderate, reaching a maximum of 37.4% cell death at 200 µg/mL (Fig. 75).

By interfering with the bioactive substances, possibly through redox cycling, Cu may limit the amount of cellular damage that the compounds might cause. Copper may also reduce the anticancer potential via modulating oxidative stress.

Iron (Fe): Anticancer potential of *A. nilagirica* continued to be hindered by iron, although a less noticeable decrease in cytotoxicity. Although the cytotoxicity increases with Fe concentration, it was still significantly lower than the control (89.4%) at its peak (40.4% at 200 µg/mL). Because iron is a necessary element, it may change the metabolic pathways involved in cell death processes, which in turn may change the bioavailability of the bioactive components in the leaf extract. Iron may also cause oxidative stress in cells, which could alter apoptotic pathways and reduce the ability to cause cell death (Fig. 76).

Mercury (Hg): Of the metals examined, mercury had remarkable cytotoxic-inhibiting effect. It allowed 4.68% cell death at 12.5 µg/mL and 6.37% at 25 µg/mL. Whereas effect on cytotoxicity, however, became increasingly noticeable as concentration rose, only reached a peak of 31.9% at the highest concentration. Potential of plant to cause cancer cell death may be diminished by interactions of Hg with cellular components, which may include cellular membrane rupture or inhibition of cellular repair systems (Fig. 77).

The study shows that metal ions, including mercury, iron, copper, chromium, and aluminium, may reduce anticancer potential of *A. nilagirica* by modifying its cytotoxic effects in a concentration-dependent way. These metals disrupt the bioactive compounds in the leaf extract, which may change how well they cause cancer cell death.

Table 33. Effect of aluminium, chromium, copper, iron and mercury on % Cytotoxicity of leaf extract of *Artemisia nilagirica* (Clarke) Pamp.

Drug concentration (µg/mL)	**% Cell Death**					
	Control	**Aluminium**	**Chromium**	**Copper**	**Iron**	**Mercury**
12.5	12.0±1.2	8.52±1.5	9.97±0.8	4.46±0	4.39±0	4.68±0.4
25	18.2±2.6	13.1±1.7	13.9±0.6	4.68±0.4	6.14±0	6.37±1.1
50	35.7±2.8	19.8±1.8	20.0±1.6	6.47±1.1	8.04±1.5	9.5±0.7
150	56.9±1.3	42.3±2.6	32.0±1.7	11.2±1.6	15.0±1.2	12.9±1.2
100	78.5±0.7	69.2±1.5	48.0±0.8	17.1±1.3	18.3±1.4	20.4±1.6
200	89.4±2	81.6±1.1	72.1±2.8	37.4±1.8	40.4±2.2	31.9±1.2

Values given are mean of 3 replicates ±S.E

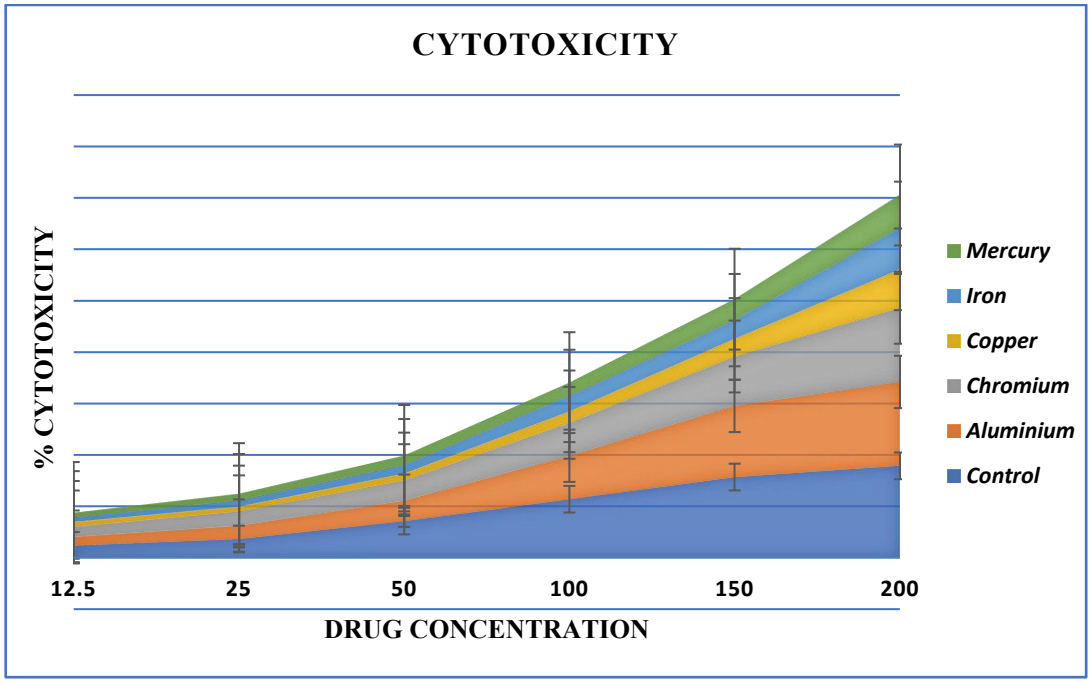


Fig. 71 Effect of aluminium, chromium, copper, iron and mercury on % Cytotoxicity of leaf extract of *Artemisia nilagirica* (Clarke) Pamp.

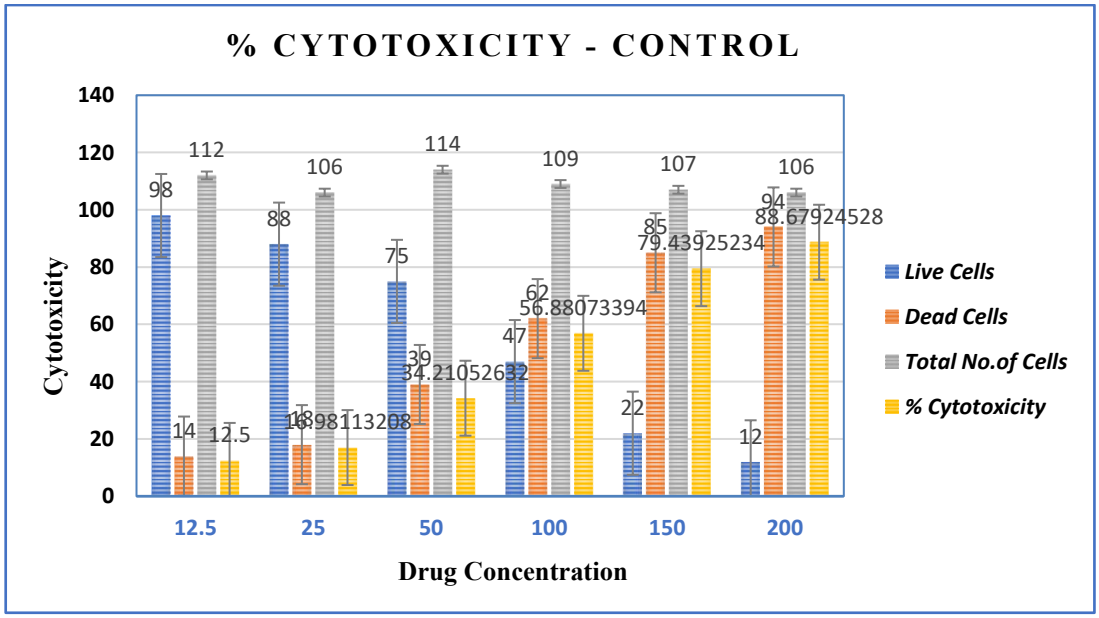


Fig. 72 Percentage cytotoxicity of leaf extract of *Artemisia nilagirica* (Clarke) Pamp. at different concentrations.

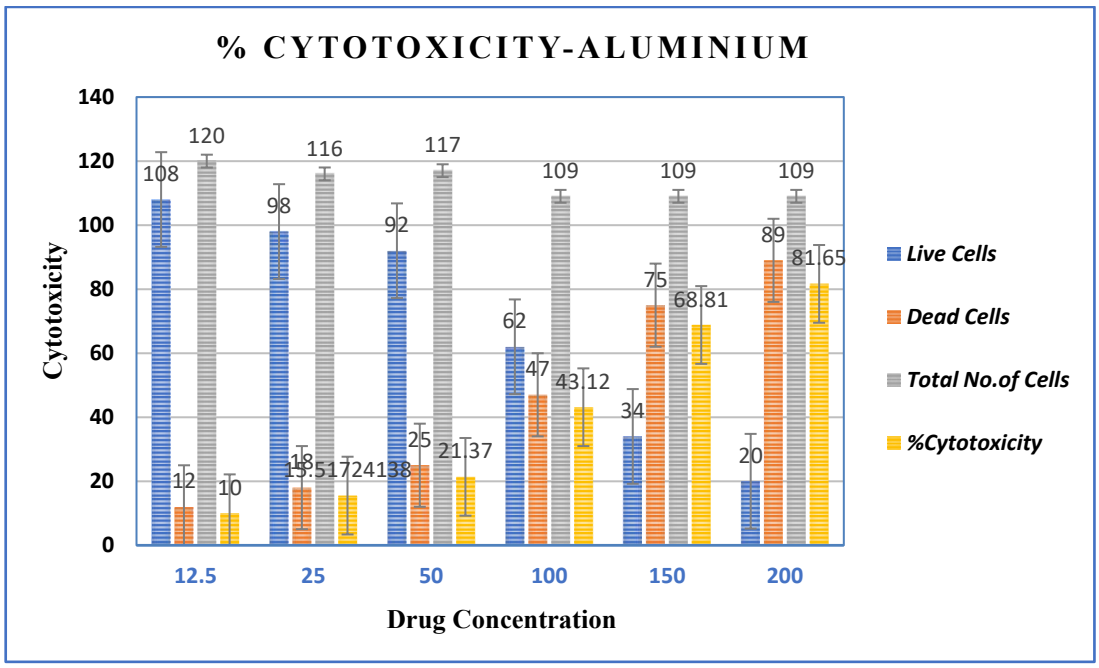


Fig. 73 Percentage cytotoxicity of leaf extract of *Artemisia nilagirica* (Clarke) Pamp. treated with aluminium.

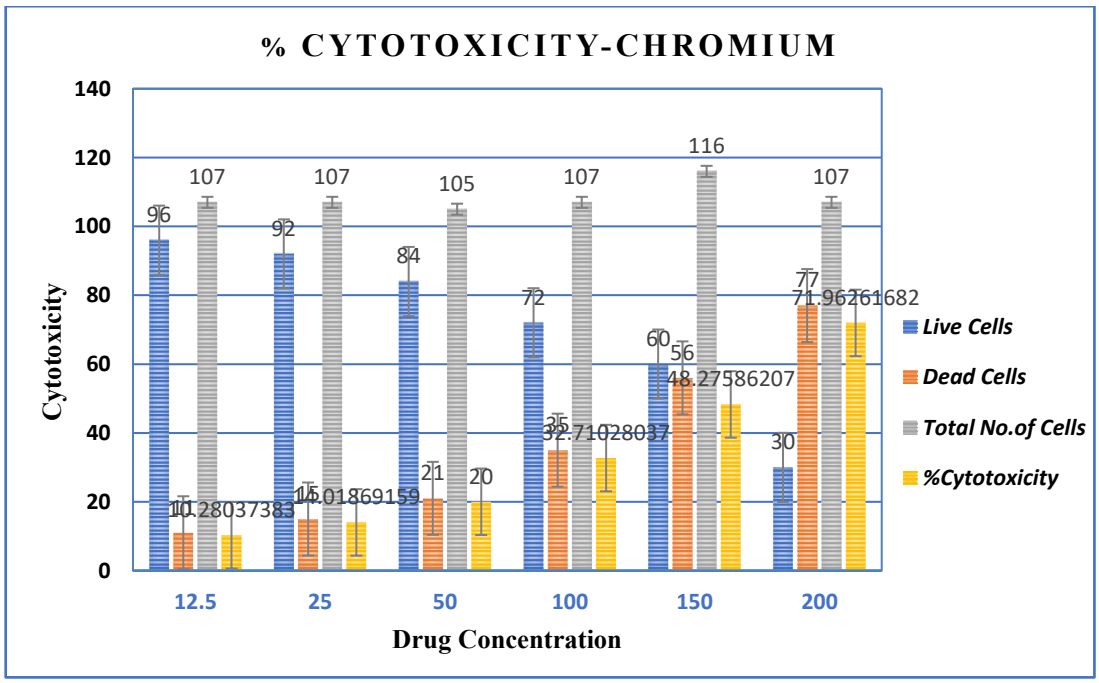


Fig. 74 Percentage cytotoxicity of leaf extract of *Artemisia nilagirica* (Clarke) Pamp. treated with chromium.

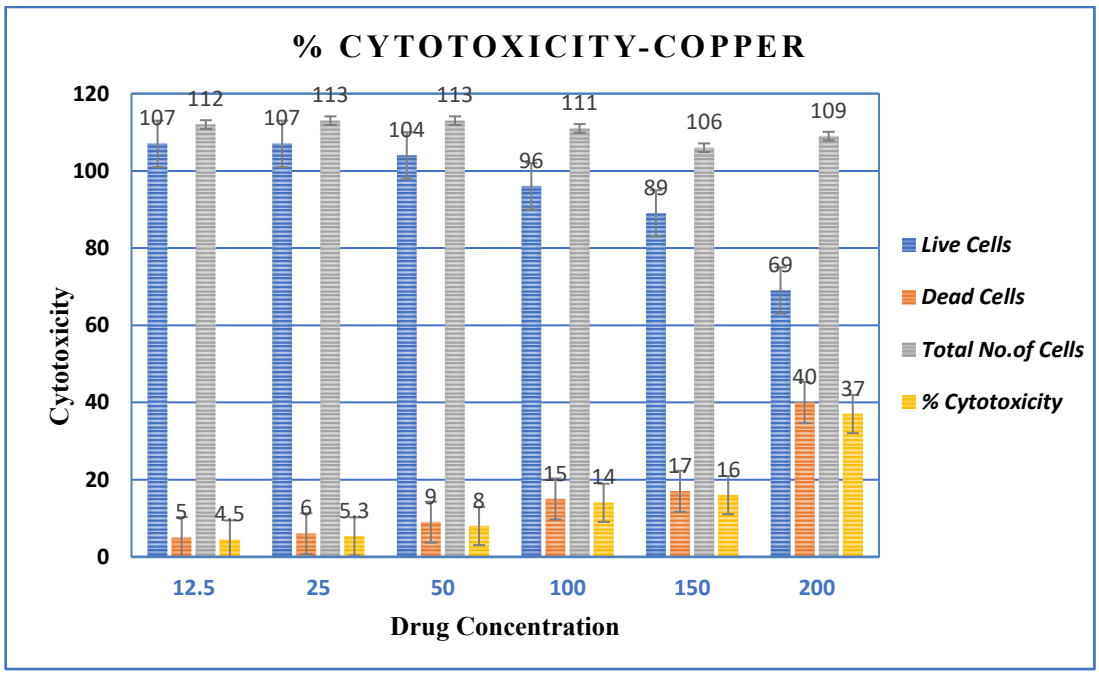


Fig. 75 Percentage cytotoxicity of leaf extract of *Artemisia nilagirica* (Clarke) Pamp. Treated with copper.

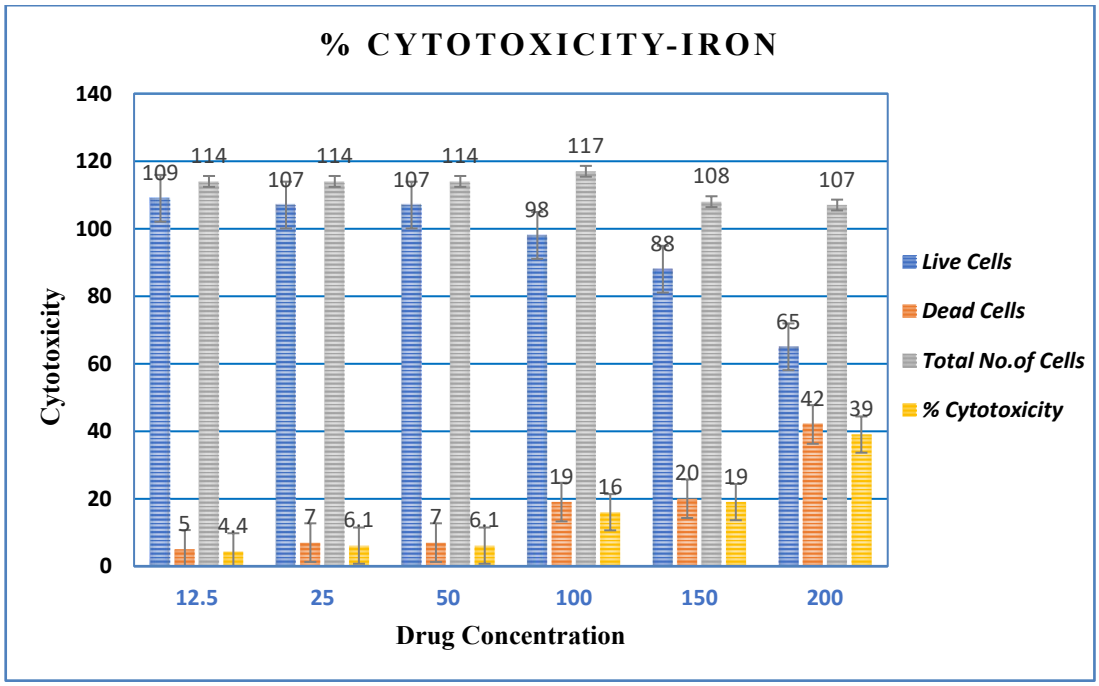


Fig. 76 Percentage cytotoxicity of leaf extract of *Artemisia nilagirica* (Clarke) Pamp. treated with iron.

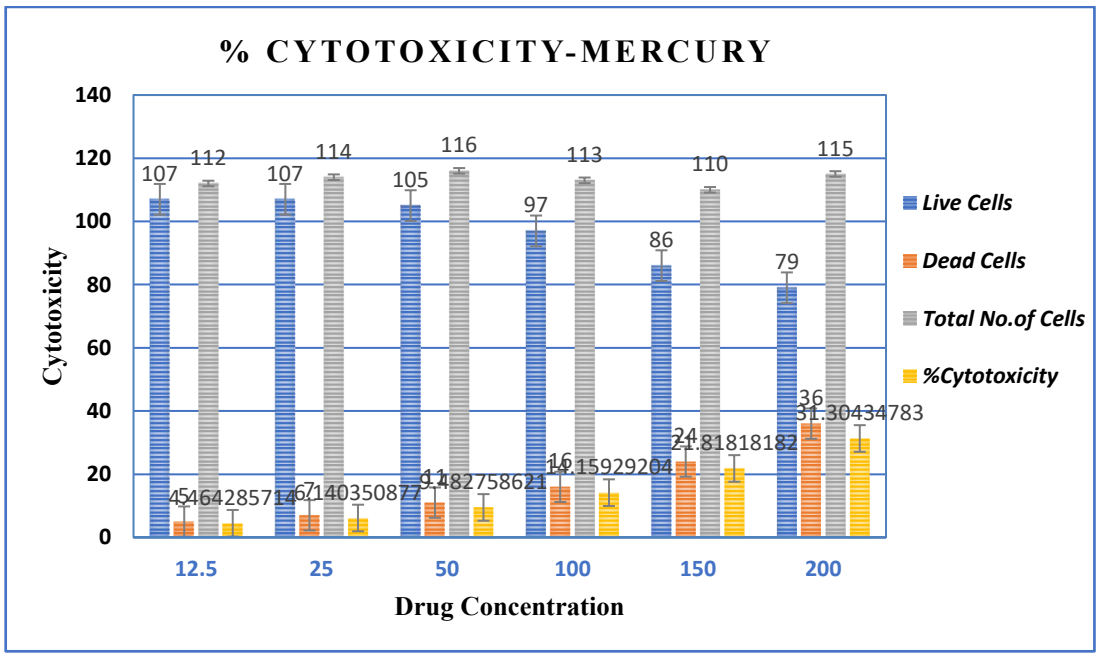


Fig. 77 Percentage cytotoxicity of leaf extract of *Artemisia nilagirica (*Clarke) Pamp. treated with mercury.

CHAPTER 5
DISCUSSION

The impact of heavy metals like Al, Cr, Cu, Fe and Hg on *Artemisia nilagirica* was examined through screening experiments using varying concentrations of the salts of aluminium (AlCl3), chromium (K2Cr2O7), copper (CuSO4), iron (NaFeEDTA) and mercury (HgCl2). It was determined that 80 µM Al, 70 µM Cr, 50 µM Cu, 70 µM Fe and 10 µM Hg showed toxicity symptoms that resulted in 50% growth retardation. The species and metal have an impact on the sensitivity and/or tolerance of plants to heavy metals, according to a comprehensive study by Foy *et al.* (1978). According to the authors, reference trials with a range of values that show around 50% growth retardation while retaining plant growth and survival should be utilized to establish the optimal concentrations for assessing the toxicity of selected heavy metals (Tables. 2-6).

Growth retardation is one of the most obvious effects of heavy metal poisoning on plants. When compared to the control, the growth pattern and rate of *A. nilagirica* grown in Hoagland nutrient medium containing known amounts of Al (80 µM), Cr (70 µM), Cu (50 µM), Fe (70 µM) and Hg (10 µM) showed notable differences in root and shoot length, leaf area and total biomass productivity. The toxicity thresholds of each metal are implicitly revealed by the striking variations in quantities that cause nearly identical development retardation and survival maintenance. The concentrations of metals chosen for the culture tests to cause growth retardation that is roughly comparable while maintaining plant life varies greatly, indicating the varying degrees of toxicity of each metal to *A. nilagirica*.

Morphological criteria such as root length, shoot length and leaf area are used to evaluate the growth pattern of the heavy metals chosen for treatments on *A. nilagirica* in this study.

Impact of Heavy Metals on Root Growth in *Artemisia nilagirica*

The detrimental effects of metals such Al, Cr, Cu, Fe and Hg are highlighted by the observed decrease in root growth in *A. nilagirica* after heavy metal exposure (Fig.3 & Table.7). This is consistent with research showing that heavy metals affect vital physiological functions in plants, such as the uptake of nutrients and water, the control of oxidative stress, and the integrity of the cell wall (Huang and Cunningham, 1996; Singh, 2005; Hussain *et al.*, 2010; Solanki and Dhankhar, 2011; Pantoja, 2021).

The present study reveals retardation of normal root growth associated with Al toxicity. This is in agreement with the view of Horst *et al.* (2010). According to the authors, aluminium mostly affects root meristems, preventing extension and resulting in structural defects. In crops like rice, higher amount Al has also been demonstrated to affect cell wall polysaccharides, decreasing root elongation and flexibility (Yang *et al*., 2008). Oxidative stress caused by Cr produces reactive oxygen species (ROS) that harm enzymes and membranes in cells. Furthermore, Cr inhibits elongation via influencing mitotic activity at the terminals of roots (Kopittke *et al*., 2008). The same trend is exhibited by *A. nilagirica* upon Cr toxicity.

Copper contamination caused severe reduction in root length of *A. nilagirica.* This aligns with the perspectives of Munzuroglu and Geckil (2002), states that, Cu impairs enzymatic processes, especially in root cortical cells, which results in decreased root growth. Excess iron causes oxidative damage and metabolic abnormalities even if it is necessary. According to studies on cucumbers, elevated iron interferes with proton pumps and lowers the amount of ATP available in root cells (Kabala *et al*., 2008). Similar trend is displayed by *A. nilagirica* towards Fe toxicity.

The most hazardous was Hg, which cause severe growth retardation in roots of *A. nilagirica.* This is well explained by Hossain et al., (2012) that, Hg interfered with cellular detoxification mechanisms and enzymatic activity, preventing root elongation. It attaches to sulfhydryl groups of proteins, upsetting metabolic processes and making oxidative stress worse.

Effects of Heavy Metals on Shoot Growth in *Artemisia nilagirica*

The effects of Al, Cr, Cu, Fe and Hg on stem elongation in *A. nilagirica* were examined in this study. With differing levels of toxicity linked to each metal, the data clearly show an inhibitory influence on stem growth (Fig.4 & Table.8). With a stem length of 29.02 cm by day 20, Al had the least inhibitory effect and was nearly identical to the control. However, long-term exposure might affect vascular function, which lowers the availability of nutrients and water for stem elongation (Kochian *et al*., 2015). Stem elongation was moderately inhibited by Cr exposure, resulting in an ultimate length of 23.2 cm. Disruptions in photosynthetic processes and oxidative stress are associated with Cr^{6+} toxicity. Additionally, chromium suppresses the movement of nutrients from roots to shoots, which indirectly affects the growth of shoots (Shanker *et al*., 2005). The amount of energy needed for cell division and elongation is limited by the decline in photosynthetic efficiency.

The shoot length was only 21.84 cm by day 20 due to the substantial toxicity of Cu, an essential micronutrient that becomes toxic when present in excess (Yruela, 2009). ROS damage lipids, proteins and DNA, which hinders cellular respiration and energy metabolism required for elongation. Copper accumulation in vascular tissues also hinders the transport of nutrients and water, which further restricts growth. By day 20, excess Fe had decreased stem elongation to 22.48 cm. Although Fe is necessary for metabolism, at high concentration it produces hydroxyl radicals, which harm cells (Kabala *et al*., 2008). Proton pumps in the plasma membrane are inhibited by Fe toxicity, which affects cytoplasmic pH and nutrient uptake-two processes essential for cell proliferation in elongating tissues.

Mercury demonstrated the most toxicity, limiting shoot elongation to 20.3 cm by 20^{th} day. This result is corroborated with the following findings. Enzymatic processes necessary for cell wall synthesis and cell division are disrupted by high reactivity of Hg with sulfhydryl groups in proteins (Hossain *et al.*, 2012). Mercury also inhibits aquaporins, which decreases water intake, which is essential for turgor-driven stem elongation. Mercury exposure produces ROS, which worsen oxidative damage and cause cellular dysfunction (Qian *et al*., 2021).

Different metals have different physiological and biochemical effects, which is reflected in their differing levels of toxicity. Because of their strong attraction to cellular constituents and ability to produce oxidative damage, Hg and Cu caused most significant growth retardation. Al had comparatively minor effects on shoot elongation, whereas Fe and Cr showed moderate toxicity. The mobility and chemical reactivity of these metals in plant tissues are consistent with this hierarchy.

Effects of Heavy Metals on Leaf Area in *Artemisia nilagirica*

Plant growth is negatively impacted by heavy metals, and one important indicator of toxicity is the effect on leaf area. The detrimental effects of Al, Cr, Cu, iron Fe and Hg on *A. nilagirica* leaf area are highlighted in this study. Disruptions in physiological and biochemical processes necessary for photosynthesis and growth are reflected in the reported decreases in leaf area (Fig.5 & Table.9).

The leaf area was reduced the least by Al (80 µM), with growth reaching 9.98 cm^2. Leaf growth is indirectly limited by Al toxicity, which mainly impacts root systems by changing calcium signalling and nutrient absorption. Unfortunately, compared to other metals, its effects on leaf expansion were less severe, most likely because of its limited translocation to aerial tissues (Kochian *et al*., 2015).

The development of the leaf area was severely hindered by Cr (70 µM), reaching only 7.14 cm^2. Chromium-induced toxicity mostly damages chlorophyll and decreases stomatal conductance. In addition to limiting nutrient intake, effect of Cr on root function indirectly restricts leaf growth (Shanker *et al*., 2005). Diminished growth of the leaves was probably caused by oxidative damage, reduced cell division and cell growth, and decreased photosynthesis.

The leaf area was reduced to 6.42 cm^2 by Fe (70 µM) toxicity. Excess Fe causes hydroxyl radicals to be produced by the Fenton reaction, which causes oxidative stress and cellular damage, even though Fe is necessary for electron transport and chlorophyll synthesis. Cellular functions necessary for leaf growth and photosynthesis are inhibited by this toxin (Kabala *et al*., 2008).

Cu and Hg exhibited the strongest inhibitory effects on leaf area. Cu restricted growth to 5.75 cm², whereas Hg decreased the final leaf area to 6.4 cm² at a dose of 10 µM. Due to their strong ability to produce ROS, both metals can lead to oxidative stress and harm to biological components such as membranes and chloroplasts. Reduced turgor pressure results from interference of Hg with aquaporin-mediated water transport, whereas excess Cu impairs photosynthetic efficiency by disrupting electron transport in photosystems (Qian *et al*., 2021; Yruela, 2009).

Photosynthetic capacity is immediately impacted by reduced leaf area, which restricts the generation of carbohydrates and the buildup of biomass. A decrease in cell division, elongation, and differentiation as a result of oxidative damage and disturbed cellular homeostasis is indicated by the reduction of leaf expansion under heavy metal stress. Furthermore, the diverse ways that metals behave and react in plant tissues are reflected in the varying levels of toxicity that have been reported.

Effect of Heavy Metals on Tolerance Index in *Artemisia nilagirica*

The effect of heavy metal stress on physiological and metabolic functions of *A. nilagirica* is reflected in the decrease in the tolerance index (TI) (Fig.6 & Table.10). The different levels of toxicity displayed by each metal affected the capability of plants to adapt and flourish in challenging environments. A more thorough examination of the impacts of Al, Cr, Cu, Fe and Hg is provided below, along with insights from current scientific research.

Tolerance Index gradually decreased as a result of Al stress, reaching 71.33 on day 20. Although Al is generally not regarded as hazardous at low concentrations, its buildup in acidic soils can significantly hinder nutrient uptake and root elongation by interfering with the rhizosphere supply of Ca and Mg. Al toxicity impairs cell wall flexibility, especially in the roots, which indirectly lowers the transport of nutrients and water to the shoots and leaves and ultimately affects overall growth, according to recent research by Liu *et al*. (2023).

Chromium toxicity has been closely associated with its capacity to cause oxidative stress through the overproduction of ROS, as evidenced by its TI of 67.33

on day 20. Chromium damages photosynthetic pigments and stomatal conductance, which directly prevents biomass development and production, according to research by Gao *et al*. (2023). Further aggravating cellular damage, Cr can also combine with vital macromolecules like proteins and nucleic acids to create complexes.

Despite being a necessary element, Cu was harmful in high concentrations, causing TI to fall to 66.15. High Cu concentration interferes with metabolic processes, including the electron transport chain in mitochondria and chloroplasts, its function in enzymatic reactions is negatively impacted. According to a Zhang *et al*. (2022) study, High copper content damages photosystem II effectiveness and promotes lipid peroxidation of membranes, which significantly reduces growth tolerance.

By day 20, Fe toxicity had caused a TI of 68.3, which was explained by the Fenton reactions that produced hydroxyl radicals and harmed cellular structures. Iron is an essential nutrient, but in high concentration it disturbs redox state out of balance, which damages the integrity of the chloroplasts and the photosynthetic apparatus. Recent research by Sharma *et al*. (2023) indicates that Fe-induced oxidative stress may also affect the control of phosphorus and nitrogen metabolism, which would increase the toxicity.

Mercury had a major effect on TI, indicating that even at low quantities, it has a high potential for phytotoxicity. Mercury affects metabolic pathways and enzymatic activity by breaking down the thiol groups in proteins. Plants are susceptible to oxidative damage because of strong affinity of Hg for sulphur compounds, which disrupts glutathione metabolism and antioxidant defence (Park *et al*., 2023).

Effect of Heavy Metals on Stomatal Index in *Artemisia nilagirica*

In *Artemisia nilagirica*, the stomatal index (SI) of the upper and lower epidermis (UE and LE) responded differently to heavy metal treatments, the degree of these alterations varied according to the type of metal and the length of exposure (Fig.7 & 8; Table.11).

SI decreased slightly for both UE and LE after- Al treatment. According to Ma *et al*., (2021), that this reaction may be related to disruption of cell wall characteristics

and its suppression of cell division in the epidermis by aluminium. Auxin distribution, which controls stomatal development, may be impacted by Al stress, as evidenced by the reduced stomatal differentiation seen in a number of plant species (Yang *et al*., 2020).

SI was considerably elevated by Cr exposure, particularly in the LE. According to Sundaramoorthy *et al*. (2022), Cr stress is known to cause oxidative damage and interfere with cellular signalling pathways that affect the patterning of epidermal cells. According to reports, Cr stress causes increased stomatal density as a compensatory mechanism for decreased carbon dioxide uptake and poor photosynthesis (Lopez-Luna *et al*., 2021). Its function in improving transpiration efficiency under stress may be the reason for the more noticeable effect on the LE.

Both UE and LE had higher SI values after copper treatment, with the LE showing a more pronounced increase. Reactive oxygen species (ROS) are known to be overproduced in response to Cu poisoning, which disrupts the development of stomata and the differentiation of epidermal cells (Ali *et al*., 2022). In situations where copper-induced photosynthetic inhibition is present, this rise might constitute a physiological reaction to preserve gas exchange. In addition, iron led to modest increases in SI, which were more pronounced in the LE. Iron is a necessary nutrient, but too much of it can cause oxidative stress and interfere with stomatal control (Wang *et al*., 2021). As plants try to adjust to stress by controlling transpiration and gas exchange, iron excess may result in altered stomatal development.

With a sharp increase in values over time, Mercury had the most noticeable effect on SI, particularly in the LE. By attaching to thiol groups in proteins, changing the activities of enzymes, and producing oxidative stress, mercury poisoning seriously impairs cellular functions (Ghosh *et al*., 2023). An overstimulation of stomatal patterning pathways in response to significant cellular damage is probably the cause of the unusual increase in SI after mercury exposure.

The findings indicate that SI, especially in the LE, increased most significantly as a result of mercury exposure. This is consistent with previous studies that show how Hg affects stomatal growth as a part of a stress-adaptive response by plant. Hg is

known to cause oxidative stress, which upsets cellular equilibrium and causes stomatal density to rise. It is believed that this reaction improves transpiration and gas exchange to offset metabolic alterations brought on by stress.

As a detoxifying technique, phytovolatilization involves plants transforming ingested mercury into less harmful volatile forms, mostly elemental mercury (Hg^0), which are then expelled through stomata. Because it lessens intracellular Hg accumulation, this process is important in animals that can withstand mercury. According to studies, the release of Hg^0 is facilitated by the increased stomatal density during Hg stress, making it an essential survival mechanism. Wu *et al*. (2023), for example, showed that Hg stress in *Arabidopsis* enhanced phytovolatilization efficiency by increasing stomatal density. Similarly, research on Oryza sativa and Brassica juncea showed that stomatal number and mercury detoxifying capacity were positively correlated (Gupta *et al*., 2022; Zhao *et al*., 2021).

The formation of ROS and altered phytohormonal signalling, specifically involving cytokinin and abscisic acid (ABA), are connected to the mercury-induced increase in SI. While cytokinin encourages stomatal patterning, ABA controls stomatal closure during stress. Increased stomatal differentiation results from disruption of these mechanisms by mercury (Ghosh *et al*., 2023). Additionally, Hg stress changes the expression of genes that regulate stomatal development, including MUTE and SPCH (SPEECHLESS), which helps explain the observed increases in stomatal density (Zhang *et al*., 2023).

The substantial rise in SI during mercury stress highlights the dual function of metal in detoxification and physiological adaptation. Potential of *A. nilagirica* for phytoremediation is demonstrated by phytovolatilization, which is made possible by increased stomatal density. In addition to reducing mercury toxicity, this mechanism shows how adaptable the species is to contaminated surroundings.

Scanning Electron Micrographs - Stomata

The noticed differences in stomatal behaviour and structural adaptations of *A. nilagirica* during heavy metal treatments demonstrate the physiological responses to

environmental stress by the plant. Analysis using SEM showed that the type of metal exposure had a substantial impact on the distribution patterns and stomatal aperture (Fig. 9 &10).

Stomata in control plants seemed nearly closed, indicating a physiological state that is ideal for gas exchange and water saving in typical circumstances. This result is consistent with other research that highlights the function of stomatal closure in controlling transpiration and preserving homeostasis in non-stressful circumstances, such as that conducted by Hetherington and Woodward (2003). In the absence of outside stimuli, the plant functioning is probably at its baseline in this closed state.

Stomata showed less or no opening when exposed to Cr and Al, indicating a protective reaction to these harmful elements. Dehydration and oxidative stress are known to result from interference of Al with root water uptake and ion transport (Poschenrieder *et al*., 2008). Similar to this, chromium interferes with photosynthesis and causes the buildup of ROS, which makes stomatal closure necessary to reduce further ion entry and water loss (Shanker *et al*., 2005). limited stomatal aperture in *A. nilagirica* may therefore be a survival tactic to lessen the harmful effects of these metals.

However, in contrast to the other treatments, plants treated with Hg displayed stomata that were widely open. This behaviour might be related to the phytovolatilization of Hg, a detoxifying process in which plants emit volatile mercury through their stomata. The function of stomata in mercury volatilization has been previously studied by Meagher and Heaton (2005), suggesting that this process aids in lowering intracellular accumulation and toxicity. Adaptive response of the plant to mercury stress is demonstrated by the stomatal opening that was seen in *A. nilagirica* during Hg treatment, which strongly supports this detoxification process.

In contrast to the control, stomata that were partially open were the result of Cu and Fe treatments. Despite being necessary minerals, Cu and Fe are also hazardous at greater doses because they can cause ROS and interfere with cellular processes (Yruela, 2005; Briat *et al.*, 2015). Disturbances in guard cell function and epidermal

cell growth, which are frequently documented under heavy metal stress, are reflected in the uneven stomatal distribution seen in Cu-treated plants and the partial stomatal opening after Fe treatment.

Studies by Broadley *et al*. (2007), which characterize stomata as crucial interfaces for gas exchange and water management, are consistent with the alterations in stomatal structure shown under heavy metal stress. These findings highlight the dual function of stomata as both ion uptake inhibitors and detoxification promoters via mechanisms like phytovolatilization.

Impact of Heavy Metals on Anatomy of *Artemisia nilagirica* (Clarke) Pamp.

The morphological changes or impacts of these specific metals: Al, Cr, Cu, Fe and Hg are also associated with the anatomical changes observed in light and scanning electron microscopy studies. Metal deposition in plants altered the structure of the roots, stem and leaves (Adejumo *et al*., 2021).

The Al treatment caused damage to piliferous cells and the loss of root hair, resulting in substantially decreased root development (Fig. 11- A). These results had been observed in plants exposed to Al before (Linderberg and Greger, 2002). According to authors, crop productivity was influenced by nutritional deficits that resulted in less inhibition of root development because of Al toxicity. Al also causes distinct endodermis with a discernible cortical area and a specific thickening of the cell wall, which alters the structure of the roots. The endodermis of the root thickens as a result of propensity of Al to bind to membrane proteins (Linderberg and Griffiths, 1993) and form cross connections between pectin and cell wall proteins (Chang *et al*., 2002). Changes in root morphology, such as atrophy of root hair and swelling of root tips, as well as inhibition of root growth, have also been connected to Al toxicity in plants (Huang *et al*., 2014). According to Wang *et al*. (2016), Al can seriously injure plants, particularly the roots, at micromolar quantities. According to Riyaz *et al*. (2018), the morphology of the roots mostly controls tolerance to Al and the root tip is essential for detecting Al toxicity. To reduce the damage caused by Al buildup, the plants use a variety of physiological strategies (Kochian *et al*., 2015; Riaz *et al*., 2018).

There are more xylem vessels in the stem of *A. nilagirica* that has been treated with Al, according to its anatomy (Fig.12 -D).

The Al treatment caused a number of anatomical changes in the plants. Rice poisoning reduced the diameter of the roots and shoots, reduced the number of metaxylem vessels in the root, and caused the stomata in the leaves to close (Samad *et al*., 2019). It was found that the midrib region of the lower epidermis and the vasculature shrank in size as a result of the Al treatment in the leaves. Silva *et al*. (2016) found that in the Al-sensitive sunflower cultivar, the Al stress led to increased parenchyma layers and leaf blade thickness as well as lignification of the root tissues. These changes in plant anatomy can be used as markers of Al sensitivity in sunflowers. Ciamporova (2002) describes morphological alterations in the roots after Al treatment, including thickening, disruption of the peripheral tissues of root and the appearance of lateral roots closer to the root tip.

In chickpea plants exposed to Al, the size and number of vessels within the root, the length of the main root and the number of lateral roots all decreased. The leaf had fewer palisade parenchyma cells and the stem had more sclerenchyma cells (Samad *et al*., 2021). Corn plants grown in Al solutions exhibited fewer lateral roots, slower root growth and morpho-anatomical alterations in the leaf, including a thinner cuticle layer and smaller metaxylem and protoxylem diameters in the vascular bundle (Batista *et al*., 2013). The *Stenocalyx dysentericus* seedlings showed resistance to Al with increased root relative elongation and internal detoxifying mechanisms observed in the root tissue (Rodrigues *et al*., 2016). Water mimosa plants exhibited yellowing leaves, weaker roots, stele area contraction and a change in the colour of the leaf tissue when exposed to Aluminium sulphate.

Chromium treatment resulted in notable structural alterations in the roots, stem and leaves of *A. nilagirica*. There is a discernible stelar region composed of large xylem vessels and pith in the Cr-treated plant. It has been demonstrated that Cr stress damages the piliferous layer of roots and results in the loss of root hair (Fig. 13-A). SEM images revealed that the depositions had changed the most. SEM images of the control root and shoot displayed the characteristic epidermis, cortex, vascular region

and pith; however, upon exposure to Cr, these tissues underwent deformation. This outcome is consistent with research on plants such as Cicer arietinum and Vigna radiata (Chandra *et al*., 2010; Medda and Mondal, 2017).

The root, stem, and leaves of *A. nilagirica* underwent significant structural alterations as a result of chromium treatment. The plant treated with Cr has a noticeable stelar zone made up of big xylem vessels and pith. *A. nilagirica* suffers damage to its piliferous layer of roots and loses its root hair when under Cr stress (Fig.13- B). The depositions were the most noticeable alteration, according to SEM images. The cortex, vascular region, pith and epidermis were all clearly visible in the SEM images of control root and shoot; however, these tissues were distorted after being exposed to Cr. This conclusion is supported by reports from plants such as *Cicer arietinum* and *Vigna radiata* (Chandra *et al*., 2010; Medda and Mondal, 2017).

Chromium treatment caused significant structural changes in the root, stem and leaves of *A. nilagirica.* There is a conspicuous stelar region with large xylem vessels and pith when *A. nilagirica.* is treated with Cr. Chromium stress has been found to cause *A. nilagirica* to ruin its piliferous layer of roots and lose its root hair. SEM images showed that the depositions were the most noticeable alteration. The distinguishing characteristics of the cortex, vascular region, pith, and epidermis were visible in the scanning micrograph of the control root and shoot; however, these tissues were distorted upon exposure to Cr. Similar findings have been reported for plants such as *Cicer arietinum* and *Vigna radiata* (Chandra *et al*., 2010; Medda and Mondal, 2017).

Compared to the leaf, the root and stem suffered more damage; there were some tissue depositions and a considerable distortion of the cell structures (Fig.12 E). This is consistent with Wakeel *et al*. (2020) findings. The reduction of Cr (VI) to Cr (V) during the symplast-mediated endodermis transit could be one reason for the anomalies in the roots. The cells of the root cortex then hold onto Cr (V).

Chromium may migrate from the root to the stem as a result of ongoing exposure to Cr. Higher Cr concentrations can result in increased ROS generation, oxidative damage at the cellular level, and stem morphological defects (Shanker *et*

al., 2005). According to Shanker *et al*. (2004), cell dysfunction brought on by Cr buildup in roots and the extraction of Ca^{2+} ions from binding sites in cell walls and plasma membranes can also lead to harm to root and stem tissues. Accordingly, poor translocation from the root to the shoots and the sequestration of Cr in the root vacuoles alter the structure of the root (Shanker *et al*., 2004). This is related to the reduced root growth in *A. nilagirica.*

Chromium may transfer from the root to the stem as a result of ongoing exposure to Cr. Increased ROS generation and anomalies in stem morphology can result from oxidative damage caused by increased Cr concentrations at the cellular level (Shanker *et al*., 2005). Cell dysfunction brought on by Cr buildup in roots and the removal of Ca^{2+} ions from binding sites in cell walls and plasma membranes can also damage root and stem tissues (Shanker *et al*., 2004). Due to the sequestration of Cr in the root vacuoles and insufficient transport from root to shoots, this can be associated with reduced root growth in *A. nilagirica* (Shanker *et al*., 2004).

Scanning electron micrographs showed specific masses on the mesophyll cells of the Cr-treated leaves (Fig.19- F). The barrier of the apoplast to water flow is increased by cell wall thickening caused by metal deposits and/or incrusting elements in the cell walls (Rucinska-Sobkowiak, 2016). The SEM-EDX data indicates that the macro and microelement content of the shoot is lower than that of the roots, which will ultimately lead to less water and soluble mineral nutrients being transferred from the root to the shoot.

The root, stem and leaf tissues of Parkinsonia aculeata plants treated with Cr exhibited thicker xylem walls (Zhao *et al*., 2011). *Cicer arietinum* exhibited clear structural alterations in response to Cr stress, such as an increase in the xylem wall thickness and diameter (Medda and Mondal, 2017). *Zea mays* seedlings also displayed indications of cell structural abnormalities and inclusions of Cr deposits in the xylem wall and inner parenchyma cells (Singh *et al*., 2015). According to Purohit and Varghese (2003), responses of tomato and brinjal plants to Cr stress were indicated by structural alterations in the xylem and thickening of the xylem wall. Our results are in agreement with these findings.

Following copper treatment, *Artemisia nilagirica* exhibited angular, thick-walled xylem vessels, a damaged piliferous layer and no root hairs at all (Fig.14- A). Cu poisoning damages roots because copper is an essential metal that is involved in many metabolic processes. According to Sheldon and Menzies (2005), symptoms might vary from a disturbance of the piliferous layer of root and a reduction in the growth of root hair to more severe root structure deformation. According to Marques *et al*. (2018) and Hossain *et al*. (2020), higher concentrations (sub-lethal) of Cu affect root growth and morphology. According to Bochientio *et al*. (2015) and Cai *et al*. (2014), Cu treatment results in a decline in root biomass and volume, which are directly linked to cell division.

Following Cu stress, there seems to be a decrease in root growth due of a decrease in cell division, which causes the cell walls of the roots to thicken (Batool *et al.*, 2015). The presence of highly stained patches or particles scattered throughout the cells and appearing embedded is a noticeable anatomical feature of *A. nilagirica* roots exposed to Cu (Fig.14- B). These spots show the localized and stained Cu content in the root. The impact of Cu treatment on plant anatomy varied. *Alternanthera tenella* exhibited smaller stem and leaf tissue cells, more druse crystals and fewer active reaction centers when exposed to high copper concentrations (Martins *et al*., 2020).

According to Gong *et al*. (2019), spinach seedlings exhibited changes in cell ultrastructure, such as weaker chloroplast membranes and cell wall disintegration, during exposure to increased Cu concentrations. In spring barley, too much copper caused the cytoplasmic and cell wall membranes to break down, the stele diameter to decrease, and the root epidermis to deteriorate (Minkina *et al*., 2020). In response to higher Cu concentrations, *Billbergia zebrina* showed morphological changes in its roots, such as thicker exodermal cell walls and an increased stomatal index (Martins *et al*., 2016). According to De Souza *et al*. (2017), *Erythrina fusca* exposed to high Cu concentrations showed changes in thylakoid membranes and cell plasmolysis in the root cortical area.

Essential oils (EOs) are oily, fragrant liquids derived from aromatic plants. Essential oils are naturally occurring plant components that capture the flavour and

perfume of the plant. They are primarily found in leaves and flowers. *A. nilagirica* is rich in essential oils. According to Sharifi-Rad *et.al*. (2016), EOs are complex mixtures of volatile phytoconstituents, primarily terpenes, that may be extracted from various plant sections utilizing techniques including steam distillation and hydro distillation. EOs have been used in ancient medical and healing systems due to their diverse array of bioactive phytoconstituents, which include anti-inflammatory, anti-cancer, antioxidant, and antibacterial properties (Moccia *et al*., 2020). EOs are utilized extensively in many different industries, such as pesticides, cosmetics, food preservation, and aromatherapy, in addition to their medical uses. EOs can be extracted using both conventional and innovative methods, but the latter is more effective, time-efficient, and environmentally friendly. EOs are vital to plants in general because they provide them with their unique flavour and aroma, as well as a number of health benefits and applications.

External secretory tissue found outside of plants includes glandular trichomes (secretory glandules or bristles), non-glandular trichomes, and epidermal papillae (Ascensao and Pais, 1998; Turner *et al*., 2000; Baran *et al*., 2010; Rezakhanlo and Talebi, 2010; Kremer *et al*., 2014). Schizogenous pockets, also called secretory pockets, secretory canals, and intracellularly secreting cells are all examples of the internal secretory tissue of plant (Asbahani *et al*., 2015).

Secretory tissues have been found in a number of families, including the Lauraceae, Asteraceae, Rutaceae, Myrtaceae, Poaceae, Cupressaceae and Piperaceae (Bruneton 1999). Secretory glandules are particular histological structures that create, collect, and store essential oils (EOs) (Bouwmeester *et al*., 1995; Bruneton, 1987). Svoboda and Greenaway (2003) established the existence of two types of secretory glandules: those located inside internal organs with endogenous secretion and those found on the outside of plants with external secretion. They are also present in the cytoplasm of specific secretory cells in one or more plant organs.

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Talebi, 2010; Kremer *et al*., 2014). Schizogenous pockets, also called secretory pockets, secretory canals and intracellularly secreting cells are all examples of the internal secretory tissue of plant (Asbahani *et al*., 2015).

One characteristic of leaf anatomy of *A. nilagirica* exposed to Hg, is the production of numerous epidermal hairs on the upper and lower epidermis, most of which mature into trichomes (Fig.15- E). Similar to the structural changes of plants under mercury stress, detoxifications are closely related to changes in leaf structure. The structure of the leaf epidermal patterns is evident from SEM analyses (Fig.22- E). The thin-walled mesophyll cells rupture, leaving some lacuna in the mesophyll tissue. The functional aspects of the structural alterations in the stem and leaf that are linked to the detoxifying mechanism of Hg toxicity are covered in the sections that follow.

Impacts of aluminium, chromium, copper, iron and mercury on elemental distribution in root, stem, and leaf of *Artemisia nilagirica* (Clarke) Pamp.

The analysis of the elemental distribution (EDXMA) in *A. nilagirica* tissues upon treatment with various metals reveals significant alterations in the accumulation of specific elements, reflecting the plant responses to metal stress. The changes observed in the root, stem and leaf tissues of *A. nilagirica* suggest a complex interaction between the plant and the heavy metals, which can have profound implications for the physiological processes of the plant.

Effect of Aluminium

Aluminium is a well-known toxic metal that affects plant growth and development, primarily by altering nutrient uptake and causing oxidative stress (Wang *et al*., 2021). In the current study, the root tissues showed an increase in Al content, along with decrease in other elements. This suggests that Al may interfere with K and Cu uptake, as previously reported in studies showing that Al toxicity often disrupts ion homeostasis in plants, especially by decreasing the uptake of K and Ca (Singh *et al*., 2017). In the stem tissues, Al induced a significant decrease in Ca, which could be related to the fact that Al competes with essential cations like Ca for binding sites in the cell wall and plasma membrane (Miyasaka *et al*., 2020). Additionally, the leaf

tissues showed a decrease in K and Cu, which may indicate that Al impacts the translocation of these elements within the plant, affecting overall nutrient balance and photosynthetic efficiency (Kochian *et al*., 2020).

Effect of Chromium

Chromium exposure in plants often leads to oxidative stress and alterations in the metabolism of essential nutrients (Vazquez *et al*., 2021). In the present study, Cr treatment significantly increased Cr content in root tissues, which is consistent with previous findings where Cr accumulates in roots as plants attempt to sequester and detoxify it (Li *et al*., 2020). The observed decrease in K and Ca in the root tissues suggests that Cr may disrupt the uptake or transport of these essential nutrients, which are vital for cell wall structure and energy transfer. In the stem tissues, the increase in Cr content was also significant, with minor reductions in K and S. This is consistent with the finding that Cr exposure can alter the metabolism of macronutrients and disturb enzyme activities associated with S metabolism (Srivastava *et al*., 2020). In the leaf tissues, Cr treatment resulted in an increase in P, which may indicate a compensatory mechanism to support plant metabolism under metal stress. These results underscore the toxic effects of Cr on nutrient uptake and metabolism, as well as its ability to disrupt normal plant functioning.

Effect of Copper

Copper is an essential micronutrient, but at high concentrations, it becomes toxic, causing oxidative stress and damage to cellular components (Zhao *et al*., 2020). In the root tissues, Cu treatment resulted in a significant increase in Cu content, along with a slight decrease in K and Fe. Cu toxicity has been shown to impair K uptake and disrupt ion balance by inhibiting K channels in the plasma membrane (Marschner, 2012). The increase in Cu content in the stem and leaf tissues reflects the inability of plant to limit Cu accumulation, as the metal translocate from roots to shoots, potentially causing further physiological disruption. This translocation is commonly observed in Cu-treated plants, where Cu accumulates in the vacuoles of leaf cells, potentially interfering with photosynthetic efficiency (Vassilev *et al*., 2019). The slight reduction in Fe content observed in the root tissues may reflect competition

between Cu and Fe for binding sites, as both metals are crucial for redox processes within the plant (Tian *et al*., 2021).

Effect of Iron

Iron is an essential nutrient for plants, playing a key role in chlorophyll synthesis and electron transport in photosynthesis (Yuan *et al.*, 2020). In this study, Fe treatment resulted in a significant increase in Fe content in the root, stem and leaf tissues, suggesting that *A. nilagirica* is able to accumulate Fe in response to external Fe supply. This response is consistent with the ability of many plants to regulate iron uptake through increased ferric reductase activity and synthesis of iron-chelating compounds (Nijhawan *et al*., 2019). The minimal effects on other elements suggest that Fe accumulation occurs without significant disruption to the plant nutrient homeostasis, supporting the notion that Fe is critical for plant health and metabolic processes (Wang *et al*., 2018).

Effect of Mercury

Mercury is a highly toxic heavy metal that can accumulate in plant tissues and disrupt cellular processes by forming bonds with S and N-containing molecules, leading to protein denaturation (Samarakoon *et al*., 2021). The results from the present study show an increase in Hg content in the root, stem, and leaf tissues, suggesting that *A. nilagirica* is capable of accumulating and translocating Hg to aboveground tissues. The increase in Na in the root, stem and leaf tissues may be a stress response, as Hg exposure is known to disrupt ion transport systems, potentially leading to increased Na uptake (Tang *et al*., 2020). The reduction in Cu content in the leaf tissues may reflect Hg-induced oxidative stress and metal displacement, as Hg binds to thiol groups in proteins, impairing metal homeostasis (Pilon-Smits *et al*., 2019). These findings suggest that Hg exposure has a detrimental impact on nutrient balance and can cause ion imbalance, contributing to plant stress and metabolic disruptions.

Effects of aluminium, chromium, copper, iron and mercury on dry weight percentage of *Artemisia nilagirica* (Clarke) Pamp.

The present study evaluates the growth responses of *A. nilagirica* to the exposure of metals such as Al, Cr, Cu, Fe and Hg. These metals are known to affect plant growth by interacting with various biochemical and physiological processes. Our results indicate distinct growth patterns in root, stem and leaf tissues, suggesting the varying impacts of each metal on plant development.

Al exposure generally resulted in moderate growth in both the root and stem, with slightly less pronounced effects on leaf growth. This finding aligns with existing studies that report Al as a potent phytotoxic metal. High concentrations of Al can interfere with nutrient uptake, causing stunted root growth, which, in turn, affects overall plant biomass. The effect of Al on root development is particularly significant because it disrupts root cell elongation by accumulating in root tips, leading to a decrease in root volume and, consequently, the ability of plant to absorb water and nutrients (Mousavi *et al*., 2011). While the stem showed moderate growth, leaf development was less responsive, reflecting the cascading effects of root damage on overall plant development.

In terms of dry weight, Al toxicity often leads to a decrease in plant biomass, especially in root tissues, as excessive Al can impair cell division and elongation (Liu *et al*., 2013). As a result, although there was an increase in dry weight over time, the rate of increase was less pronounced compared to the control group. This slower growth can also be attributed to the inverse relationship between dry weight and moisture content, as the reduction in root function due to Al exposure limits the water uptake by plant, thereby decreasing moisture content and increasing the concentration of dry matter in tissues (Tamas *et al*., 2017).

Chromium exposure showed similar patterns of moderate growth, with steady increases in root and stem weight, though less so in leaf weight. Cr is another metal that can inhibit plant growth by interfering with cellular respiration and inhibiting chlorophyll production (Sharma *et al*., 2014). It has been shown to accumulate in roots and stems, where it disrupts cellular metabolism and reduces the ability of plant to

generate energy for growth. The result is a moderate increase in dry weight, but at a slower rate compared to the control group, as plants struggle to cope with chromium-induced stress. This was reflected in the leaf tissue, where the growth rate was slower, possibly due to impact of Cr on photosynthesis and its interference with leaf structure.

The relationship between dry weight and moisture content is often inverse in plants exposed to metals like Cr. When a plant is under stress from Cr, it might show a higher dry weight percentage due to the reduced water content in tissues. This is because Cr inhibits root water absorption, which leads to dehydration, concentrating the solutes within the plant tissues, and increasing dry matter content (Gul *et al*., 2017).

Copper had the most noticeable inhibitory effect on growth, especially in leaf tissues, with minimal increases in both root and stem dry weight. Cu is an essential micronutrient for plants, but at elevated concentrations, it becomes toxic, causing oxidative stress, inhibiting photosynthesis, and damaging cellular membranes (Baker *et al*., 2017). Excessive Cu accumulation in plant tissues can result in chlorosis, particularly in the leaves and a reduction in overall plant biomass. In this study, the minimal increase in copper-treated plants might reflect these toxic effects, particularly on photosynthetic processes, which are critical for biomass production.

The inverse relationship between dry weight and moisture content is evident in copper-treated plants. As Cu disrupts the ability of plant to maintain cellular functions, including water transport, moisture content is reduced, leading to higher dry weight percentages. Cu-induced stress typically causes a decrease in transpiration, further lowering water uptake and concentrating solutes in plant tissues, which increases the dry weight percentage (Pichler *et al*., 2012).

Iron treatment had a more positive impact on growth, particularly in root and stem tissues, with moderate growth in the leaves. Fe is an essential micronutrient that plays a key role in chlorophyll synthesis and electron transport during photosynthesis. Adequate iron levels can enhance plant growth, increase biomass and improve overall health (Davis *et al*., 2014). In this study, Fe promoted growth in both the root and stem, likely due to its essential role in nutrient assimilation and energy production.

However, the leaf tissue showed less pronounced growth, which could be due to limited impact of Fe on leaf development when compared to its effects on root and stem tissues.

Iron typically supports better hydration and nutrient uptake, leading to balanced water content and dry weight. The dry weight increase in iron-treated plants was more aligned with the control, suggesting that iron does not drastically reduce water content, maintaining a relatively stable moisture to dry matter ratio (Ricci *et al*., 2015).

Mercury exposure had the most detrimental effect on plant growth across all tissues. Hg is a highly toxic metal that disrupts cellular functions, particularly by inhibiting enzyme activity and causing oxidative damage (Nogueira *et al*., 2018). In this study, Hg-treated plants showed minimal growth, especially in leaf tissues. This aligns with previous findings where Hg was shown to inhibit seed germination, root elongation, and overall plant biomass due to its toxicity (Brun *et al*., 2016). The poor growth in Hg-treated plants likely results from its interference with water uptake and cellular respiration, leading to dehydration and reduced cell turgor, which limits both water content and dry weight accumulation.

The inverse relationship between dry weight and moisture content was also evident in Hg-exposed plants. The reduced water content in tissues of Hg-treated plants resulted in an increase in dry weight percentage, as the reduced ability of plant to take up water concentrated solutes in its tissues (De Souza *et al*., 2013).

Effect of Heavy Metal Toxicity on Pigment Content in *Artemisia nilagirica*

The data presented in Table. 16 reveals how heavy metals, such as Al, Cr, Cu, Fe and Hg, affect chlorophyll (Chl *a*, Chl *b* and total chlorophyll) content and carotenoid content in *A. nilagirica*. For plants to absorb light and convert energy, photosynthetic pigments are essential. Metal stress, which manifests as oxidative stress, enzyme inhibition and damage to photosynthetic equipment, interferes with the stability and production of pigments. The results are in line with past studies that highlight how harmful heavy metals are to chloroplast function.

Chlorophyll *a* Content

Under mercury stress, chlorophyll *a*, the main pigment for photosynthesis, showed notable decreases. By day 20, the effects of mercury were most noticeable, as Chl *a* level dropped from 2.01 ± 0.106 mg/g DW to 1.96 ± 0.013 mg/g DW. This decline is consistent with findings that Hg degrades chlorophyll by causing severe oxidative damage and protein denaturation (Patra *et al.*, 2021). According to Sharma *et al*. (2020), Cu and iron also caused notable decreases, most likely as a result of their role in producing ROS that hinder the stability and production of chlorophyll. On the other hand, the impacts of Al and Cr were rather mild; their final Chl levels were 5.02 ± 0.033 and 4.74 ± 0.112 mg/g DW, respectively. This could be explained by partial tolerance of *A. nilagirica* to certain metals through the activation of antioxidant defences.

Chlorophyll *b* Content

Similar patterns to those of chlorophyll *a* were seen in the accessory pigment chlorophyll *b*, although the drop was not as noticeable. Hg has a substantial inhibitory effect on chloroplast function; by day 20, Chl *b* had decreased to 0.56 ± 0.141 mg/g DW (Gupta & Nayek, 2022). Chlorophyllide an oxygenase and other enzymes involved in Chl *b* synthesis were suppressed, as seen by the significant decreases in copper and iron. Chl *b* levels decreased less sharply in response to Al and Cr, with final values of 2.24 ± 0.18 and 2.02 ± 0.11 mg/g DW, respectively, suggesting differential toxicity.

Total Chlorophyll Content

Trends in the total chlorophyll concentration were in line with those of its constituent parts. It sharply dropped to 2.52 ± 0.116 mg/g DW during Hg stress, supporting studies that mercury degrades chlorophyll pigments by interfering with photosystem II and the ultrastructure of chloroplasts (Singh *et al*., 2022). Significant decreases were also caused by copper and iron, which ended at 2.96 ± 0.025 and 3.24 ± 0.124 mg/g DW, respectively, underscoring the negative consequences of metal-induced oxidative stress. Higher levels of total chlorophyll were maintained by the

Al and Cr treatments (7.26 ± 0.115 and 6.76 ± 0.215 mg/g DW, respectively), indicating reduced pigment inhibition.

Carotenoid Content

The reactions of carotenoids, which are essential for photoprotection and ROS scavenging, to metal stress differed. Cu, Fe and Hg induced significant decreases in carotenoid levels, whereas treatments with Al and Cr kept them comparatively steady at 3.39 ± 0.003 and 2.86 ± 0.010 mg/g DW. Mercury had the lowest end levels at 2.03 ± 0.044 mg/g DW, while copper decreased the carotenoid content to 2.11 ± 0.320 mg/g DW. This is consistent with research showing that heavy metals accelerate oxidative degradation and interfere with carotenoid production (Mandal *et al*., 2021).

Pigment content is affected by heavy metals in a number of ways. Iron, copper and mercury are known to produce ROS in large quantities, which can cause oxidative damage to pigment molecules and chloroplast membranes (Gill & Tuteja, 2019). Furthermore, heavy metals interfere with enzyme pathways like phytoene synthase and δ-aminolaevulinic acid dehydratase that are necessary for the formation of carotenoid and chlorophyll. The reduction in pigments under heavy metal stress also indicates poor nutrient intake, especially for nitrogen and magnesium, which are essential for the production of chlorophyll.

It is interesting to note that treatments with Al and Cr led to a considerably lesser decrease in pigment levels, which may indicate that *A. nilagirica* has partial tolerance mechanisms. These could include the storage of metals in vacuoles to lessen cytoplasmic toxicity and increased activity of antioxidant enzymes like catalase and superoxide dismutase, which ameliorate ROS-induced damage (Zhang *et al*., 2020).

Impact of Heavy Metals on Total Soluble Sugar Content in *Artemisia nilagirica*

The information demonstrates how soluble sugars accumulate differently in root, stem and leaf tissues of *A. nilagirica* when exposed to heavy metal stress (Table. 17). Because heavy metals cause disturbances in physiological and biochemical processes, the observed trends highlight the function of carbohydrates in plant stress response by reflecting changes in carbohydrate metabolism across tissues.

All treatments showed a steady rise in the amount of soluble sugar in roots, although the most notable increase was observed with mercury (10 µM) (8.16 mg/g DW at day 12). This points to an adaptation strategy in which carbohydrates reinforce root cell structures under stress and preserve water potential by acting as Osmo protectants. In plants under mercury stress, the subsequent drop after day 12 indicates either cellular damage or the exhaustion of reserves. Similar patterns were noted by Liu *et al*. (2022), who showed that under metal toxicity, roots store soluble carbohydrates to fight oxidative damage brought on by ROS.

Sugar accumulation was higher in the stems than in the roots, and the greatest levels were once again induced by mercury treatment (16.48 mg/g DW at day 12). The higher sugar content may indicate improved transport to lessen oxidative damage in root and leaf tissues, as stems are essential for the translocation of photosynthates. Significant increases in sugar were also brought on by copper and chromium, indicating their impact on the distribution and redistribution of carbohydrates. Stems frequently act as short-term sugar storage facilities during heavy metal stress, bolstering their function in promoting metabolic stability throughout the plant (Raza *et al*., 2023).

Particularly under mercury stress, leaf tissues showed the largest accumulation of soluble sugar, reaching a peak of 19.45 mg/g DW on day 12. This reaction is probably related to photosynthetic disruption brought on by heavy metals, in which sugars build up as a result of compromised carbon consumption pathways. According to Zhang *et al*. (2023), disruption of stomatal function by Hg may also make sugar accumulation worse. It is also known that higher sugar levels in leaves improve osmotic adjustment and serve as signalling molecules that trigger genes that respond to stress (Gupta *et al*., 2022).

The findings of Wang *et al*. (2021), who noted that during heavy metal stress, leaves prioritize sugar production to sustain cellular turgor and antioxidant responses, are in line with this hierarchical sugar allocation.

Among all metals, mercury had the biggest impact on the level of soluble sugar. Its recognized effects on photosynthetic inhibition and stomatal conductance,

which interfere with carbon metabolism and cause sugar accumulation, are consistent with this. Additionally, as demonstrated by Sharma *et al*. (2022), mercury-induced oxidative stress triggers sugar-mediated antioxidant responses.

Their crucial involvement in adaptive strategies of *A. nilagirica* is highlighted by the notable tissue-specific alterations in soluble sugar concentration under heavy metal stress. Strong metabolic interference by mercury is highlighted by its noticeable impacts, which calls for strong sugar-mediated defences.

Impact of Heavy Metals on Starch Content in *Artemisia nilagirica*

One important environmental stressor that drastically changes plant metabolism of carbohydrates, especially starch content, is heavy metal toxicity. *A. nilagirica* demonstrated tissue-specific decreases in starch levels with various heavy metal treatments in the current investigation (Table. 18). These results, which are in line with new discoveries in plant stress physiology, demonstrate the disruption of starch production, accumulation, and degradation brought on by metals such as Al, Cr, Cu, Fe and Hg.

When exposed to heavy metals, starch levels in root dramatically dropped. Over the course of 20 days, aluminium (80 µM) gradually decreased the amount of photosynthate translocation and enzyme inhibition in the starch biosynthesis pathway, causing a drop from 2.55 ± 0.05 to 2.25 ± 0.12 mg/g DW. The trend for chromium (70 µM) was comparable but marginally milder. According to research by Ahmad *et al*. (2020), the observed decrease is probably the result of chromium toxicity interfering with carbohydrate partitioning. Because of its capacity to cause oxidative stress, harm chloroplasts, and interfere with enzymes involved in starch synthesis, such as ADP-glucose pyro phosphorylase, mercury (10 µM) had the greatest detrimental effect, lowering starch levels from 2.84 ± 1.40 to 2.04 ± 0.06 mg/g DW (Liu *et al*., 2019).

Under heavy metal treatments, the starch content of stems generally decreased. A larger fall from 4.62 ± 0.58 to 3.63 ± 0.15 mg/g DW was induced by Hg stress, whereas mild reductions were caused by Al and Cr. This is consistent with research

by Verma *et al*. (2021), who found that Hg stress prevents stems from accumulating starch because it decreases the conversion of sucrose to starch and increases the breakdown of starch to satisfy higher energy requirements. In line with research by Zhao *et al*. (2022), which demonstrates that copper toxicity alters chloroplast ultrastructure, resulting in lower photosynthetic efficiency and starch biosynthesis, copper stress also markedly reduced stem starch.

Under high metal stress, starch content in leaves substantially reduced; mercury had the most noticeable effect, dropping from 6.61 ± 0.24 to 4.63 ± 0.52 mg/g DW. According to Chen *et al*. (2020), this may be because mercury causes oxidative damage to chloroplasts, which lowers the amount of photosynthetic starch produced. According to Singh *et al*. (2021), copper and chromium also markedly decreased the amount of starch in the leaves, which is in line with their recognized effects on inhibiting photosynthesis and improving respiratory metabolism.

A number of physiological disturbances, including as reduced photosynthesis, altered enzymatic activity, and the mobilization of stored starch to meet the elevated respiratory needs during stress, are reflected in the decrease in starch content throughout tissues under heavy metal stress (Ahanger *et al.*, 2022). Strong affinity of Hg for thiol groups, which inhibits enzymes essential for starch production, is the primary cause of its toxicity. While stem and leaf starch decreases are mostly caused by reduced photosynthesis and elevated oxidative stress, the observed tissue-specific starch reductions indicate that root starch is more directly impacted by impeded carbohydrate translocation.

Impact of Heavy Metal Toxicity on Total Protein Content in *Artemisia nilagirica*

The complex effects of heavy metal exposure on the overall protein composition of *A. nilagirica* roots, stems, and leaves are shown by the findings in Table. 19. Over time, different plant tissues exhibit varying protein responses to heavy metals such as Al, Cr, Cu, Fe and Hg, indicating a complex interplay between metabolic adaptation and stress.

With a protein content peaking at day 8 (23.62 ± 0.15 mg/g DW) and then sharply dropping by day 20, roots show a high sensitivity to Hg stress. Oxidative stress, which impairs cellular processes and produces reactive oxygen species, is the reason why Hg can change the amounts of root proteins. Excess ROS has been found to cause proteolysis and hinder protein synthesis, which may account for the observed decrease following an initial adaptive response (Farid *et al*., 2022). Moderate peaks are seen in other metals, such as Cu and Fe, with subsequent falls suggesting a similar imbalance brought on by oxidative stress. Because nitrogen absorption is a crucial precursor for protein synthesis, treatments with Al and Cr cause relatively smaller protein peaks (Mishra *et al*., 2021).

The protein content of stems treated with Hg reaches its maximum at day 8 (25.34 ± 1.21 mg/g DW), and then starts to decrease on day 20 (to 20.82 ± 1.02 mg/g DW). Peak levels of protein caused by Cu and Fe are somewhat lower, and by day 20, they have significantly decreased. According to Bashri *et al*. (2020), the initial increase in protein levels could have been triggered by metal-induced overexpression of stress-responsive proteins that reduce metal toxicity, such as heat shock proteins and metallothionein. However, extended exposure might overpower detoxifying systems, which would lower the production of proteins. Their milder effects are a result of their interference with the enzymatic pathways that are involved in the metabolic activity of plant cells.

The most notable protein buildup in leaves is caused by Hg, which peaks at 29.74 ± 0.14 mg/g DW on day 8 before gradually declining. Since leaves are the main locations for photosynthesis and protein turnover, the high protein levels in leaves under mercury exposure suggest a substantial stress response. Heavy metals can increase stress-responsive proteins including glutathione-S-transferase and superoxide dismutase, which are essential for ROS detoxification, according to proteomic research (Singh *et al*., 2023). The activation of defensive metabolic pathways is reflected in the identical trends that Cu and Fe show, with initial increases followed by mild decreases. Compared to Hg, the impacts of Al and Cr are less

noticeable, indicating that the processes involved in protein synthesis in leaves are not as severely disrupted.

Following an initial increase, the protein level in all tissues decreases, suggesting that extended exposure to heavy metals impairs the ability of plants to maintain protein homeostasis. This is in line with research by Zhou *et al*. (2021), which found that extended exposure to heavy metals causes ribosomal dysfunction and protein degradation. Differential metabolic activity and the potential for metal buildup in roots, stems, and leaves are highlighted by tissue-specific differences in protein response.

Effect of Heavy Metals on Proline Content in *Artemisia nilagirica*

Plants under heavy metal stress undergo significant physiological and metabolic alterations, which frequently result in oxidative and osmotic stress. As an osmolyte, antioxidant, and protein and membrane stabilizer, proline accumulation is a crucial adaptive response in such circumstances. As shown in Table. 20, the current study focuses on the varying impacts of Al, Cr, Cu, Fe and Hg on proline accumulation in roots, stem and leaves of *A. nilagirica.* These results are integrated with findings from contemporary literature in this debate.

Proline levels in all tissues are constant under control settings, indicating that there is no stress. Nonetheless, proline content rises noticeably with heavy metal exposure, with tissue-specific variations. Proline accumulation is constantly highest in leaves, followed by stems and roots. These results are consistent with those of Gill and Tuteja (2010), who observed increased proline synthesis in abiotically stressed metabolically active tissues like leaves.

The highest proline accumulation is induced by Hg (10 μM) in all tissues; on day 20, the proline concentration of leaves peaks at 3.518 mg/g dry weight. This is consistent with research showing that mercury poisoning causes extreme oxidative stress, which calls for high proline levels for scavenging ROS and protecting cells (Sharma and Dietz, 2009). major proline buildup is also stimulated by Cu and Cr, indicating that these elements have a major effect on cellular redox equilibrium.

Compared to Cu and Hg, Fe and Al cause insignificant increases in proline, perhaps because they interfere with cellular redox states less strongly.

Enzymes like Δ^1-pyrroline-5-carboxylate synthase (P5CS) are upregulated under heavy metal stress, and proline breakdown pathways are suppressed. Proline's function in reducing osmotic stress by preserving turgor pressure and scavenging ROS is supported by the higher quantities of proline found in this investigation. This is in line with studies conducted by Hayat *et al*. (2012), which emphasize dual roles of proline in antioxidation and Osmo protection.

Protein and membrane oxidative damage is linked to copper poisoning. Copper-induced proline generation in *Arabidopsis* was shown by Pena *et al*. (2011), and the increased proline levels seen in copper-treated plants support their findings. According to Shanker *et al*. (2005), exposure to Cr increases proline buildup, which is consistent with its interference with photosynthetic electron transport and development of oxidative stress. Proline levels are raised by iron and Al toxicity, despite with less noticeable consequences; this could be a reaction to metal-induced disturbances in cellular homeostasis and nutrition uptake (Kumar *et al*., 2020).

Proline plays a crucial function in *A. nilagirica* under heavy metal stress, as this study shows, with mercury having the greatest detrimental consequences. Each tissue has a different amount of proline, which reflects its metabolic activity and capacity for stress response. According to recent studies, these results offer important new information about how plants adapt to heavy metal stress.

Impact of Heavy Metal Toxicity on Total Phenolics Content in *Artemisia nilagirica*

Table. 21 shows that when exposed to heavy metals such Al, Cr, Cu, Fe and Hg the phenolic content in roots, stems and leaves of *A. nilagirica* significantly increases. The production of lignin and cell wall fortification may be significantly impacted by this reaction, which points to an adaptive mechanism to combat oxidative stress and structural damage brought on by metal toxicity.

As shown in Table. 21, biochemical defence mechanisms by *A. nilagirica* against oxidative damage are reflected in the observed increase in total phenolics content during heavy metal stress. ROS produced under metal stress must be countered by phenols, which are important secondary metabolites. Their function as antioxidants includes metal chelation and ROS scavenging, which lessen cellular damage and promote stress tolerance. Interestingly, lignin production, which strengthens cell wall structures and increases stress resilience, is correlated with the higher phenolic content found in roots, stems and leaves.

With leaf phenolic levels reaching an elevation of 37.48 mg/g dry weight by day 20, the rise in phenolics content during mercury exposure was especially noticeable. The phenylpropanoid pathway, which controls the synthesis of phenolics and lignin, has been shown to be upregulated by mercury-induced ROS overproduction (Ahammed *et al*., 2021). Significant phenolic formation was also generated by Cu and Cr, which showed similar tendencies. According to Zhou *et al*. (2020), copper exposure increased phenolic content and lignin deposition in Populus species, indicating that copper, which is known to catalyse ROS via redox cycling, requires an antioxidative response.

It is well-known that under heavy metal stress, phenolic buildup and lignin synthesis are correlated. The polymer lignin, which is made from phenolic precursors, strengthens cell walls and restricts the absorption of metals. Singh *et al*. (2022) reported that *Arabidopsis thaliana* treated to cadmium showed increased lignification under stress. In order to limit metal access and reduce oxidative damage, roots, stems, and leaves of *A. nilagirica* have thicker cell walls, which are probably the result of lignin deposition fuelled by increased phenolic synthesis.

Despite being less effective than Cu or Hg, Fe and Al also produced notable phenolic reactions, especially in leaves, which are essential for the detoxification of photosynthetic ROS. According to Zhu *et al*. (2022), *Brassica napus* leaves that were exposed to light-induced oxidative stress and heavy metals consistently had the highest levels of phenolic compounds. The slight response of root and stem further

illustrates their different functions in metal uptake and translocation, with lignin buildup reducing metal mobility.

The importance of phenols in metal stress tolerance is highlighted by their dual function as structural elements and antioxidants. The structural and metabolic changes seen in *A. nilagirica* are supported by the overexpression of the phenylpropanoid pathway and the lignin production that follows. This study supports the findings of Pourcel *et al*. (2020), who highlighted the critical role phenolics play in improving cell wall stiffness and reducing cellular dysfunction brought on by stress.

Effect of Heavy Metals on MDA Content in *Artemisia nilagirica*

The oxidative damage brought on by exposure to Al, Cr, Cu, Fe and Hg is highlighted by the increased malondialdehyde (MDA) level in *A. nilagirica* during heavy metal stress that was noted in this study. A crucial biomarker for oxidative stress in plants, MDA, a byproduct of lipid peroxidation, gives information on the degree of membrane damage brought on by ROS. The findings, which are presented in Table. 22, are in line with other studies that demonstrated the harmful impacts of heavy metals on the physiological and biochemical functions of plants.

The cellular redox balance is upset by heavy metals, which causes an overabundance of ROS, including hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and superoxide radicals (O_2^-). The peroxidation of polyunsaturated fatty acids brought on by these ROS attack on cellular membranes results in the generation of MDA. According to research by Shahid *et al*. (2022), heavy metals like copper and mercury can dramatically increase ROS levels, which causes lipid peroxidation in a variety of plant species.

According to its recognized high toxicity, mercury produced the greatest MDA levels in the root, stem, and leaf tissues in our investigation. The formation of ROS is made worse by propensity of Hg to attach to thiol groups in proteins and interfere with the activities of antioxidant enzymes (Verma *et al*., 2021). According to Zhou *et al.* (2020), copper also caused significant oxidative damage, as seen by higher MDA levels. Cu can engage in Fenton processes to produce hydroxyl radicals.

Tissue-specific reactions to heavy metal stress are highlighted by the varying MDA accumulation in roots, stems and leaves. Because of their greater metabolic rates and exposure to external factors like light, which can increase the formation of ROS, leaves consistently showed the highest MDA levels (Pourcel *et al*., 2020). Despite being the main location for heavy metal uptake, root tissues showed relatively lower MDA levels. This could be because to chelating agents like phytochelatins or localized detoxification processes (Singh *et al*., 2022).

A situation where ROS production overwhelms the antioxidant defence system, which includes enzymatic antioxidants like SOD, CAT and peroxidase (POD), is reflected in the rise in MDA levels. Long-term exposure to heavy metals reduces ability of antioxidants to scavenge them, which causes oxidative damage and an increase in MDA accumulation, according to Ahammed *et al*. (2021).

The ability of various metals to cause oxidative stress varies. For instance, Al, despite being less redox-active, caused ROS indirectly by interfering with signal transduction and cellular metabolism, which in turn affected MDA levels. MDA levels were shown to be considerably raised by Cr, a known oxidative stressor, most likely as a result of the production of Cr (VI), a potent oxidant (Zhu *et al*., 2022). According to Pourcel *et al*. (2020), iron, although a necessary micronutrient, increased MDA because it participated in the Fenton reaction when present in excess.

In addition to lipid peroxidation, the rise in MDA concentration also points to ion leakage, poor membrane integrity, and disturbed cellular processes. As shown in other stress situations, increased lipid peroxidation can interfere with respiration, photosynthesis, and the movement of nutrients (Sharma *et al*., 2022). Strong impact of heavy metals on leaf MDA levels may also be related to their obstruction of electron transport chains and deterioration of photosynthetic pigments.

Bio accumulation pattern in *Artemisia nilagirica* (Clarke) Pamp.

This study investigates the bioaccumulation and translocation of Cu and Hg in *A. nilagirica*, with an emphasis on understanding the plant tolerance mechanisms under metal stress. The results reveal that the plant is capable of accumulating and

translocating Cu and Hg efficiently, demonstrating traits consistent with hyperaccumulation. Additionally, the study observes significant physiological changes in the plant, such as increased trichome formation and alterations in stomatal behaviour. These responses are crucial for mitigating metal toxicity and are indicative of complex tolerance strategies, including metal sequestration and phytovolatilization. The discussion below integrates these findings with relevant research, highlighting the role of trichomes, stomatal modifications, and potential phytovolatilization mechanisms.

Bioaccumulation refers to the process by which a plant absorbs and concentrates metals from its environment, often in amounts higher than found in the surrounding soil or water. In this study, *A. nilagirica* exhibited clear patterns of bioaccumulation for copper and mercury, as evidenced by the steady increases in Bio-concentration Factor (BCF) and Translocation Factor (TF). Both copper and mercury had TF values greater than 1, indicating that the plant was not only able to accumulate these metals but also translocate them effectively from the roots to the aerial parts.

Research on hyperaccumulators, such as *A. nilagirica*, supports these findings. Hyperaccumulating plants, which can accumulate metals to concentrations that are several orders of magnitude higher than those found in non-hyperaccumulating species, are often characterized by high TF values (Baker and Brooks, 1989). The ability of *A. nilagirica* to translocate Cu and Hg efficiently suggests that the plant is a hyperaccumulator of these metals, consistent with the findings of Clemens (2001) and Verbruggen *et al*. (2009), who describe the high efficiency of metal uptake and translocation in hyperaccumulating plants.

One of the primary mechanisms by which plants mitigate metal toxicity is the sequestration of metals within specialized cellular compartments, such as vacuoles, or within structures like leaf trichomes. In *A. nilagirica*, a significant increase in both glandular and non-glandular trichomes was observed in response to Cu and Hg toxicity. This observation suggests that trichomes play a key role in sequestering excess metal ions and preventing their harmful effects on plant metabolism.

Trichomes are known to act as physical barriers to metal ions, trapping them on the leaf surface and thus reducing the internal burden of these metals (Seregin and Ivanov, 2001). Glandular trichomes, in particular, have been shown to accumulate toxic metals like Cu and Zn by storing them in their glands or vacuoles (Rajkumar *et al*., 2012). Non-glandular trichomes, which lack the ability to store metals in vacuoles, may serve as a physical deterrent, preventing further metal uptake by trapping particles on the plant surface (Zhou *et al*., 2015). The increase in trichome density in *A. nilagirica* under metal stress may thus be an adaptive response to mitigate the toxic effects of copper and mercury by both sequestering and physically blocking the entry of excess metal ions.

The observed increase in trichome formation is in line with findings by Vassilev *et al*. (2005), who noted that metal stress often induces trichome formation as a strategy to sequester excess metals and prevent them from entering the plant vascular system. Similarly, Baker *et al*. (2000) highlighted the role of trichomes in the immobilization of metal ions, suggesting that the increase in trichome number in *A. nilagirica* reflects an adaptive response to cope with Cu and Hg toxicity.

Another striking physiological response observed in *A. nilagirica* was the widening of the stomatal apertures under Cu and Hg toxicity. Stomatal behaviour plays a crucial role in regulating plant water loss and gas exchange, and modifications in stomatal aperture have been implicated in the ability of plant to manage metal stress. In the case of metal stress, the widening of stomatal apertures may facilitate increased transpiration, aiding in the expulsion of excess water and potentially contributing to the removal of metal ions (Sharma and Dubey, 2005).

One notable consequence of stomatal modifications is the potential for phytovolatilization, particularly for mercury. Phytovolatilization is a process by which plants absorb volatile elements, such as Hg, and release them into the atmosphere in a less toxic form. The observed increase in stomatal aperture in *A. nilagirica* may enhance this process, allowing the plant to expel Hg through transpiration (Ernst, 2005). Pilon-Smits *et al*. (2009) documented that certain plants can volatilize mercury through their stomata, a process that involves the reduction of

inorganic Hg^{2+} to its volatile elemental form Hg^0, which is then released into the atmosphere.

This process is consistent with the findings of Zhao *et al*. (2017), who proposed that the ability of plants to increase stomatal aperture under metal stress can enhance metal volatilization. In the case of mercury, this physiological modification could be an effective mechanism for detoxifying the plant and reducing the toxic burden of Hg. The increased stomatal aperture observed in *A. nilagirica* may thus indicate a strategy for both enhancing transpiration and facilitating the volatilization of mercury, thereby contributing to the ability of plant to tolerate high mercury levels.

Effect of Heavy metals on SOD and Catalase

The results in the Table. 25; Figs. 54 -56 illustrate the effect of different heavy metals (Al, Cr, CU, Fe, Hg) on the SOD activity in *Artemisia nilagirica*, revealing a clear enhancement of SOD activity across all tissues (root, stem and leaf) in response to metal-induced oxidative stress. This increase suggests a protective antioxidant response mechanism activated by the plant to counteract oxidative damage from ROS generated by metal toxicity. The upregulation of SOD activity is a well-documented stress tolerance strategy in plants, as SOD is a key enzyme involved in ROS detoxification.

Aluminium toxicity in plants is primarily associated with the production of ROS, leading to oxidative damage in cellular components. In the present study, *A. nilagirica* shows a progressive increase in SOD activity in response to Al exposure, especially in leaf tissues, which is consistent with findings from recent studies. For instance, Sharma and Dubey (2005) observed that Al stress in *Brassica juncea* led to a significant increase in SOD activity, indicating a robust antioxidant response to mitigate oxidative stress (Sharma & Dubey, 2005). This increasing SOD activity suggests that the plant enhances its capacity to detoxify superoxide radicals, which are abundantly produced in the presence of Al. Additionally, Khan *et al*. (2020) also reported that plants under Al stress upregulate antioxidant enzymes like SOD to combat ROS-mediated cellular damage (Khan *et al*., 2020).

Chromium exposure is another major source of ROS in plants. In this study, a significant rise in SOD activity was observed in *A. nilagirica*, particularly in the root and leaf tissues. This result aligns with the findings of Rao *et al*. (2016), who reported that Cr stress in *Vigna radiata* led to an enhanced activity of antioxidant enzymes, including SOD (Rao *et al*., 2016). Similarly, Chandra and Yadav (2018) noted that Cr-induced oxidative stress in plants triggers the upregulation of SOD as part of the plant defence mechanism to protect against ROS-induced damage (Chandra & Yadav, 2018). The higher SOD activity in the leaf indicates that oxidative stress in photosynthetically active tissues is particularly high under Cr stress, and the plant responds by boosting its antioxidant defences.

Copper toxicity is known to generate ROS through Fenton-like reactions, which can cause significant oxidative damage to plant cells. In this study, *A. nilagirica* exhibited a marked increase in SOD activity in the root and leaf tissues, consistent with previous reports. Kramer *et al*. (2017) demonstrated that Cu stress leads to a significant increase in SOD activity in *Arabidopsis thaliana*, suggesting that SOD plays a crucial role in mitigating Cu-induced oxidative stress (Kramer *et al*., 2017). Similarly**,** Yadav *et al.* (2020) reported that copper exposure in *Vigna mungo* resulted in increased SOD activity, highlighting the role of this enzyme in plant responses to heavy metal stress (Yadav *et al*., 2020). The observed increase in SOD activity in *A. nilagirica* suggests an efficient detoxification of Cu-induced ROS and an enhanced antioxidative defence mechanism, especially in the leaf, which may face higher oxidative pressure.

Mercury is one of the most toxic heavy metals, inducing oxidative stress by generating ROS. The present study found a substantial increase in SOD activity in *A. nilagirica*, particularly in the stem and leaf tissues, indicating a strong response to mercury-induced oxidative stress. Similar findings were reported by Sharma *et al*. (2019), who observed that Hg exposure led to a significant increase in SOD activity in *Zea mays*, indicating that Hg-induced oxidative stress activates antioxidant defence systems (Sharma *et al*., 2019). Furthermore, Verma *et al*. (2020) highlighted that Hg stress triggers an increase in SOD activity in various plant species, reinforcing the

idea that SOD is essential for mitigating Hg-induced oxidative damage (Verma *et al*., 2020).

The results from Table. 26; Figs. 57-59 demonstrate how different heavy metals impact the catalase activity in *A. nilagirica* tissues, showing the plant response to oxidative stress. Catalase is a crucial enzyme involved in the detoxification of ROS, and its activity often increases in response to metal-induced stress (Sharma & Dietz, 2009). In this study, impact of Al, Cr, Cu, Fe, Hg on catalase activity examined, but the responses varied depending on the metal and tissue type.

Aluminium exposure led to a notable increase in catalase activity, particularly in root and stem tissues. This is consistent with previous studies, where Al-induced oxidative stress caused an increase in antioxidant enzyme activity, including catalase, in various plant species (Aravind *et al*., 2015). The transient peak in catalase activity in the leaf tissue followed by a decline suggests an initial stress response, followed by a possible toxic effect or adaptive response, similar to findings by Razi and Nasser (2011), who observed a similar pattern in Al-treated plants.

Chromium exposure also induced a moderate increase in catalase activity, especially in the leaf, indicating a significant oxidative stress response. Cr has been shown to increase ROS production, which activates antioxidant defence systems, including catalase, to neutralize these reactive species (Siddiqui *et al*., 2011). In this study, the moderate increase in catalase in roots and stems suggests a more subtle stress response compared to other metals, which aligns with studies where Cr toxicity was less severe but still prompted antioxidant enzyme activity (Bansal *et al*., 2013).

Copper caused a significant increase in catalase activity in the stem and leaf, with a more moderate increase in the root. Cu toxicity leads to the generation of ROS, and plants often enhance catalase activity as part of their defence mechanisms to mitigate oxidative damage (Verma *et al*., 2016). The robust response in leaf and stem tissues may reflect the higher metabolic activity in these tissues, which are more directly exposed to Cu ions, as observed in other studies on Cu-induced oxidative stress in plants (Arefian *et al*., 2021).

Iron exposure led to a marked increase in catalase activity across all tissues. Fe-induced oxidative stress has been well documented in plants, as excess iron can catalyse the formation of ROS, leading to lipid peroxidation and cellular damage (Hernandez *et al*., 2013). Similar to the results in this study, other research has shown that iron toxicity increases catalase activity in order to protect the plant from oxidative damage (Gao *et al*., 2020). The consistent rise in catalase activity across root, stem, and leaf tissues in this study highlights the active defence mechanism by plant against iron-induced stress.

Mercury exposure resulted in the highest catalase activity, indicating the most severe oxidative stress among all the metals tested. Mercury is a potent inducer of ROS and its toxicity has been linked to substantial increases in antioxidant enzyme activities, including catalase (Pereira *et al*., 2017). The dramatic rise in catalase activity in root, stem and leaf tissues of *A. nilagirica* suggests that the plant is under considerable oxidative stress. This finding is consistent with other studies showing that Hg exposure can significantly elevate catalase activity as part of the plant defence strategy (Zhang *et al*., 2014).

The results from this study provide insight into how different heavy metals affect catalase activity in *Artemisia nilagirica*, reflecting the adaptive responses of plant to oxidative stress. Among the metals tested, mercury caused the highest increase in catalase activity, while copper and iron also induced significant increases, especially in leaf and stem tissues. Aluminium and chromium showed more moderate effects, with Al in particular causing a transient response in leaf tissue. These findings suggest that plants activate antioxidant enzymes like catalase to counteract the oxidative damage caused by heavy metal exposure, and the degree of this response is influenced by both the type of metal and the tissue exposed.

GCMS ANALYSIS

The GCMS profile of *A. nilagirica* highlights a rich variety of metabolites, some of which are known for their medicinal properties. 3-Thujanol, a major compound in the control, has been documented for its antimicrobial and anti-inflammatory properties. Studies have shown that it exhibits significant antibacterial

activity (Chung *et al*., 2015) and possesses potential as a therapeutic agent for managing inflammation (Hwang *et a*l., 2017). Similarly, Phytol, another abundant compound, has been extensively studied for its antioxidant and anticancer activities. Research by D'Arc *et al*. (2019) demonstrated that Phytol possesses strong free-radical scavenging properties and can inhibit the growth of cancer cells, making it a valuable compound in cancer therapy. Furthermore, Caryophyllene oxide (14.31%) is recognized for its anti-inflammatory and analgesic effects, as noted by Gertsch *et al.* (2008), who found it effective in modulating the endocannabinoid system. Its potential in reducing inflammation and pain has garnered interest for therapeutic use in conditions like arthritis and other chronic inflammatory diseases.

In addition, (-)-Beta-caryophyllene (2.30%) has been shown to have significant anti-inflammatory properties, particularly through its action on CB2 receptors (Gertsch *et al*., 2008). This feature makes it a potential therapeutic agent for managing various inflammatory diseases. Germacrene-D (0.61%), although less abundant, has been reported to exhibit antitumor and antimicrobial effects (Park *et al*., 2019). Alpha-humulene (0.47%) is another compound in the control sample known for its anti-inflammatory and anticancer properties. It has been shown to inhibit the growth of cancer cells in vitro and in vivo (Shao *et al*., 2020).

In the Aluminium-treated sample, several notable shifts in metabolite abundance suggest potential alterations in metabolic pathways due to Al toxicity. The decrease in 3-Thujanol (from 27.72% to 24.56%) and the complete absence of (+)-Cedrol (0.43%) and Ledenoxide-(ii) (1.18%) may indicate a disruption in the biosynthesis of monoterpenes and sesquiterpenes. Such disruptions can occur under oxidative stress, where toxic agents like Al interfere with enzyme activity, leading to the degradation or altered synthesis of certain compounds (Wu *et al*., 2017). Cedrol, known for its anti-inflammatory, antifungal and antimicrobial properties (Bharati *et al*., 2019), could lose its potential in the presence of Al-induced stress, impairing its therapeutic applications.

Interestingly, the emergence of new compounds, such as (-)-Isocaryophyllene (3.18%) and Thunbergol (2.84%), indicates that Al exposure could stimulate the

formation of novel metabolites. (-)-Isocaryophyllene is a sesquiterpene with demonstrated anti-inflammatory and analgesic activities (Bhat *et al*., 2014), and its increased concentration suggests that Al may trigger pathways that enhance its biosynthesis. Similarly, Thunbergol, a compound not present in the control, has been suggested to have anti-inflammatory and anticancer properties (Li *et al*., 2015), indicating that it could play a role in mitigating Al-induced stress at the cellular level.

The increased levels of Germacrene-D (from 0.61% to 3.61%) in the Al-treated sample might also reflect the plant response to stress. Studies have demonstrated that Germacrene-D has antioxidant properties (Park *et al*., 2019), suggesting that its accumulation could be part of a protective mechanism against the oxidative stress induced by Al. On the other hand, Andrographolide (1.80%) is significantly reduced in the Al-treated sample (0.87%). Andrographolide is well known for its hepatoprotective, anti-inflammatory, and anticancer effects (Pavithra *et al.,* 2020), and its reduction could indicate impairment in its biosynthesis due to Al toxicity.

The reduction of Alpha-humulene (from 0.47% to 0.35%) may reflect the adaptive response of plant to environmental stress, as this compound is known for its antimicrobial and anti-inflammatory properties (Shao *et al*., 2020). Such a decrease in levels may impair the ability of plant to combat oxidative damage and microbial infections, which is crucial for maintaining its natural defences.

Chromium toxicity is a significant environmental stressor that can cause alterations in plant metabolism, particularly in the synthesis of secondary metabolites. The GC-MS analysis of *A. nilagirica* shows notable changes in its metabolite profile when exposed to Cr, leading to a reduction in the number of secondary metabolites and the emergence of new ones. Secondary metabolites play a crucial role in the defence mechanisms of plants, and changes in their composition can influence both the ability of plant to cope with stress and its medicinal potential.

In the control plant, a total of 26 metabolites is present, including compounds like Cedrol, Ledenoxide-(ii), Isoaromadendrene Epoxide, and Andrographolide, which are known for their therapeutic properties. Cedrol, a sesquiterpene, has been

shown to have anti-inflammatory, antifungal, and antimicrobial properties (Bharati *et al.*, 2019). Its complete absence in the Cr-treated plant suggests that Cr exposure may impair the biosynthesis of this compound. Similarly, Ledenoxide-(ii), a sesquiterpene epoxide, is known for its anti-inflammatory and antimicrobial effects, and its absence may indicate a reduction in the ability of plant to combat oxidative stress and microbial infections under metal stress (Shao *et al*., 2020).

Moreover, Isoaromadendrene Epoxide (2.72% in control) and Andrographolide (1.80% in control) are also absent in the Cr-treated plant. Isoaromadendrene Epoxide is known for its antifungal and antimicrobial properties, and Andrographolide, a major bioactive compound in *A. nilagirica*, is renowned for its hepatoprotective, anti-inflammatory, and anticancer effects (Pavithra *et al.*, 2020). The absence of these compounds may impair the medicinal potential of plant, as they play a significant role in therapeutic applications.

Chromium-induced oxidative stress can interfere with the enzymatic pathways involved in the synthesis of these secondary metabolites. Metal toxicity often disrupts the normal metabolic processes in plants, leading to reduced synthesis of key bioactive compounds. This is consistent with the findings of previous research, where heavy metals, including Cr, were shown to inhibit the production of secondary metabolites like flavonoids, terpenes, and alkaloids, which are crucial for plant defence and human health (Sharma and Dietz, 2009). The decrease in these metabolites suggests that Cr exposure may inhibit the pathways responsible for their synthesis, thus compromising the natural defence of plant mechanisms and reducing its therapeutic properties.

Interestingly, the Cr-treated plant also exhibits the emergence of new compounds not present in the control sample. These include Cis-Z-alpha bisabolene epoxide (6.49%), Stigmasta-5,22-dien-3-ol (4.38%), and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (4.5%). The formation of these compounds in response to Cr stress suggests an adaptive mechanism by which the plant synthesizes new secondary metabolites to cope with metal toxicity.

Cis-Z-alpha bisabolene epoxide, a sesquiterpene, is known for its antimicrobial and antifungal properties. Its increased presence may indicate a

response to the increased oxidative stress caused by Cr exposure. Sesquiterpenes like Cis-Z-alpha bisabolene epoxide are involved in plant defence mechanisms and are known to exhibit significant antioxidant and antimicrobial activities, which could help the plant mitigate the effects of Cr toxicity (Bhat *et al*., 2014). This suggests that Cr stress might stimulate the biosynthesis of specific compounds that help the plant deal with the oxidative damage caused by the metal.

Stigmasta-5,22-dien-3-ol, a sterol, is also formed in the Cr-treated plant. Sterols are important components of plant membranes and have been shown to play a crucial role in stabilizing membrane structure under stress conditions. Additionally, sterols possess antioxidant properties and can contribute to metal stress defence by protecting cellular structures from oxidative damage (Zhang *et al*., 2019). The increased presence of Stigmasta-5,22-dien-3-ol could thus be an adaptive response to improve membrane integrity and protect against Cr-induced oxidative stress.

The presence of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, a long-chain alcohol, in the Cr-treated plant is another notable adaptation. This compound is often associated with the regulation of oxidative stress and has been shown to exhibit antimicrobial and antioxidant properties (Zhao *et al*., 2017). Its formation may help the plant neutralize reactive oxygen species (ROS) generated by chromium exposure, enhancing the ability of plant to survive in contaminated environments.

The reduction in the abundance of Phytol (21.37% in control, 21.29% in Cr-treated plant) and (-)-Beta-caryophyllene (2.30% in control, 10.01% in Cr-treated plant) suggests that chromium has a selective impact on specific compounds. Phytol is widely recognized for its antioxidant and anticancer activities (Arc *et al*., 2019). The slight reduction in its abundance in the chromium-treated plant may reflect a moderate decrease in its synthesis due to oxidative stress. On the other hand, the increase in (-)-Beta-caryophyllene, a sesquiterpene with anti-inflammatory and analgesic properties (Gertsch *et al*., 2008), may indicate that Cr stress selectively stimulates the production of certain compounds that aid in the defence mechanisms of plant.

Copper is an essential micronutrient for plants, but in excess, it can induce toxicity that leads to oxidative stress and the alteration of metabolic processes. In this study, the GC-MS analysis of *A. nilagirica* exposed to copper reveals the synthesis of new secondary metabolites while also showing the loss or reduction of others. Secondary metabolites are crucial for the plant defence against abiotic stresses such as metal toxicity, and they also possess medicinal properties that can be beneficial to human health. The changes in the metabolite profile due to Cu exposure suggest adaptive mechanisms that might influence the medicinal value of plant.

The copper-treated *A. nilagirica* plant displayed the emergence of several new compounds, which were absent in the control. Geranyl linalool isomer b (2.74%) is one such compound. Geranyl linalool is a monoterpene known for its potent antioxidant, antimicrobial and anti-inflammatory properties (Liu *et al*., 2016). The formation of this compound in response to copper exposure could be part of the plant defence mechanism to combat oxidative stress caused by metal toxicity. It has also been shown that linalool-related compounds can improve the plant tolerance to heavy metals by reducing the formation of ROS, thus minimizing cellular damage (Alvarez *et al*., 2019). The presence of Geranyl linalool isomer b suggests that copper may stimulate the synthesis of compounds with antioxidative capabilities to help the plant mitigate the adverse effects of metal stress.

Another newly formed compound in the Cu-treated plant is Valerenol (1.22%). Valerenol is a sesquiterpenoid that is known for its sedative, anxiolytic, and antioxidant effects (Zhao *et al*., 2016). It is a constituent of *Valeriana officinalis*; a plant widely used in traditional medicine for treating insomnia and anxiety. The appearance of Valerenol in the Cu-treated plant could be viewed as a beneficial adaptation to enhance the medicinal properties of plant under stress conditions. Given its well-documented therapeutic effects, this compound may contribute to the overall medicinal value of *A. nilagirica*, especially under Cu-induced stress conditions. The production of Valerenol highlights the ability of plant to produce bioactive compounds that can potentially be used in medicinal formulations.

The synthesis of 8,11,14-Docosatrienoic acid, Methyl ester (1.32%) is another notable addition. This compound, also known as methyl linolenate, is a fatty acid ester that has shown significant anti-inflammatory and antimicrobial activities (Tan *et al*., 2015). Fatty acids like 8,11,14-Docosatrienoic acid have been linked to various health benefits, including modulation of immune responses and reduction of oxidative stress. The presence of this compound in the Cu-treated plant suggests that copper exposure might enhance the synthesis of compounds that have an immunomodulatory role, which could be beneficial for the plant's survival and stress tolerance.

Several compounds that are present in the control sample are lost or significantly reduced in the Cu-treated plant. Cedrol (0.43% in control), a sesquiterpene alcohol with antifungal, antimicrobial, and anti-inflammatory properties (Bharati *et al*., 2019), is completely absent in the Cu-treated plant. The loss of Cedrol suggests that Cu toxicity may disrupt the biosynthesis of certain terpenoids. Given the medicinal significance of Cedrol, this loss could decrease the potential of plant to fight infections and inflammation, two major therapeutic areas where this compound has demonstrated efficacy.

Similarly, Isoaromadendrene Epoxide (2.72% in control), known for its antimicrobial and antioxidant activities, is also missing from the Cu-treated plant. The absence of this compound could be a result of metabolic disruptions caused by copper stress, as plants may redirect their metabolic pathways to produce other compounds in response to the stressor. The loss of Isoaromadendrene Epoxide may, therefore, decrease the ability of plant to fight bacterial and fungal infections, weakening its medicinal potential in this regard.

Furthermore, Andrographolide (1.80% in control), a well-known bioactive compound with anti-inflammatory, anticancer, and hepatoprotective properties (Pavithra *et al*., 2020), is absent in the copper-treated plant. Andrographolide is crucial for a variety of therapeutic effects, including boosting immune function and treating liver diseases. Its absence suggests that copper exposure negatively affects the synthesis of this potent therapeutic compound. This loss likely reduces the overall

medicinal value of *A. nilagirica* under copper toxicity, as Andrographolide plays a key role in the plant health-promoting properties.

The impact of Cu on the medicinal potential of *A. nilagirica* appears to be a mixed one. On one hand, the synthesis of new bioactive compounds such as Geranyl linalool isomer b, Valerenol, and 8,11,14-Docosatrienoic acid, Methyl ester suggests that Cu may stimulate the plant to produce compounds that can help alleviate the effects of metal toxicity through their antioxidant, anti-inflammatory and antimicrobial properties. These newly synthesized compounds may enhance the plant defence system against Cu-induced oxidative stress and may also contribute positively to the overall medicinal profile of plant.

On the other hand, the loss of important compounds like Cedrol, Isoaromadendrene Epoxide, and Andrographolide suggests that copper toxicity impairs the ability of plant to produce certain key secondary metabolites with proven medicinal benefits. The absence of these compounds may decrease the medicinal potential of plant, particularly in areas such as antimicrobial and anti-inflammatory treatments.

The GC-MS analysis of *A. nilagirica* exposed to iron revealed the emergence of several new secondary metabolites. One of the newly synthesized compounds is Spathulenol (2.58%), a sesquiterpene alcohol known for its antimicrobial, antifungal, and anti-inflammatory properties (Baser *et al*., 2005). Spathulenol is recognized for its ability to inhibit microbial growth and reduce inflammation, making it a valuable compound for treating infections and inflammatory diseases. The increased synthesis of Spathulenol in response to iron stress suggests that the plant may be producing this compound as part of a defence mechanism against metal-induced oxidative stress and microbial pathogens. This adaptation could enhance the medicinal properties of plant, particularly in the areas of antimicrobial and anti-inflammatory treatments.

Another new compound formed under iron toxicity is 2-Beutenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yi) (2.37%), which, although less studied, belongs to a group of organic compounds that may have antioxidant and anti-inflammatory effects (El-Sayed *et al.*, 2011). Given the oxidative stress induced by excess iron, the

synthesis of such compounds may help in neutralizing ROS and reducing oxidative damage, thereby improving the ability of plant to tolerate environmental stress. The presence of this compound suggests that *A. nilagirica* may increase the production of specific antioxidants to cope with the excess Fe, which could bolster its role as a therapeutic agent in combating oxidative stress-related diseases.

However, Fe toxicity also led to the loss of several key secondary metabolites that are known for their therapeutic properties. For instance, Caryophyllene oxide (5.22%) and Andrographolide (1.80%), which were present in the control plant, were absent in the Fe-treated plant. Caryophyllene oxide is a sesquiterpene oxide known for its anti-inflammatory, antimicrobial and anticancer properties (Pratiwi *et al*., 2019). Its absence in the Fe-treated plant suggests that iron toxicity may disrupt the metabolic pathways that lead to its synthesis, potentially reducing the medicinal efficacy of plant in treating inflammation and infections.

Similarly, Andrographolide, a well-documented anti-inflammatory, anticancer and hepatoprotective compound, was also lost under iron exposure (Pavithra *et al*., 2020). Andrographolide plays a crucial role in liver protection, immune modulation and reducing chronic inflammation, making it an important compound in the medicinal potential of *A. nilagirica*. The loss of Andrographolide due to Fe toxicity could severely compromise the ability of plant to serve as a treatment for liver diseases, cancer, and inflammatory disorders.

Additionally, Cedrol (0.43%), a sesquiterpene alcohol known for its antifungal and anti-inflammatory effects, was also lost under iron stress (Liu *et al*., 2016). The absence of Cedrol may lead to a reduction in the ability of plant to fight fungal infections and reduce inflammation. This loss further diminishes the medicinal value of *A. nilagirica*, particularly in therapeutic areas related to fungal infections and inflammation.

The overall impact of iron toxicity on the medicinal potential of *A. nilagirica* is a combination of both positive and negative changes. On one hand, the synthesis of Spathulenol and 2-Beutenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl) suggests an adaptive response to oxidative stress, potentially enhancing the ability of

plant to cope with metal toxicity and providing additional antimicrobial and anti-inflammatory benefits. These newly formed compounds could potentially improve the therapeutic properties of plant in combating infections, inflammation and oxidative stress-related conditions.

On the other hand, the loss of important compounds like Caryophyllene oxide, Andrographolide and Cedrol significantly reduces the overall medicinal value of plant, particularly in treating liver diseases, cancers and inflammatory conditions. The absence of Andrographolide, in particular, is a major setback, as this compound is highly valued for its broad-spectrum medicinal applications. The loss of Caryophyllene oxide and Cedrol further diminishes the potential of plant in treating infections and inflammation.

Under mercury stress, several new compounds were detected in *A. nilagirica*, which may be indicative of the attempt of plant to adapt to metal toxicity. Isothujol, a newly formed compound, has demonstrated antioxidant and antimicrobial properties, which could help the plant mitigate oxidative damage caused by mercury (Rao *et al*., 2017). The formation of Isothujol under Hg stress suggests an enhanced defence mechanism against ROS generated during metal toxicity. Since Hg exposure is known to induce oxidative stress, the presence of Isothujol might help neutralize free radicals, thereby protecting the plant from further damage and potentially improving its medicinal properties related to oxidative stress-related ailments.

Another new compound, 9-Acetyl-S-octahydrophenanathrene, is noted for its neuroprotective and anti-inflammatory activities (Kim *et al*., 2019). Its formation in the presence of mercury could represent a plant response to reduce inflammation and protect against cellular damage caused by the metal. Mercury is known to induce neurotoxic effects and the synthesis of 9-Acetyl-S-octahydrophenanathrene could potentially provide therapeutic benefits in neurodegenerative conditions or inflammatory diseases.

Globulol, a compound formed in response to mercury toxicity, is recognized for its antimicrobial and anti-inflammatory activities (Yuan *et al*., 2020). The presence of Globulol suggests that the plant is gearing up to resist infections and inflammation,

which are common consequences of metal-induced stress. The enhanced production of Globulol could also reflect an adaptive response to the microbial and oxidative stresses induced by Hg exposure, which may improve the medicinal potential of plant in treating infections and inflammation.

In contrast, several important bioactive metabolites were lost under mercury stress, which could significantly affect the medicinal value of *A. nilagirica*. Caryophyllene oxide, which was present in the control, is known for its anti-inflammatory, anticancer and antimicrobial properties (Kuo *et al*., 2017). Its absence in the mercury-treated plant suggests a reduced potential to combat inflammation and microbial infections. The loss of Caryophyllene oxide may reduce the ability of plant to function as a therapeutic agent in conditions requiring anti-inflammatory and antimicrobial treatment.

Similarly, Andrographolide, a key compound with proven antiviral, anticancer and anti-inflammatory properties (Narayana *et al*., 2015), was absent in the Hg-treated plant. Andrographolide is an important therapeutic molecule in *A. nilagirica*, and its loss could significantly diminish the medicinal potential of plant, particularly in the treatment of viral infections and chronic inflammatory conditions. Spathulenol, which also has notable antimicrobial and antioxidant properties (Liu *et al*., 2019), was lost in the presence of mercury, indicating a compromised ability to resist oxidative damage and microbial infections.

The absence of these compounds further emphasizes the detrimental impact of mercury toxicity on the medicinal properties of plant. The loss of key metabolites like Caryophyllene oxide, Andrographolide and Spathulenol could lead to a significant reduction in the therapeutic potential of the plant, especially in combating cancer, inflammation, and infections.

Mercury-induced stress led to a decrease in the overall diversity of bioactive compounds in *A. nilagirica*. While the plant did produce some new compounds with potential antioxidant, antimicrobial, and anti-inflammatory properties, such as Isothujol, Globulol, and 9-Acetyl-S-octahydrophenanathrene, these newly synthesized metabolites might not entirely compensate for the loss of important

compounds like Caryophyllene oxide, Andrographolide, and Spathulenol. The loss of these key bioactive metabolites could result in a reduced capacity of the plant to treat a wide range of diseases, including cancer, infections, and inflammation.

Mercury toxicity, therefore, appears to negatively affect the medicinal potential of *A. nilagirica*, primarily due to the loss of essential therapeutic compounds. While new metabolites that may help in coping with metal stress are produced, their overall medicinal value is still uncertain and likely does not match the range of therapeutic effects provided by the lost compounds. Thus, the overall medicinal potential of the plant is likely diminished under mercury stress.

FTIR ANALYSIS

Impact of Aluminium Toxicity on Interaction with Functional Groups - FTIR Analysis

The interaction between Al and important functional groups linked to proteins and cell wall components changed slightly in plants treated with Al, according to Fourier Transform Infrared (FTIR) analysis. This suggests that exposure to Al causes slight biochemical changes in plant tissues. Similar absorption bands were seen in the FTIR spectra of the control and Al-treated plants, indicating that Al poisoning does not significantly affect the main structural components of plant. Nevertheless, minute alterations in the functional groups reveal information about the defences against Al stress by plant.

The cellulose, hemicellulose and pectins in the cell wall are characterized by the absorption bands at 3397 cm^{-1} and 2923 cm^{-1}, which are ascribed to hydroxyl (OH) stretches and carboxyl (COOH) groups. These bands are essential for preserving the structural integrity and hydration of the plant cell wall. These groups help the plant create cross-linked structures, which are necessary for preserving the strength of the cell wall, and they are involved in hydrogen bonding inside polysaccharides (Mishra *et al*., 2020).

These absorption bands were comparable in the control and Al-treated plants, according to the FTIR analysis, indicating that exposure to Al had no discernible

effect on the functional integrity of the cell wall polysaccharides. The slight alterations in the FTIR spectrum imply that Al toxicity may not result in significant disruptions to the cell wall matrix at the levels investigated in this study, even though it is known that Al ions can bind with carboxyl groups in pectin and other cell wall components, causing cross-linking or modification of the cell wall structure (Yang *et al*., 2020). This result is consistent with earlier research that found that mild Al poisoning did not significantly alter the structure of polysaccharides (Zhang *et al*., 2019).

It is significant to remember that Al is known to interact with the hydroxyl and carboxyl groups of cell wall, possibly forming Al-pectin complexes that alter the cell wall permeability and plasticity (Yuan *et al*., 2021). The FTIR bands in this study are similar, though, which implies that these interactions may be more subtle and do not cause significant observable shifts in the absorption bands at the Al concentrations under investigation.

The N-H bending and C=O stretching vibrations of amide bonds in proteins, most especially polypeptides, are represented by the band at 1635 cm^{-1}. The existence of proteinaceous structures in plant tissues is indicated by this band. According to the FTIR spectra, the amide I band at 1635 cm^{-1} remained constant in plants treated with Al when compared to the control, indicating that the protein structure of plant was not substantially altered by Al treatment.

It has been demonstrated that Al toxicity results in oxidative stress, which damages proteins by causing denaturation and aggregation (Li *et al*., 2020). The stability of the amide band seen here, however, suggests that Al ions had little effect on the secondary structure of the plant proteins under investigation. The absence of a noticeable change in this absorption band might also indicate that peptide bonding and protein folding held up well to exposure to Al. This result is in contrast to research where increased amounts of Al toxicity caused protein conformational changes, possibly resulting in the modification or degradation of amino acid residues like cysteine and glutamate (Sharma *et al*., 2019).

Al may nevertheless have an impact on protein function by attaching to particular metal-binding sites or thiol groups inside proteins, which could change enzyme activity or compromise the performance of stress-related proteins, even if no discernible changes in the amide I band were found in this investigation. The adaptive responses of plant, such as the activation of antioxidant defence systems or metal-binding proteins, may have contributed to the overall protein structure being preserved under mild Al exposure, according to the FTIR data.

The overall slight changes seen in the FTIR spectra imply that exposure to Al caused minor, non-significant modifications in the functional groups connected to the proteins and polysaccharides in the plant cell wall. According to Gomes *et al*. (2020), Al toxicity is known to cause oxidative stress, which impacts a number of plant biochemical pathways, including the generation of ROS and the ensuing cellular damage. To lessen the stress caused by Al, the plants may have engaged defence systems, according to the FTIR spectra.

According to recent research, ability of plant to tolerate Al involves the activation of processes that modify their cell walls. For example, the production of organic acids, such as citric acid, which can chelate Al ions and stop them from attaching to the cell wall (Zhou *et al*., 2020). Furthermore, plants may produce more metal-binding proteins, like phytochelatins and metallothioneins, which can absorb excess Al and shield the plant from its harmful effects (Barcelo *et al*., 2019). The absence of notable structural alterations in the FTIR spectrum raises the possibility that these processes were in place in the plants treated with Al, limiting extensive alterations to the protein and cell wall structures.

Al treatment may have caused slight changes in the biochemical makeup of plants without producing major structural disturbances, as evidenced by the FTIR analysis of plants treated with Al, which showed slight changes in the functional groups linked to proteins and cell wall components. The hydroxyl, carboxyl, and amide bands remained unaltered, suggesting that the protein structure and cell wall integrity were mainly preserved. This could be because defensive mechanisms were triggered in reaction to the toxicity of Al. To evaluate the long-term effects of Al on

plant biochemistry and physiology, more research is required, particularly with regard to greater Al concentrations or longer exposure periods.

Impact of Chromium Toxicity on Functional Groups - FTIR Analysis

Significant changes in the spectrum bands corresponding to important functional groups were found in the FTIR analysis of the control and Cr-treated plants, offering insights into the biochemical changes brought on by Cr exposure. The findings show that Cr has an impact on the structural elements of plants, such as proteins, polysaccharides, and the cell wall, which may change how the plant uses energy and how healthy it is overall.

In the Cr treated plant, the band that was seen at 3397 cm^{-1} in the control plant- which was ascribed to hydroxyl (OH) stretching vibrations from proteins, carbohydrates, and polyphenols-shifts to 3400 cm^{-1}. This change implies that exposure to Cr may modify the functional groups of plant or cause modifications in hydrogen bonding. Because of their high reactivity, Cr ions can interact with the hydroxyl groups in proteins and polysaccharides in the cell wall, changing the structure of the cell wall (Sayed *et al*., 2021). The plant may be more susceptible to oxidative stress and have a harder time maintaining turgor pressure and other cellular activities as a result of the changed hydrogen bonding, which may also impact the structural integrity of cell wall and capability to retain water (Zhang *et al*., 2021). This change in the OH stretch also suggests that Cr may alter the plant metabolic pathways in response to stress, which could reorganize the cell structure of the plant.

The N-H bending and C=O stretching vibrations in protein peptide bonds are indicated by the amide I and amide II bands at 1635 cm^{-1}. The 1635 cm^{-1} band did not significantly move or change in intensity between the control and Cr-treated plants in this investigation, indicating that Cr treatment does not significantly alter the protein backbone or secondary structure. It is crucial to remember that Cr exposure may still affect the functionality and activity of proteins, possibly through interactions with sulfhydryl groups or other functional sites in proteins, even though no structural change is visible in the FTIR spectra (Chandra *et al*., 2022). Even if these structural

alterations are not visible in the amide I/II areas of the FTIR spectrum, heavy metal poisoning in plants frequently results in protein denaturation or deactivation through oxidative damage or direct binding to protein functional groups.

Silica (SiO_2) is responsible for a new band that appears in the FTIR spectra of the Cr-treated plants at 1020 cm^{-1} and is not present in the control plants. This band implies that silica has a role in how the plant reacts to exposure to Cr. According to Zhao *et al*. (2020), silica deposition in plant cell walls has been found to be a defence mechanism against a number of environmental challenges, including heavy metal toxicity. By creating stable compounds with metals like Cr, silica can decrease the mobility of heavy metals in plants, lowering their bioavailability and potentially lessening their harmful effects. According to earlier research, silica accumulation increases a resistance by plant to metals, such as Cr, by trapping the metal ions in the cell wall and preventing them from entering the internal tissues of the plant (Song *et al.*, 2021). According to this research, Cr-induced silica deposition in plant cell walls may be essential for lowering Cr toxicity and improving plant resistance to stress.

The observed spectrum changes imply that the structure of the plant cell wall and the function of its proteins are affected by Cr poisoning. The change in the hydroxyl stretch suggests that Cr may disrupt the water-binding ability and polysaccharide network, both of which are essential for preserving the turgidity and integrity of the cell wall. In order to lessen the harmful effects of Cr, the plant may start protective processes including silica deposition, as shown by the appearance of a silica-related band. By acting as a barrier surrounding the plant cell wall, silica may lower the amount of Cr ions available and stop more plant cell damage.

Although Cr impacts the structural integrity of plant, it may not always result in immediate changes to the overall protein structure, as evidenced by the lack of noticeable changes in the amide bands at 1635 cm^{-1}. However, greater research on protein function and activity may shed light on the subtle impacts of chromium on plant physiology, especially with regard to damage caused by oxidative stress or enzyme inhibition.

Impact of Copper Toxicity on Interaction with Functional Groups - FTIR Analysis

Several notable spectrum changes are revealed by the FTIR analysis of the control and Cu-treated plants, indicating that Cu ions interact with important functional groups, especially those present in proteins and peptides. Plant metabolism and stress responses are eventually impacted by these interactions, which change the chemical makeup and functional activity of plant biomolecules. As will be covered below, the alterations in the FTIR spectra that are indicative of Cu toxicity have the potential to interfere with regular physiological functions of plant.

The N-H stretching band at 3397 cm^{-1}, which is normally linked to the presence of primary and secondary amines or amide groups in proteins, completely vanished after the Cu treatment, which was its most notable effect. By creating coordination compounds with amine groups, Cu has been shown to interact with nitrogen-containing macromolecules, especially proteins (Shao *et al*., 2020). Disappearance of this band raises the possibility that Cu may have attached to the nitrogen atoms in amines or amides, interfering with natural hydrogen bonding and conformation of proteins.

According to earlier research, Cu can alter the secondary structure of proteins, frequently by causing oxidative stress or displacing metal cofactors from enzymes, both of which compromise the integrity of the protein structure (Zhang *et al*., 2019). Therefore, the absence of this N-H stretching vibration may be a sign of Cu-induced denaturation or changes in protein structure, which could affect plant cellular processes and enzyme activity.

The change in the amide I and II bands from 1635 cm^{-1} in the control plant to 1610 cm^{-1} in the copper-treated plant was another noteworthy finding. The bending vibrations of the N-H and C=O functional groups in proteins are linked to the amide I and II bands (Lee *et al*., 2021). Changes in the structure of protein or chemical nature are indicated by a shift to a lower wavenumber, which could be brought on by unsaturated C=C bonds or the coordination of copper ions with the peptide bonds (Bai

et al., 2020). This change implies that Cu is interacting with both carbon-carbon double bonds and functional groups that include nitrogen, which is probably changing the overall structure of the protein. Plants exposed to toxic quantities of Cu may experience decreased metabolic activities as a result of copper binding to proteins, which may alter the natural structure and function of enzymes (Zhang *et al*., 2018).

This protein structural alterations brought on by metal binding have a long history in the literature. For instance, oxidative damage and protein misfolding brought on by copper exposure are associated with decreased plant growth and productivity in metal-stressed environments (Vazquez *et al*., 2021). Therefore, the change in the amide bands may be a reflection of harmful effects of Cu on plant biological processes, such as protein synthesis and enzyme function.

Between the control and copper-treated plants, there was no change in the C-H bending vibration at 1385 cm^{-1}, which is ascribed to the terminal methyl groups in aliphatic ketones. This finding implies that the aliphatic ketones and related chemicals found in the plant tissue, especially those with terminal methyl groups, were not substantially impacted by the copper treatment. This result is in line with research that indicates copper mostly interacts with nitrogenous chemicals, proteins, and amino acids, with little to no effect on lipids or some other biomolecules (Rodriguez *et al*., 2021). Persistence of the C-H bending band also raises the possibility that copper toxicity may affect particular molecular structures, such as proteins and peptides, that are involved in metal binding and stress response rather than having an equal effect on all plant metabolites.

Interactions of Cu with different functional groups, especially nitrogen and carbonyl groups, are consistent with the mounting evidence that copper poisoning causes oxidative stress in plants. ROS, which can harm cellular constituents such lipids, proteins, and nucleic acids, are known to be produced by copper ions (Tewari *et al*., 2019). According to Vazquez *et al*. (2021), the changes in protein-related functional groups seen in the FTIR spectra in this investigation most likely represent the oxidative damage brought on by ROS, which can result in protein oxidation, aggregation, and the disturbance of regular cellular processes. The concept that

copper-induced toxicity is mediated by metal-protein interactions that impact enzyme performance and general cellular homeostasis is further supported by ability of Cu to form coordination bonds with amino acids including histidine, cysteine, and glutamine (Wang *et al*., 2022).

Impact of Iron Toxicity on Interaction with Functional Groups - FTIR Analysis

The absence of notable alterations in the absorption bands at important wavelengths in the FTIR spectrum indicates that iron toxicity has little effect on the functional groups connected to the plant cell wall polysaccharides and proteins, according to the results of the FTIR analysis conducted for this study. In particular, the FTIR spectrum of iron-treated plant lack or diminished absorption bands at 3397 cm^{-1}, 2923 cm^{-1}, and 1635 cm^{-1} suggests that exposure to iron may not result in significant changes to the main constituents of the plant cell wall and protein structures. This contrasts with other metals, such copper, which interact with functional groups in proteins and polysaccharides to cause major structural and metabolic changes in plants (Sanchez-Rodriguez *et al*., 2017; Singh *et al*., 2021).

The hydroxyl (OH) and carboxyl (COOH) groups found in cellulose, hemicellulose, pectins, and other polysaccharides that make up the plant cell wall are linked to the 3397 cm^{-1} and 2923 cm^{-1} bands in the FTIR spectrum, respectively (Santos *et al*., 2020). These groups are essential to the capability of plant to interact with water and other macromolecules as well as its structural integrity. The findings of the study demonstrate that the FTIR spectra of the Fe-treated plant exhibits a considerable reduction in or absence of certain absorption bands. This implies that the main components of the cell wall are not significantly degraded or altered by Fe toxicity. On the other hand, heavy metals such as Cu or Cd frequently caused the components of cell walls to break down or their functional groups to change, which resulted in decreased cell wall integrity and plant stress (Lopez-Millan *et al*., 2020; Pal *et al*., 2022).

Recent studies have shown that Fe can bind to cell wall components like pectin, which may affect cell wall growth or cross-linking, even though this finding

may indicate that Fe does not severely destroy polysaccharides in the plant (Roberts *et al*., 2021). Impact of Fe on polysaccharide structures, however, might be less severe than those of other heavy metals, indicating a more subtle manner of toxicity, based on the minor changes in the FTIR spectra. Instead of directly changing the cell wall structure, Fe toxicity may affect cell wall metabolism at the level of enzyme activity.

Usually seen in the amide I and II areas of the FTIR spectrum, the 1635 cm^{-1} band represents N-H bending and C=O stretching vibrations from peptide bonds in proteins (Papageorgiou *et al*., 2020). This band indicates the existence and structural soundness of proteins in the tissue of the control plants. The absence or large reduction of this band in the spectra of the Fe treated plants, however, indicates that iron treatment does not substantially alter the protein structure of the plant in a way that would be detected by FTIR analysis. This contrasts with other heavy metals, such as Cu, which interacts with amine and carboxyl groups to cause protein denaturation, altering protein structure and perhaps causing polypeptide chains to aggregate (Pasternak *et al*., 2020).

Fe may not directly disrupt peptide bonds or protein folding, as evidenced by the lack of appreciable changes in protein structures of Fe treated plant. While Fe does interact with certain Fe-binding proteins and enzymes involved in redox reactions or chlorophyll biosynthesis, recent research on Fe toxicity has shown that these interactions might not substantially alter the overall structure of the plant proteome (Hernandez *et al*., 2021). Rather than creating broad protein denaturation or conformational changes that may be detected by FTIR, iron poisoning may result in functional alterations in enzymes or other proteins that are essential for plant metabolism.

According to Sanchez-Rodriguez *et al*. (2017), Fe is a crucial micronutrient needed for a number of physiological functions in plants, such as respiration, photosynthesis, and enzyme activity. Iron can, however, become toxic at high amounts, resulting in oxidative stress and the generation of reactive oxygen species (ROS). The absence of notable alterations in the FTIR spectrum in this investigation

implies that Fe toxicity does not cause the same degree of disturbance in cell wall composition and protein structure as other heavy metals, even though Fe-induced oxidative stress can cause growth inhibition, chlorosis, and damage to cell membranes (Wang *et al*., 2020). This might be the case because Fe toxicity may not directly change the structural elements of the plant, but rather mainly impact metabolic functions like electron transport or redox reactions.

Impact of Mercury Toxicity on Interaction with Functional Groups - FTIR Analysis

The FTIR spectra of the control and plants treated with Hg showed distinct changes in the absorption bands corresponding to the major functional groups in the plant cell wall and proteins. The control plant spectrum displays typical absorption bands associated with functional groups in cellulose, hemicellulose, pectins and proteins, which are important structural components of the plant. The Hg-treated plant, on the other hand, shows altered intensities or shifts in these bands, indicating potential changes in the biochemistry of plant as a result of Hg exposure.

Key Absorption Bands in the FTIR Spectrum was 3397 cm^{-1} and 2923 cm^{-1}: OH, and COOH Groups of Cellulose, Hemicellulose, Polysaccharides and Pectins. The band at 3397 cm^{-1} is characteristic of hydroxyl (OH) stretching vibrations, which are present in the cellulose, hemicellulose and pectin components of the cell wall (Santos *et al*., 2020). This is a crucial feature for hydrogen bonding and water retention in the plant cell wall. The band at 2923 cm^{-1} corresponds to C-H stretching vibrations, specifically from alkyl groups like those found in methyl and methylene groups of cellulose and hemicellulose (Zhou *et al*., 2020). These polysaccharides provide structural support and are integral to the overall strength and rigidity of the plant cell wall. The Hg treatment resulted in a shift or reduction in the intensity of these absorption peaks, indicating that Hg may alter the composition or integrity of the cell wall components. This could suggest disruption of polysaccharide structure or changes in the water-binding capacity of the cell wall, potentially affecting cell wall elasticity and overall plant turgidity (Wang *et al*., 2021). Hg has been shown to interact with the hydroxyl and carboxyl groups, disrupting hydrogen bonding or cross-

linking, which may compromise the structural integrity of cell wall (Nakamura *et al*., 2021).

The band at 1635 cm^{-1} corresponds to amide I and amide II bands, which are associated with N-H bending and C=O stretching vibrations in peptide bonds within plant proteins (Li *et al*., 2022). This is a critical region for detecting changes in the secondary structure of proteins, such as α-helices and β-sheets, which are essential for proper protein folding and function. In the case of the plants treated with Hg, there may be a shift or decrease in intensity of the 1635 cm^{-1} band, indicating that mercury exposure might alter protein structure or protein-protein interactions. Hg is known to interact with thiol groups and other functional groups in proteins, leading to protein denaturation and aggregation (Pal *et al*., 2021). The observed shift suggests that Hg might cause conformational changes or reduced stability of proteins, impairing their functions in various physiological processes.

The band at 1385 cm^{-1} corresponds to C-H bending vibrations from terminal methyl groups of aliphatic ketones and other organic compounds in the plant (Kumar *et al*., 2021). This band is often attributed to alkyl groups, such as those found in lipids and other cellular components. The intensity of this band may also be altered upon mercury exposure, suggesting that Hg could be affecting lipid metabolism or the composition of membrane lipids. Hg ions are known to interact with lipid bilayers, causing membrane destabilization and alterations in lipid composition, which can lead to membrane permeability changes (Kumar *et al.*, 2021). This alteration may reflect membrane damage or disruption of cell signalling pathways, impacting cellular homeostasis and overall plant growth.

These FTIR spectral changes are consistent with previous research that has shown that heavy metal exposure, particularly Hg, can lead to alterations in structural components such as polysaccharides, lipids, and proteins, thereby affecting plant structural stability and metabolic activity.

Metal Toxicity on the Anticancer Potential of *Artemisia nilagirica*

The information in Table. 33 shows how the cytotoxicity caused by the leaf extract of Artemisia nilagirica at various concentrations is influenced by metal toxicity (Al, Cr, Cu, Fe and Hg). The findings demonstrate the different effects of various metals, which, depending on the metal and its concentration, can either reduce or increase the anticancer activity of extract. Interaction of these metals with bioactive compounds of *A. nilagirica* seems to lessen the potential of plant to kill cancer cells.

As anticipated, the cytotoxicity of the leaf extract of *A. nilagirica* rises with increasing extract concentrations (from 12.5 µg/mL to 200 µg/mL) in all treatments. This cytotoxic response was changed, nevertheless, by the presence of metal ions, indicating a potential interaction between the metals and the plant extract's bioactive ingredients. Metals dramatically alter the bioactivity of plant extracts, according to similar findings in another plant-based anticancer research (Mishra *et al*., 2013; Ozer *et al*., 2015). The interaction of metals with cellular processes involved in apoptosis may be the cause of this modification (Shrivastava *et al*., 2017).

Aluminium exhibits a definite suppressive effect on cytotoxic potential of *A. nilagirica*. In comparison to the control (8.52% and 13.1%, respectively), Al exposure significantly reduces cell death at lower dosages (12.5 and 25 µg/mL). The cytotoxicity of Al is lower (81.6%) than that of the control (89.4%) even at 200 µg/mL. This observation is in line with research that indicates Al can change metal ion homeostasis and cell signalling pathways, interfering with cell apoptotic processes (Barbieri *et al*., 2016). According to Mansouri *et al*. (2020), Al has been demonstrated to bind to proteins and enzymes that regulate cell death, decreasing the bioavailability of anticancer drugs.

At lesser quantities, Cr also lessens cytotoxic impact of *A. nilagirica* (9.97% at 12.5 µg/mL and 13.9% at 25 µg/mL). The effect of Cr on cell death diminishes with increasing concentration; at 200 µg/mL, the maximal cell death rate was 72.1%, which is still less than the control (89.4%). This decrease in cytotoxicity may be the result of propensity of Cr to cause DNA damage and oxidative stress, which ironically may trigger repair processes that shield cells from the effects of anticancer drugs (Patocka *et al*., 2014; Ghosh *et al*., 2015). The bioactive molecules in the extract may be

inhibited as a result of these interactions, which would limit their ability to fully combat cancer.

The adverse effect of Cu on cytotoxicity was severe than that of Al and Cr. Cu produced comparatively less cytotoxicity at higher doses than the control; at 200 µg/mL, the maximum cytotoxicity was 37.4%. According to Davis *et al*. (2018), redox-active characteristics of Cu have the potential to decrease the effectiveness of anticancer drugs by catalysing oxidative processes that modify cellular communication pathways. According to Ullah *et al*. (2016), although Cu is a necessary cofactor for a number of enzymes, too much of it in the body might cause problems with cellular metabolism and lessen anticancer properties of *A. nilagirica.*

Though it still had an adverse effect on the anticancer capability of extract, iron shown a less noticeable decrease in cytotoxicity. Iron considerably decreased cytotoxicity at its maximum (40.4% at 200 µg/mL) in comparison to the control (89.4%). Iron is known to catalyse Fenton reactions that produce extremely reactive hydroxyl radicals, which could hinder the capacity of extract to cause oxidative stress in cancer cells (Carocho *et al*., 2018). Moreover, too much Fe may trigger defensive mechanisms in cells, like Fe-responsive proteins, which may lower bioavailability of the chemical compounds performing anticancer property of the plant (Ghosh *et al*., 2015).

Of the metals studied, Hg showed the least inhibitory effect on cytotoxicity. Mercury caused 31.9% cell death at the maximum dose (200 µg/mL), which was much less than the control (89.4%). Because mercury can damage cell membranes and prevent cell repair processes, it may interfere with the cytotoxicity of extract and hence lessen the effectiveness of anticancer treatments (Mansi *et al*., 2017). Moreover, the primary mechanism of toxicity by Hg is its capacity to attach to protein thiol groups, which may interfere with cellular signalling pathways implicated in apoptosis (Dorea *et al*., 2016).

The results of this investigation indicate that the presence of specific metals, including Al, Cr, Cu, Fe and Hg may reduce the cytotoxic effects of *A. nilagirica* extract, hence inhibiting its anticancer potential. There are a number of reasons for this detrimental effect:

Metals may attach themselves to the active ingredients of plant extract, decreasing their bioavailability or changing how they interact with apoptotic cell targets (Mansouri *et al*., 2020). Other research on plants and metals have found similar interactions (Mishra et al., 2013; Ozer *et al*., 2015). The ability of the extract to cause oxidative stress, a crucial mechanism of anticancer activity, may be hampered by the participation of metals like Cu and Fe in redox reactions (Ullah *et al*., 2016; Carocho *et al*., 2018). The metals under investigation may trigger defensive mechanisms in cells, like the metal-responsive transcription factor (MTF-1), which could stop the active ingredients of leaf extract from causing the greatest amount of cytotoxicity (Patocka *et al*., 2014). An excessive amount of metal ions, including Fe and Cu can raise oxidative stress, which may harm anticancer drugs or prevent them from working as intended (Carocho *et al*., 2018).

These findings emphasize how crucial it is to take biological or ecological metal pollutants into account when assessing the anticancer potential of plant-based extracts. To maximize the therapeutic utility of *A. nilagirica* and other plant-based treatments, more investigation is required to examine the molecular mechanisms behind these interactions.

CHAPTER 6
SUMMARY AND CONCLUSION

Artemisia nilagirica (Clarke) Pamp. locally known as karppoora thulasi, is an aromatic shrub belongs to the family Asteraceae, used in traditional medicine for the treatment of various ailments. The present research aims to examine how certain selected heavy metals such as aluminium, chromium, copper, iron and mercury affect the medicinal plant *A. nilagirica*. To assess the effects of heavy metals on growth, physiology, and metabolic changes in general and medicinal properties in particular, a comparative study was conducted on parameters such as bioaccumulation pattern, anatomical/histochemical changes in the root, stem, and leaves of plants grown under Hoagland nutrient solution and exposed to known quantities of Al, Cr, Cu, Fe and Hg.

The control group consisted of plants grown on Hoagland medium at half strength without any metal treatment. After 4, 8, 12, 16 and 20 days of heavy metal treatment, analyses of several structural and functional features in the root, stem, and leaves were conducted. Rooted cuttings were cultivated in various concentrations of aluminium chloride ($AlCl_3$), potassium dichromate ($K_2Cr_2O_7$), copper sulphate ($CuSO_4$), Sodium iron ethylenediamine tetra acetic acid (NaFeEDTA) and mercuric chloride ($HgCl_2$) in order to standardize the concentrations of heavy metals that cause toxicity symptoms and cause roughly 50% growth retardation. For the investigation, the metal salt concentrations where the rooted propagules of *A. nilagirica* survived but showed around 50% growth retardation were chosen. The trial-and-error method was used to determine the ideal concentrations. The standardized heavy metal concentrations such as, 80 µM of $AlCl_3$, 70 µM of $K_2Cr_2O_7$, 50 µM of $CuSO_4$, 70 µM of NaFeEDTA, and 10 µM of $HgCl_2$ were applied to *A. nilagirica* plants in order to conduct additional study.

Growth of plants were assessed in terms of root length, shoot length and leaf area. In order to evaluate tolerance potential, the tolerance index was also computed and used as a metric. Protein, phenolics, proline, sugar, starch, chlorophyll and

carotenoid pigments were also analysed quantitatively. Since heavy metal toxicity causes ROS to be produced, the role of scavenging enzymes including catalase, superoxide dismutase activity and MDA formation was also investigated in plant organs, including the root, stem and leaves.

Additionally, a scanning electron microscopy (SEM) study was performed to verify the anatomical changes in the various plant components. Heavy metal ion distribution and particular elemental allocation pattern were also ascertained using the SEM-EDX (SEM coupled to Energy Dispersive X-ray analysis) technique. The ability of *A. nilagirica* to bioaccumulate heavy metals was examined using ICP-OES. Given that this plant has therapeutic properties, the distribution and occurrence of bioactive secondary metabolites were further investigated using GC-MS analysis. Impact of heavy metal toxicity on specific functional groups is determined by FTIR-spectrometer. In vitro Cytotoxicity studies for understanding anticancer potential of *A. nilagirica* leaf extract and to analyse how heavy metals alters it by employing Dalton's Lymphoma Ascites Cells (DLA).

After analysing, contrasting and disputing the observation data using relevant recent literature, the following findings are reached.

- The morphology and growth retardation of *A. nilagirica* were found to be substantially comparable to those of various Al, Cr, Cu, Fe and Hg concentrations. However, the quantity needed to produce evident poisoning symptoms varied significantly, indicating that tolerance potential of plant changed based on the metal. Because of their diverse chemical characteristics and ways of interacting with biological structures, these metals have different impacts. Due to their higher capacity to produce ROS and greater affinity for essential cellular components, mercury and copper exhibited the most notable growth inhibition.

- The study reveals that heavy metal exposure severely inhibits root growth in *A. nilagirica*, with Hg exerting the greatest toxic effect, followed by Cu, Fe, Cr and Al. This indicates that the species is highly susceptible to heavy metal stress, which can hinder its growth and survival in contaminated environments. Reduced stem elongation negatively impacts biomass and ecological fitness, potentially

impairing its adaptability to polluted habitats. Decreased leaf area under heavy metal stress limits photosynthetic capacity, affecting carbohydrate production and biomass accumulation. The reduction in leaf expansion suggests oxidative damage and disruption of cellular homeostasis, leading to impaired cell division and elongation. The varying levels of toxicity observed reflect the unique reactivity and mechanisms of action of each metal in plant tissues.

- In *A. nilagirica*, the study shows that heavy metals have different effects on stomatal growth. Fe and Al have minimal impact, whereas Cr, Cu and Hg have a major effect, especially on the lower epidermis. These results emphasize the importance of stomatal regulation in plant responses to exposure to heavy metals and highlight the ability of *A. nilagirica* to develop metal-specific stomatal responses when exposed to various metal stresses. The increase in SI under mercury stress highlights the capability of plant for detoxifying and physiological adaptability, with increased stomatal density and aperture size facilitating phytovolatilization. Our knowledge of stomatal function in heavy metal tolerance and detoxification is advanced by this adaptation, which points to the phytoremediation potential of *A. nilagirica*, especially in areas contaminated with mercury.

- Anatomical observations, such as impaired growth, loss of root hairs, disruption of root structure and damage to vascular tissues, suggest that Hg and Cu are more toxic than Al, Fe and Cr despite Cu being an essential element. These findings highlight that, although the morphological changes caused by heavy metals may be minimal, Cu and Hg induce more severe anatomical disruptions in the plant.

- SEM analysis of the leaf surface shows the growth of glandular bladder-like trichomes and multicellular uniseriate long hairs. Size and quantity differ among treatments. Leaf treatment with Cu and Hg produced the greater number of trichomes. Additionally, these structures are meant to trap metal ions and serve as a channel for the release of volatile metal forms, especially mercury. Compared to the distribution of other elements, the weight percentages of Al, Cr, Cu and Hg in their respective treatments increased significantly, confirming the accuracy of

the SEM-EDX investigation. The elemental composition of *A. nilagirica* root, stem and leaf tissues was affected differently by the metal treatments. Outcome of the study reflected how the plant responded to stress caused by the metal. While exposure to Al, Cr, Cu, Fe and Hg affected elements such as K, Ca and Mg, each metal caused a different pattern of element redistribution that affected nutrient absorption, translocation, and overall plant metabolism. These results highlight the intricate relationships that exist between plants and heavy metals, as plants try to accumulate metals in their tissues while preserving the equilibrium of nutrients when they are under stress.

- The study shows that different metal treatments had distinct effects on the dry weight of *A. nilagirica* tissues. While Fe, Cr and Al had moderate to minor impacts, showed little inhibition on root growth, stem growth, and leaf development, copper and mercury caused the most severe growth inhibition, particularly in leaf tissues. These findings suggest that although Al, Cr and Fe either have a minimal influence on growth, Cu and Hg impede plant development, especially in the leaves. An inverse link between dry weight and moisture content was demonstrated by metal stress, which caused dehydration and a rise in solute concentration in plant tissues, resulting in a greater dry weight % despite overall growth reduction.

- According to the results, pigment concentration in *A. nilagirica* is negatively impacted by heavy metals, with the most noticeable decreases occurring in Hg, Cu and Fe. The results emphasize that oxidative damage and disruption of photosynthetic machinery are important causes of pigment degradation. Partial pigment retention was possible with Al and Cr, despite their continued inhibitory effects, suggesting possible tolerance mechanisms. These results emphasize how important it is to understand the toxicity of specific metals and develop strategies to make plants more resistant to metal stress.

- The findings show a tendency for stress-induced sugar buildup that is followed by a decrease with extended exposure. The most noticeable effects were caused by Hg, indicating that in *A. nilagirica*, it has a significant influence on osmotic

control and sugar metabolism. These patterns show how the plant body reacts to heavy metal stress, trying to preserve its energy stores and osmotic equilibrium before succumbing to toxicity after prolonged exposure. The study emphasizes how severely heavy metal stress affects starch metabolism in *A. nilagirica*. The results are in line with recent research showing how vulnerable carbohydrate storage is to exposure to heavy metals. Enhanced starch hydrolysis is the direct cause of increased sugar level. The development and survival of plants in contaminated settings are more broadly affected by this disturbance.

- The observed protein dynamics under heavy metal stress show a balance between the damage caused by toxicity and the adaptive mechanisms of the plant. Prolonged exposure causes metabolic disturbances and protein breakdown, whereas acute exposure promotes protein synthesis as part of the stress response. *A. nilagirica* has an advanced defence mechanism against heavy metal stress, as evidenced by the enhanced phenolics and suggested lignin production. Because phenolics and lignin not only reduce oxidative stress but also stop metal transfer, these findings help to clarify the potential of plants for phytoremediation and offer information for sustainable environmental management.

- Proline may be essential for osmoregulation, which helps to lessen the toxicity of heavy metal ions in plants, as evidenced by the persistent buildup of proline in the plants exposed to metal toxicity. Furthermore, *A. nilagirica* has low levels of antioxidant enzyme activity, indicating that proline serves as an efficient antioxidant in these plants. This suggests that proline may play a major role in the scavenging of ROS in response to heavy metal stress to protect cell organelles.

- MDA levels were consistently highest in leaf tissues across all treatments, followed by stems and roots, according to overall patterns. This pattern might be explained by the increased metabolic activity and environmental exposure of leaves, which probably intensifies oxidative stress. Cr, Fe and Al were the next most severe increases in lipid peroxidation among the heavy metals examined, followed by Cu and Hg. These findings emphasize oxidative burden of *A.*

nilagirica caused by heavy metals, emphasizing their effects on lipid peroxidation and membrane integrity.

- The bioaccumulation profiles of each metal varied and were found to be influenced by the absorption, translocation and accumulation processes in *A. nilagirica*. Cu and Hg accumulation was at its highest while others were at its lowest. (hyper accumulator - Cu and Hg and metal stabilizer for others Al, Cr and Fe).

- Exposure to heavy metals causes substantial changes in metabolic profile of *A. nilagirica*, which impacts the production of secondary metabolites that may have therapeutic uses. The biosynthesis of a number of secondary metabolites is disrupted by Al toxicity; however, the development of novel molecules such as (-)-Isocaryophyllene and Thunbergol indicates an adaptive response to stress. Although exposure to Cu leads to the synthesis of novel compounds, the loss of significant therapeutic metabolites lowers the total medicinal efficacy of plant. In addition to increasing stress tolerance, Fe exposure promotes the synthesis of beneficial compounds. However, it also reduces the total therapeutic potential by reducing important bioactive molecules, particularly those associated with liver-protective, anti-inflammatory, and anticancer properties. The creation of novel molecules such as isothujol and globulol is induced by exposure to Hg, which may mitigate metal toxicity. However, the loss of beneficial chemicals such as (+)-Cedrol, and 9,12- Octadecadienoic acid, methyl ester may reduce the therapeutic value of the plant. Overall, metal stress modifies pharmacological profile of *A. nilagirica*, possibly decreasing its therapeutic potential.

- FTIR study demonstrates that the molecular structures of plant are impacted differently by various heavy metals. Functional groups in proteins and polysaccharides are not significantly affected by Al, indicating that metabolic alterations are minor. A new silica band (1020 cm^{-1}) is one of the minor changes that Cr causes, signifying metabolic changes. More noticeable alterations brought on by Cu include changes in amide vibrations and the elimination of the N-H stretching band, which are indicative of interactions with protein structure and

plant macromolecules. Fe suggests metabolic alterations unrelated to primary components because it has little influence on important functional groups. The cellular structure of plant is damaged when Hg changes the functional groups in proteins and cell walls.

- Depending on the metal and its quantity, the results clearly demonstrates that the presence of these metals modifies the cytotoxic effects of *A. nilagirica* leaf extract on cells. It is to be assumed that cytotoxicity increases in proportion to the concentration of the plant extract. The overall anticancer efficiency of extract may be diminished depending on the apparent metal-specific influence on its cytotoxic effects.

The study provides evidence that metal ions, such as Al, Cr, Cu, Fe and Hg, can alter the cytotoxic effects of *A. nilagirica*, hence reducing its anticancer potential. The potency to kill cancer cells is altered by metals when they come into touch with bioactive components. With its strong anticancer properties, control plant extract offers a natural alternative to synthetic medicines. Moreover, Cu, Fe and Hg lessen the impact of cell death; nevertheless, their effects are more pronounced than those of Al and Cr, suggesting that they would not be as effective in cancer treatment. These findings underline the need for additional investigation into the molecular mechanisms behind these interactions and the development of countermeasures, both of which will eventually boost therapeutic potential of *A. nilagirica* in anticancer treatments.

CHAPTER 7
RECOMMENDATIONS

1. The concentration of heavy metals in the natural habitat of *Artemisia nilagirica* can be measured to assess the threat of contamination.

Subsequent studies could delve further into the assessment of the concentration of heavy metals in the natural habitat of *Artemisia nilagirica* through detailed soil analysis. Conducting systematic soil tests in areas where the plant naturally grows will help determine the extent of environmental contamination and identify specific metals that pose a significant threat to its growth, physiology, and medicinal value. This approach would provide critical insights into the correlation between soil metal load and plant health, enabling the formulation of region-specific conservation and remediation strategies. Moreover, such data can serve as a baseline for environmental monitoring, facilitating the sustainable utilization of *A. nilagirica* in phytoremediation and phytostabilization approaches for the restoration and management of polluted ecosystems.

2. Investigating the Molecular Mechanisms of Heavy Metal Toxicity and Tolerance

Future research could go deeper into the molecular mechanisms underlying metal toxicity and tolerance in *Artemisia nilagirica*, even if the study concentrates on the physiological responses of this plant to different heavy metals. In particular, a gene expression analysis might be carried out to determine the responses of important stress-related genes to various metal treatments. Furthermore, examining the function of transporters, chelating agents, and metal-binding proteins in reducing metal stress may shed light on how *A. nilagirica* controls or tolerates metal exposure at the cellular level. This could potentially improve our understanding of its adaptation tactics.

3. Bioprospecting and Synthesizing Anticancer Drugs from *Artemisia nilagirica*

Future studies should concentrate on separating and synthesizing bioactive components in *A. nilagirica* for the development of cancer drugs, given the promising anticancer potential of plant in its pristine habitat. Bioactive compounds including flavonoids, terpenoids and alkaloids can be extracted and their anticancer potential examined in a variety of cancer cell lines using sophisticated techniques like HPLC, mass spectrometry and NMR spectroscopy. Once found, these substances could be chemically modified to improve their targeted administration, bioavailability and efficacy. In addition to the possibility of creating new cancer treatments, this strategy highlights beneficial utilization of *A. nilagirica* in pharmaceutical applications, which aids in environmentally friendly drug discovery.

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PRESENTATIONS AND PUBLICATIONS

Fathimath Zuhra P. & Hussain K. (2024). "Copper Toxicity-Induced Physiochemical Alterations in *Artemisia nilagirica* (Clarke)Pamp." - International Conference on Frontiers in Modern Biology (FIMB-2024). Department of Zoology, Government College for Men (A), Kadapa, Andhra Pradesh & Chonnam National University, South Korea. 6th November, 2024.

Fathimath Zuhra P. & Hussain K. (2024). "Physiochemical Alterations Imparted by Iron Toxicity in *Artemisia Nilagirica* (Clarke) Pamp." - International Conference on Advances in Plant Science (APS 2024). Post Graduate Department of Botany, KAHM Unity Women's College, Manjeri & Kerala State Higher Education Council. 25th October, 2024.

Fathimath Zuhra P. & Hussain K. (2023). "Sequel of aluminium toxicity on structural and functional properties of *Artemisia nilagirica* (Clarke)Pamp."- Two-day National Seminar on Plant Biodiversity, Genetics, Breeding & Biotechnology. Department of Botany, University of Calicut, Kerala. 7 & 8 December 2023.

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Fathimath Zuhra P. & Hussain K. (2022). "Physiochemical alterations imparted by metal toxicity in *Artemisia nilagirica* (Clarke) Pamp." -International Conference cum Workshop on Physiological and Molecular Mechanisms/Markers for Abiotic Stress Tolerance in Plants. University of Calicut, Kerala, October 26 & November 4, 2022.

Workshop/Conference Attended

1. Two-day international scientific conference on environmental research: issues, challenges and strategies for sustainable development. organized by Eurasian Academy of Environmental Sciences at Karwar, Karnataka.1& 2 December 2023.

2. International Workshop on Physiological and Molecular Markers for Abiotic Stress Tolerance in Plants organized by University of Calicut during October 31-November 4 ,2023.

3. Online Workshop on Abiotic Stress Management for Climate Resilience. SRM Institute of Science and Technology, Vendgar Nagar, Chengalpet District- 603201. 11/10/2023.

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ORIGINAL ARTICLE

IRON STRESS-INDUCED CHANGES IN FUNCTIONAL GROUPS AND SECONDARY METABOLITE PROFILES OF *ARTEMISIA NILAGIRICA* (CLARKE) PAMP.: INSIGHTS FROM FTIR SPECTROSCOPY AND GC-MS ANALYSIS

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ABSTRACT : Iron toxicity is a major abiotic stress that can significantly impact plant growth and metabolic processes, especially in medicinal plants where secondary metabolites are crucial for their therapeutic properties. *Artemisia nilagirica* (Clarke) Pamp., a medicinal plant known for its antimicrobial, anti-inflammatory and antimalarial activities, is particularly sensitive to iron toxicity. This study investigates the effects of iron toxicity on the secondary metabolite profiles and chemical groups in *A. nilagirica*, utilizing Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FTIR) spectroscopy. Plants were exposed to varying levels of iron, and their extracts were analysed to determine changes in bioactive compounds. The results show that iron toxicity significantly reduces the concentration of key secondary metabolites, such as flavonoids and terpenoids, which are essential for medicinal potential of the plant. Additionally, FTIR analysis revealed alterations in the molecular functional groups associated with these metabolites. Understanding the precise effects of iron toxicity on secondary metabolite production in *A. nilagirica* is therefore essential for improving cultivation practices and preserving the medicinal efficacy of the plant. This research provides valuable insights into how environmental stressors like iron toxicity can affect the pharmacological potential of medicinal plants.

Key words : *Artemisia nilagirica,* FTIR spectroscopy, GC-MS, iron toxicity, medicinal plants, secondary metabolites.

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INTRODUCTION

Iron (Fe) is an essential micronutrient in plants, involved in various critical biochemical processes, including photosynthesis, respiration and the synthesis of key enzymes (Briat *et al*, 2020). Despite its necessity, excessive iron accumulation in plants can lead to iron toxicity, a condition that severely affects plant growth and metabolic functions (Kochian *et al*, 2015). Iron toxicity occurs when the iron uptake surpasses the plant's ability to regulate its concentrations, leading to the production of reactive oxygen species (ROS) that cause oxidative stress and damage to cellular components, such as lipids, proteins, and nucleic acids (Halliwell and Gutteridge, 2015). The impact of iron toxicity on plants has been widely studied in agricultural crops, but its effect on medicinal plants, particularly their secondary metabolites, remains poorly understood (Rai *et al*, 2020).

Artemisia nilagirica (Clarke) Pamp., commonly known as Indian wormwood, is a valuable medicinal plant that has been used in traditional medicine for its anti-inflammatory, antimicrobial and antimalarial properties (Meena *et al*, 2018; Ameen *et al*, 2019). The plant produces a variety of secondary metabolites, including flavonoids, terpenoids, and essential oils, which are largely responsible for its therapeutic effects (Bhat *et al*, 2017). However, environmental stressors, such as metal toxicity, can alter the synthesis and composition of these bioactive compounds, potentially reducing the medicinal value of plant (Zhao *et al*, 2019). Despite the recognized importance of these metabolites, the effect of Fe toxicity on the secondary metabolite profile and chemical groups in *A. nilagirica* remains largely unexplored.

Iron toxicity can lead to significant changes in the metabolic profile of plants, with potentially adverse effects